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VAAM-Jahrestagung 2016

13.–16. März in Jena

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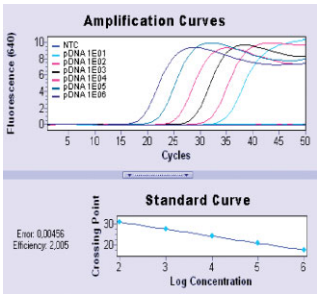

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Jahrestagung der Vereinigung für Allgemeine und Angewandte Mikrobiologie (VAAM)

Programme
Annual Conference 2016 of the Association for General and Applied Microbiology (VAAM)

13 – 16 March 2016 in Jena, Germany

Conference Chair
Prof. Dr. Axel A. Brakhage
Friedrich Schiller University Jena
Institute of Microbiology and
Leibniz Institute for Natural Product
Research and Infection Biology
Hans Knöll Institute Jena (DE)

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Programme Committee
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Organizing Society of the Conference
VAAM
Vereinigung für Allgemeine und Angewandte Mikrobiologie
Präsident: Prof. Dr. Oskar Zelder
Geschäftsstelle: Dr. Katrin Muth
Mörfelder Landstraße 125
60598 Frankfurt a. M. (DE)
Homepage: www.vaam.de

Main Programme

Abstracts

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Welcome of the President of the Friedrich Schiller University Jena

Jena has developed into a centre for microbiology: on Beutenberg Campus, microbiologists study infectious diseases and new active compounds, physicists work on novel photonic methods for pathogen diagnostics, in Lobeda physicians tackle antimicrobial resistance and in the city center microbiologists and chemists study unusual enzymatic reactions and novel mechanisms of gene regulation in fungi. I am particularly happy that the scientists succeeded in establishing a cooperative and fruitful working environment.

Often small-scale developments can make a big difference. In our case, the parable reads as follows: The Excellence Graduate School Jena School for Microbial Communication acts as a catalyst for communication between scientists – particularly those of the Friedrich Schiller University, non-university institutions and the numerous young companies. Jena is a city characterized by a tight scientific network.

Nine years ago, the Centre for Innovation Competence (ZIK) Septomics was initiated as a connecting unit between the Friedrich Schiller University Jena, the University Hospital Jena and the Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute. At the centre, scientists and physicians are working together on the investigation of sepsis – taking a special interest in the interaction of fungi with the immune system. The CSCC, the Integrated Research and Treatment Center for Sepsis Control and Care, is another initiative that was jointly launched by these partners. At the CSCC scientists are studying sepsis and its treatment, up to the inclusion of individual clinical cases. These centres are supported by basic research on invasive fungal infections within the Collaborative Research Centre/Transregio FungiNet. The Collaborative Research Centre ChemBioSys in contrast uses a variety of natural microbial communities to study chemical mediators that may be our much needed medicines of tomorrow. AquaDiva is another Collaborative Research Centre in Jena which targets microorganisms, in this case from an ecological point of view considering the ecology and biodiversity of the subterranean biogeosphere.

As you can see, the approach to microbiology in Jena is multifaceted and interdisciplinary. We hope that we can thus set a good example of how modern science is practiced and we are eager to hear and discuss the latest research results of our guests from Germany, Europe and the world!

Walter Rosenthal

Welcome of the Conference Chair

A decade has passed since we last had the chance to welcome you to our city for such a meeting. A decade in which we have not been idle: successful participation in the Excellence Initiative of the German Research Foundation with the establishment of the Jena School for Microbial Communication, large collaborative research projects have been initiated, and finally interesting scientific results at Friedrich Schiller University and non-university research institutions were achieved. But what really matters is the community among the microbiologists which enables scientific exchange and uncovers visionary research ideas. Let this year's Annual Conference of the Association for General and Applied Microbiology thrive in team spirit and fuel our creativity!

There are only selected scenes that make sports history: after a brilliant goal Miroslav Klose performs a no less brilliant somersault, Lionel Messi dribbles seemingly guided by magic towards the goal or Bastian Schweinsteiger acts as spiritus rector in the final match of the Football World Cup 2014. Those however, who pave the way for these stars mostly remain unnamed and unseen – physiotherapists, psychologists, the ball boys and girls and of course the trainer. But a team game can only be won as a team. Germany is fortunate to assemble such a successful team with many outstanding players on the international field of microbiology. Let us meet the challenge of microbial research as a team!

It is a lively and – particularly important – cooperative and friendly environment, dear colleagues, that awaits you here in Jena. Our team spirit can be seen in the realisation of projects such as the Collaborative Research Centre/Transregio FungiNet with colleagues from Würzburg, the Collaborative Research Centre ChemBioSys, the Leibniz Science-Campus InfectoOptics, and the national consortium InfectControl 2020. All of these projects take an interdisciplinary approach to microbiological issues, using novel methods. So scoring goals is not only limited to the striker – the goalkeeper can also take over.

Keeping this in mind: Welcome to the home match!

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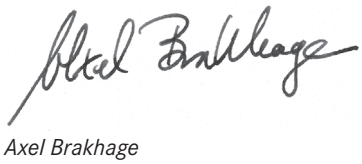

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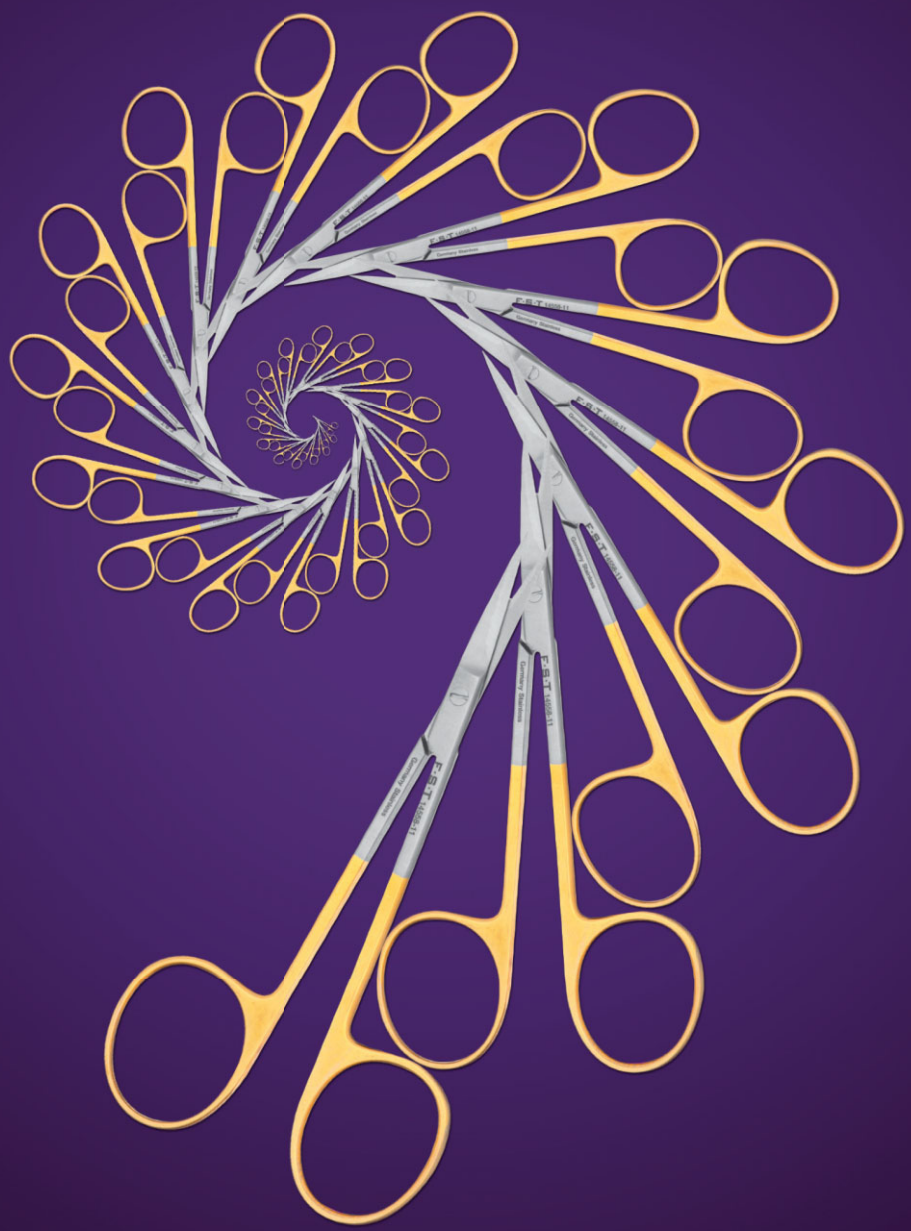

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Welcome of the President of the VAAM

In 2006 it was the first time that the VAAM conference was held in Jena. I remember it as a very well organized and highly interesting scientific conference. Therefore, I am very much looking forward to visit the second VAAM conference in Jena now in 2016. We expect 1300 participants and we can be sure that the Jena conference will continue the long series of highly attractive and successful annual VAAM meetings. For the plenary sessions the organizers have invited a variety of internationally well distinguished speakers presenting exciting topics including natural products, fungal biology and biotechnology, biodiversity and ecosystems, bio-geo-interactions and biodegradation, infection and systems biology.

It has always been a mission of the VAAM to foster young academics. Therefore, I am very happy that the VAAM annual meeting is particularly attractive for young scientists, who can present their work in short talks and in poster sessions. I am especially curious on the 'Microbe Slam', a new presentation format that we try out at the Jena conference for the first time. Furthermore, the VAAM wants to show up development perspectives for young microbiologists in the 'VAAM-Karrieresymposium', where professionals from different areas discuss their career pathways. This year it will be complemented by two other events organized for young scientist by the JSMC.

Further highlights will be the award ceremonies for the 'VAAM-Forschungspreis' and the 'VAAM-Promotionspreise'.

Last but not least I am looking forward to the VAAM annual meeting because it is an excellent opportunity to meet old friends, make new friends and to get inspired by many scientific presentations and discussions.

On behalf of the VAAM, I want to thank the conference chair Axel Brakhage and his team in particular Michael Ramm, further our congress-management firm Conventus with Alexandra Vogel as project manager and Katrin Muth from the VAAM for their great efforts and commitment in organizing the annual conference in Jena. Finally I want to thank all scientists for their contributions. I am certain that we will have an exciting conference and stimulating discussions.

Oskar Zelder

The Jena Microbiology Cluster

For millennia, mankind has conquered the earth, inhabited the uninhabitable, traversed oceans, and scaled mountains. Yet long before, microbial life on earth flourished, determining the development of flora and fauna, and far outnumbering all other species. So great is their diversity, that only a miniscule fraction of all microorganisms are known today.

Friedrich Schiller University Jena

The scientific community in Jena combines a large number of scientists who study microorganisms. Fungi, bacteria and algae are in focus: How do these microorganisms live? How are they acting in their natural habitats? And in particular: How and with whom do they communicate? The focus on microbiology is significantly shaping the research profile in Jena. The Friedrich Schiller University (FSU) Jena has committed itself to the profile line "LIFE". In addition, a number of extra university research institutes (Leibniz, Max-Planck, Fraunhofer) as well as local biotech companies and the health care industry make Jena a centre of microbiology.

Excellence graduate school Jena School for Microbial Communication

The JSMC graduate school – Jena School for Microbial Communication plays a central role. The graduate school is funded under the federal-state Excellence Initiative and offers a structured qualification and training programme for junior scientists. The JSMC addresses questions regarding microbial communication processes which influence all aspects of interactions between microorganisms and their biotic and abiotic environment.

Microbial communication and microbial communities related to natural products, infection biology and geomicrobiology

The scientists in Jena are particularly interested in microbial communities and their intra-, and interspecies interactions, in particular their relationships with humans, plants and insects. The elucidation of microbial interactions takes on many different forms: For example, the director of the Institute for Microbiology, Gabriele Diekert (Chair of Applied and Ecological Microbiology) and her team study organohalide respiration and the interactions between aerobic bacteria and lignin-degrading fungi. The Chair of Microbial Communication, headed by Erika Kothe, focuses on the interactions between basidiomycetes and forest trees, but also the environmental remediation with bacteria and fungi. Meanwhile, Johannes Wöstemeyer (Chair of General Microbiology and Microbial Genetics) is interested in understanding the developmental processes and signal transduction pathways that regulate sexual and parasitic interactions between zygomycete partners. The research group of Axel Brakhage (Chair of Microbiology and Molecular Biology) studies the infection biology of the important fungal pathogen *Aspergillus fumigatus* and also the molecular biotechnology of the production of natural products including the analysis of microbial interactions. The team of Kirsten Küsel (Chair of Aquatic Geomicrobiology) at the Institute of Ecology is interested in formation and degradation of minerals in the aquatic environment by microorganisms.

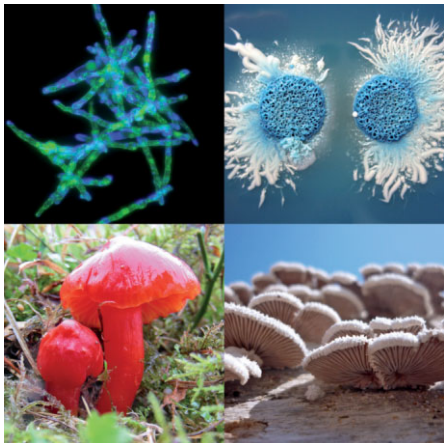
A large number of colleagues are investigating additional aspects of microbiology in connection with microbial communication, infection biology and natural product research. The research group of Thomas Winckler (Chair of Pharmaceutical Biology) studies retrotransposons in social amoeba. The team of Dirk Hoffmeister (Professor of Pharmaceutical Microbiology) is dedicated to

PhD students of the excellence graduate school Jena School for Microbial Communication benefit from a highly interdisciplinary career development programme, ambitious research projects and a thrilling cultural life of the young city.

elucidating the formation and function of natural products in basidiomycetes. The research group of Georg Pohnert (Chair of Instrumental Analytics/Bioorganic Chemistry) is interested in the chemical ecology of plankton and the defence of algae for example against bacteria by natural products. Colleagues in the Institute of Botany are also working on microorganisms. Maria Mittag (Professor of General Botany) uses the green algae *Chlamydomonas reinhardtii* to investigate the molecular mechanisms that control biological circadian clocks. The Chair of Plant Physiology, Ralf Oelmüller, studies how biomolecules that are released by fungi in the rhizosphere lead either to beneficial or pathogenic plant/fungus interactions.

Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute

During the previous decades, Jena has turned more and more into a centre of natural product research and infection biology of fungi – a development particularly influenced by the expertise of the Leibniz Institute of Natural Product Research and Infection Biology – Hans Knöll Institute – (HKI). The team of Axel Brakhage (Director HKI, Head of the Department Molecular and Applied Microbiology, and Chair of Microbiology and Molecular Biology at FSU Jena) is investigating the infection biology of the human pathogenic fungus *Aspergillus fumigatus* and the molecular biotechnology of natural products in fungi. Bernhard Hube (Department of Microbial Pathogenicity Mechanisms and Chair of Microbial Pathogenicity at FSU Jena) is studying pathogenicity using the human pathogenic yeasts *Candida albicans* and *Candida glabrata*. Peter F. Zipfel (Department of Infection Biology, Professor for Infection Biology at FSU Jena) and his team address the reactions of the complement system as part of the human immune response to bacterial and fungal infections. How, and which kind of, molecular information is exchanged during host/pathogen interactions is one of the research topics of the Department of Cell and Molecular Biology under Hans Peter Saluz (Professor for Cell and Molecular Biology at FSU Jena). Ilse Jacobsen (Research Group Microbial Immunology and Professor for Microbial Immunology at FSU Jena) develops and studies *in vivo* and *ex vivo* infection models in order to understand the reaction of the host to invading pathogen, and to look at the distribution and action of anti-infective compounds. The group of Oliver Kurzai (Professor for Fungal Septomics at FSU Jena) investigates the interaction of human cells with *Candida albicans* including the analysis of patient samples. Oliver Kurzai also heads the National Reference Centre for Invasive Mycoses.



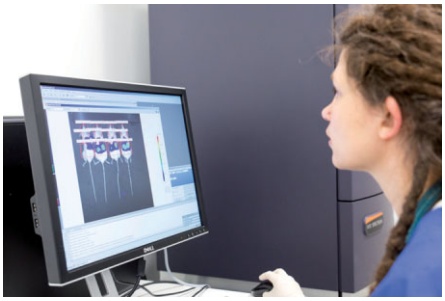
Fungi are one of the major topics of microbiological research in Jena (clockwise from upper left): *Aspergillus fumigatus*, *Candida albicans*, *Schizophyllum commune*, *Hygrocybe punicea*. © HKI (2×), FSU (2×)

While these groups focus on the infection biology of fungi, the group of Christian Hertweck (Department of Biomolecular Chemistry, and Chair of Natural Product Chemistry at FSU Jena) is concentrating on the hunt for active compounds with the goal of identifying both pharmacologically relevant substances and microbial toxins, and of understanding the production of these compounds by complex microbial consortia including anaerobic bacteria. A pillar of natural product research at the HKI is the Bio Pilot Plant, headed by Uwe Horn. The Bio Pilot Plant is unique in the academic sector, providing the technical requirements for cultivation and downstream processing of microorganisms in culture volumes of up to 2.5 m^3.

The bioinformatics and systems biology groups headed by Marc Thilo Figge (Professor of Applied Systems Biology at FSU), Reinhard Guthke (Professor of Systems Biology and Bioinformatics at FSU) and Rainer König (Professor of Systems Biology of Sepsis at UKJ), use the large amount of experimental data generated in the wet labs to develop algorithms for the modelling of infection processes and microbial interactions with the human immune system. In close collaboration with the experimental groups they furthermore analyse omics data to identify new biosynthetic activities or regulatory processes involved in microbial life cycle and multi-partner processes. The computer-based models generated by these groups are validated by laboratory experiments in iterative steps. The bioinformatics group of Stefan Schuster (Chair of Bioinformatics at FSU Jena) analyses host/pathogen interactions by evolutionary game theory and metabolic pathway analysis.

Junior Scientists

It is not only the established and experienced scientists who keep alive and advance the microbiological research in Jena. Credit for creating new impulses is due to the many junior scientists and their research groups at the university, and at the non-university research institutions on Beutenberg Campus who represent the future generation of microbiologists. The HKI Junior Research Groups, Chemistry of Microbial Communication of Pierre Stallforth, and Chemical Biology of Microbe-Host Interactions of Christine Beemelmanns, aim to elucidate microbial communication – and use specific model organisms to this end: The team of Dr Beemelmanns studies the network of termites, fungi, and bacteria, whereas Dr Stallforth studies the interactions of bacteria and the "social amoeba" *Dictyostelium discoideum*. The molecular and chemical interactions between fungi and amoeba are also studied by the group of Falk Hillmann – using the mould *A. fumigatus* and several social amoeba. Strategies for identifying and characterizing antimicrobial natural products are developed by Markus Nett and his Junior Research Group, Secondary Metabolism of predatory bacteria, at HKI. Vito Valiante starts from known biosynthesis pathways of natural products: His Research Group, Biobricks of Microbial Natural Product Syntheses, is isolating these biosynthetic pathways and transferring them to more amenable microorganisms within the framework of the Leibniz Research Cluster Biotechnology. The Junior Research Group Terrestrial Biofilms at the



Infection research in Jena ranges from the study of single molecules or cells to *in vivo* infection models combined with cutting edge imaging methods. © HKI/Schroll

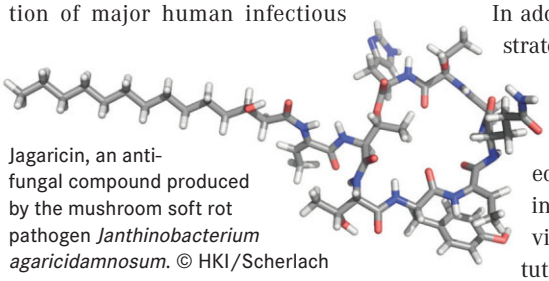
Institute of Microbiology, FSU Jena, headed by Ákos T. Kovács, has the goal of gaining a deeper understanding of the different growth strategies in biofilms (competition between growth rate and yield), employing the gram positive model bacterium *Bacillus subtilis*. The identification of novel natural products that can be synthesized by algae is the topic of the group of Severin Sasso (Juniorprofessor for Molecular Botany at FSU).

Max Planck Institute for Chemical Ecology

Together with the Friedrich Schiller University, the Beutenberg Campus with its non-university research institutes, two business incubators, and one renowned biotech company, forms the scientific epicentre of the city on the river Saale. Next to the HKI, the Max Planck Institute for Chemical Ecology is also situated on Beutenberg Campus. Research at the institute focuses on the coevolution between plants and insects, a process that naturally involves microorganisms. Wilhelm Boland (Professor for Bioorganic Chemistry at FSU Jena) and his Department of Bioorganic Chemistry are working on the defence mechanisms of plants and insects, and their interactions on different levels. The biosynthetic potential of insects is influenced and enhanced by symbiotic gut bacteria and endosymbionts. Ian Baldwin (Professor of Ecology at FSU Jena) and his team at the Department of Molecular Ecology study ecological interactions of plants, the herbivores that feed on them and the fungi and bacteria in contact with their roots. Their aim is the identification of traits that are important for an organism's Darwinian fitness. The Department of Biochemistry, headed by Jonathan Gershenzon (Professor of Plant Ecological Chemistry at FSU Jena) addresses the question how plants protect themselves from pathogens and herbivores by the production of defensive chemical compounds. Christian Kost and his Research Group, Evolutionary Ecology and Evolution, concentrate on the ecological basis of cooperative interactions of microorganisms – working at the interface of molecular microbiology and synthetic biology.

Max Planck Institute for the Science of Human History

At the Max Planck Institute for the Science of Human History archaeologists, linguists and geneticists work closely together to answer key questions of human history by using modern computational and genetic methods. The Department of Archaeogenetics, headed by Johannes Krause, focuses on host-pathogen interactions throughout history providing direct evidence for the origin, causative agents, dissemination and evolution of major human infectious disease. The results will provide a better understanding of epidemic outbreaks in the past, and comparisons of historic and modern strains will broaden our understanding of co-evolutionary changes between pathogens and their human host. A second major research topic of his group is the reconstruction of population structures of modern humans in Eurasia spanning the last 40,000 years to elucidate the different events of dispersal and population relationships.



Jagaricin, an antifungal compound produced by the mushroom soft rot pathogen *Janthinobacterium agaricidamnosum*. © HKI/Scherlach

University Hospital Jena – Infections diseases and sepsis

Thanks to the increasing understanding of microbial pathogenicity mechanisms, novel opportunities for diagnosing and treating infectious diseases arise. Several institutions of the University Hospital Jena (UKJ) are dedicated to this aspect. Sepsis research, prevention, diagnosis, and successful treatment of sepsis are at the focus of the research. The topic, which was initiated Konrad Reinhart and his colleague Michael Bauer heading the Department of Anesthesiology and Intensive Care Medicine, has developed into a central research area at the UKJ and has served as starting point for several initiatives and research programmes. The Institute of Medical Microbiology at the UKJ, headed by Bettina Löffler (Chair of Medical Microbiology), is dedicated to patient care by diagnostics and prevention of microbial infectious diseases. In addition, the team studies the infection strategies of *Staphylococcus aureus* as well as classical intracellular pathogens such as Chlamydiae and Mycobacteria – the institute is even a designated consultant laboratory for chlamydial infections. Novel diagnostic options for virus infections are tested at the Institute of Virology and Antiviral Therapy at the UKJ (Andreas Sauerbrei), with a focus on herpes, picorna, and influenza viruses.

Bacterial symbionts of the beewolf synthesize antiinfective substances as visualized by imaging mass spectrometry (LDI imaging, pseudocolour display) © MPICE/Kroiß+Kaltenpoth

Friedrich-Loeffler-Institut – Federal Research Institute for Animal Health

Two institutes of the Friedrich-Loeffler-Institut are located in Jena: The Institute for Bacterial Infections and Zoonoses headed by Heinrich Neubauer is interested in the etiology of communicable diseases of animals and on countermeasures against them. Special emphasis is given to bacteria and bacterial toxins which are transmitted from animals to humans resulting in adverse health effects or disease (zoonoses). Christian Menge and his team at the Institute of Molecular Pathogenesis study the interactions between bacterial agents causing animal diseases and zoonoses and their hosts on the molecular level with focus on Chlamydiae and bovine Mycobacteria. Currently both institutes harbour 19 National Reference Laboratories for animal infections.

Ernst Abbe Hochschule Jena – University of Applied Sciences

The research activities of Thomas Munder and his group Gene Technology/Technical Microbiology are focused on novel therapeutic strategies against tuberculosis based on protein-protein interaction studies.

Microbiologists as a team

Each scientist adds his or her specific expertise to the advancement of microbiological knowledge. The speciality in Jena is not the number of lone investigators, but the close collaboration between the individual specialists in many collaborative research projects – microbiology as interdisciplinary teamwork. These joint projects are paying tribute to the concept that progress in the areas of both basic and applied research is easier to

realise through collaboration. Two starting points led to the further development of results from basic research: Novel active compounds from microorganisms for the sake of human health, and the study of infection mechanisms and immune responses to develop novel strategies for diagnosis and therapy.

The DFG-funded **Collaborative Research Center/Transregio 124 Pathogenic fungi and their human host: Networks of interaction FungiNet** (speaker Axel Brakhage) was established by scientists in Jena and Würzburg. The goal is the elucidation of the complex mechanisms of infection by the yeast *Candida albicans* and the filamentous fungus *Aspergillus fumigatus,* and to use a systems biology approach to create a virtual infection model, serving as starting point for the development of efficient prevention and therapeutic strategies.

The aim of the **DFG Collaborative Research Center 1127 Chemical Mediators in Complex Biosystems – ChemBioSys** (speakers Christian Hertweck, Georg Pohnert) is to investigate the fundamental control mechanisms in complex biosystems, which influence our daily life. The ChemBioSys members study novel complex mediators and their places of action, the development of complex microbial communities and the processes which structure these communities and maintain the biodiversity. The long term goal lies is in the targeted manipulation of complex biosystems by chemical mediators.

Research into the microbial biodiversity is at the heart of both the **DFG Collaborative Research Center 1076 AquaDiva** (speaker Kirsten Küsel) and the **Jena Microbial Resource Collection** (JMRC, speaker Kerstin Voigt). AquaDiva is concentrating on the role of water in the soil for (microbial) biodiversity and on the contribution of this biodiversity to structuring the soil as a habitat for microbial communities. The JMRC is a joint facility of the Friedrich Schiller University Jena and the Leibniz Institute for Natural Product Research and Infection Biology (HKI). Its collection of around 50,000 fungi and bacteria and its large library of natural products is not only continuously maintained and expanded on site but also serves as an important resource for research in Jena and worldwide scientific exchange.

The Center for Innovation Competence (ZIK) Septomics headed by Oliver Kurzai (Professor of Fungal Septomics at FSU) and Hortense Slevogt (Professor of Host Septomics at UKJ) brings together basic researchers and physicians to improve sepsis diagnosis and sepsis in particular caused by fungi. The aim is to understand the complex network between pathogen and host response leading to sepsis. This aim is shared by the Integrated Research and Treatment Center for Sepsis Control and Care (CSCC) headed by Michael Bauer with a slightly different focus: The CSCC is located at the University Hospital Jena and integrates clinical research and patient care in the project. Thus, at the centre many physicians closely collaborate with natural scientists.

The HKI Bio Pilot Plant closes the gap between newly developed bioprocesses and their industrial application. © HKI/Schroll

The project consortium InfectControl 2020 – Novel Antiinfective Strategies – Science · Society · Economy unites more than forty partners from academia and industry in order to work together on the effective prevention, fast diagnosis, and efficient treatment of infectious diseases. The collaborative project which is funded under the Zwanzig20 initiative of the Federal Ministry of Education and Research brings together German research institutions, public authorities, medical institutions and companies with a focus on Eastern Germany. InfectControl 2020 uses an extensive transsectoral approach encompassing all aspects of human behaviour – in addition to classical infection biological and medical questions also problems ranging from climate change and human mobility to political counselling. The Transfer Group Antiinfectives, established by the InfectControl 2020 consortium, identifies novel substances and promotes their development to drugs. The Transsectoral Research Platform is a nationwide graduate training programme that unites the doctoral researchers of InfectControl 2020 under the umbrella of infection research. The doctoral researchers of the Transsectoral Research Platform are associated to the Jena School for Microbial Communication, benefiting from the well-established extensive qualification programme.

There are also other projects that take the leap from pure microbiology towards other disciplines. In the Leibniz ScienceCampus InfectoOptics for example scientists use methods from physics and photonics to work on the diagnosis and therapy of infectious diseases. Along the same lines, the Research Campus InfectoGnostics is a public private partnership consisting of public research institution and private biotech companies aiming at a faster and safer diagnosis of infectious diseases. The Leibniz Research Cluster is an initiative of several Leibniz Institutes which join their expertise in biotechnology and engineering sciences to expand the available spectrum of methods for compound identification. The goal is to create synthetic production units for the development of novel compounds by integrating novel technologies into existing production processes.

From basic research to product development

Motivated junior scientists and interesting research results are not the only outcome of microbiological research: The founding of new companies is another option that arises. The collaborations of the research institution in Jena led to the foundation of various start-up companies which create a lively and innovative biotech environment in and around Jena. Some of them, such as Alere Technologies GmbH, Analytik Jena AG, and Wacker Biotech GmbH to name just a few were bought by large international concerns and now secure local jobs with high added value.

Conclusion

Microbiology has become an important gravity centre in Jena with focus on microbial communication and microbial communities related to natural products, infection biology and geomicrobiology. Research is thriving with the distinct team spirit of all players and the great motivation and creativity of the junior scientists. Nationally and internationally, Jena is a good place for our science. ■

JSMC Panel discussion

The secrets to scientific success: career advice from experienced scientists

Chairs: **Natalie Töpfer, Carolin Dewald, Daniel Leichnitz, Miguel Tovar**

■ Do you wonder what a PI looks for in a postdoc? Is my research of good quality? How can I increase the impact of my research? Should I patent or publish? What are my career perspectives in microbiology?

If you have asked yourself these or related questions, you are welcome to attend the panel discussion. A panel of experienced scientists will share their career advice and perspectives for young microbiologists and engage in an interactive discussion with the audience, moderated by JSMC students. ■

Location: Hörsaal 3
Date: Monday, 14 March 2016
Time: 12.45 – 13.45 h

JSMC Lunch symposium

Microbiologists' communication: Networking and communicating your science

Participants: **Prof. Michael Poulsen, Dr. Ivan Savin**
Chairs: **Maja Rischer, René Benndorf**
How to reach the public with your science? How to build a stronger scientific network?

■ Prof. Poulssen (University of Copenhagen, Denmark) and Dr. Savin (Karlsruhe Institute of Technology, Germany) will discuss networking and public relations in science, exchanging their experiences and interacting with the participants of the workshop. In this Lunch workshop you will learn the basics and strategies for networking with PhD students, professors, companies and the public.

The Jena School for Microbial Communication (JSMC) and the company Wacker Biotech GmbH Jena will prove Lunch boxes. The number of participants is limited to 60. So first come, first served. ■

Location: Hörsaal 3
Date: Tuesday, 15 March 2016
Time: 13.00 – 13.55 h

Young scientists get-together

■ The Jena School for Microbial Communication (JSMC) invites all postdocs, PhD, master and bachelor students to a get-together on Monday, 14 March 2016. Starting at 20.00 h, with free admission for everybody, the event will take place at Rosenkeller, Johannistrasse 13 (5 min walk from the conference location). The get-together focuses on networking and encourages scientific discussions with other students from different countries in a harmonious and relaxed atmosphere. Sharing experiences and ideas can be synergistic with having fun! Please bring your name tag with you. ■

Location: Rosenkeller, Johannisstrasse 13
Date: Monday, 14 March 2016
Time: 20.00 h (meet at Venue entrance and walk to Rosenkeller) – open end

InfectControl 2020 – New Anti-infective Strategies – Science · Society · Economy

Panel discussion: New drugs and resistances

Participants: **Prof. Michael Bauer**, University Hospital Jena, **Prof. Peter Hammann**, Sanofi, **Dr. Sabina Heim**, Ascenion, **Prof. Christian Hertweck**, Hans Knöll Institute
Chair: **Axel Brakhage**, Hans Knöll Institute

■ A rapidly increasing threat is arising from new or resistant pathogens and their growing global dissemination that affects all areas of human life. This threat is further aggravated by a drastic lack of (new) effective drugs as well as insufficient preventive and diagnostic possibilities. InfectControl 2020 is an application-oriented consortium of enterprises and research institutions which aims at developing solutions to these problems ranging from the national to the global level. It is funded within the scope of the programme Zwanzig20 – Partnerschaft für Innovation initiated by the Federal Ministry for Education and Research (BMBF). InfectControl 2020 cordially invites interested researchers of all microbiological disciplines to a prominently staffed panel discussion including short keynote presentations focusing on new ways to overcome resistances by novel drugs. ■

Location: Hörsaal 3
Date: Tuesday, 15 March 2016
Time: 15.40 – 16.55 h

Venue

Friedrich Schiller University Jena
Campus Ernst-Abbe-Platz
Carl-Zeiss-Straße 3 · 07743 Jena (DE)

Address for correspondence

Conventus Congressmanagement & Marketing GmbH
Alexandra Vogel
Carl-Pulfrich-Strasse 1
07745 Jena (DE)
Phone: +49 (0)3641 31 16-361
E-Mail: vaam-kongress@conventus.de
www.vaam-kongress.de

Conference Language

Conference language is English.

Registration

Please register online at www.vaam-kongress.de.

Conference Tickets

Member [1]	275 EUR
Member Student [1, 2]	85 EUR
Member Retiree [1]	100 EUR
Non Member	345 EUR
Non Member Student [2]	110 EUR
Non Member Retiree	140 EUR

Day Tickets

Member [1]	140 EUR
Member Student [1, 2]	50 EUR
Member Retiree [1]	50 EUR
Non Member	180 EUR
Non Member Student [1]	70 EUR
Non Member Retiree	70 EUR

Social Programme[3]

Welcome Reception, 13 March 2016 – for participants	included
Welcome Reception, 13 March 2016 – accompanying person and Day Tickets	15 EUR
Mixer, 15 March 2016 – for participants	included
Mixer, 15 March 2016 – accompanying person and Day Tickets	25 EUR

[1] Members of the VAAM, GBM, DECHEMA, or DGHM
[2] Proof of status required. Please send via e-mail at registrierung@conventus.de, via Fax +49 3641 31 16-244 or postal at Conventus GmbH · Keyword: VAAM 2016 · Carl-Pulfrich-Straße 1 · 07745 Jena (DE)
[3] Registration required.

Payment/Confirmation of Payment

Please process the payment after receipt of the invoice, making reference to the invoice number. Payment via credit card is also possible (Master-/Eurocard, AmericanExpress, Visa Card). Should you transfer your invoice amount within 10 days of the start of the event, please present your transfer remittance slip at the Check-In desk as proof of payment.

Registration fees include:

- Participation in the scientific programme as well as access to the industrial exhibition
- Opening event
- Conference documents (programme, abstract book, etc.)
- Beverages within the scope of the breaks given in the programme

The conference fee also includes the provision of snacks and drinks at the welcome reception as well as buffet and beverages at the Mixer.

Check-In

The Check-In can be found on the ground floor of the conference venue Campus Ernst-Abbe-Platz, next to the main entrance.

No longer waiting lines at the counter!
With our Quick Check-In you can check in fast and comfortably by yourself. After your invoice is paid you receive a QR code (approximately 2 weeks prior to the congress itself). Please hold it under the scanner at the Quick Check-In counter on site. Your **name badge** will be printed out directly in seconds and your registration is completed.

Opening Hours

	Sunday	Monday	Tuesday	Wednesday
Industrial Exhibition	15^{00}–21^{00} h	09^{30}–19^{30} h	10^{00}–19^{30} h	10^{30}–13^{00} h
Check-In	13^{00}–19^{00} h	07^{30}–19^{30} h	08^{00}–19^{30} h	08^{30}–13^{00} h
Media Check-In	13^{00}–19^{00} h	07^{30}–18^{00} h	08^{00}–18^{00} h	08^{30}–11^{00} h

Wardrobe

The wardrobe can be found in Seminarraum 121 on the first floor and will be free of charge. Please note that the capacity is limited. We therefore kindly ask you to only use the wardrobe if absolutely necessary, e.g. preferably for your luggage.

Internet

We can offer you the following opportunities for internet usage:

Education Roaming (eduroam)
If your home university participates in eduroam and you have an internet account at your home university, you should be able to use the eduroam network at the the Friedrich Schiller University Jena.
Just follow these easy steps:

- Follow the eduroam tutorials of your home university.
- As Wi-Fi network choose eduroam.
- For user name, outer identity, inner identity and password, exclusively follow the eduroam tutorials of your home university. Please keep in mind that a short outer identity (e.g., only Muellerx) will not work in eduroam networks.

Hint: Before you use the eduroam network at the Friedrich Schiller University, we suggest to first successfully connect at least one time with the eduroam network at your home university.

Voucher
Every participant without access to the eduroam network has the opportunity to get a free voucher at the Check-In on site.

Poster Session

The poster presentations are divided in two sessions.
Monday, 15.30 – 17.30 h: All posters with even numbers (e.g. XXP02, XXP04) will be presented on 14 March 2016 from 15.30 – 17.30 h. We kindly ask all poster authors with the corresponding poster IDs to be present at their poster during this time, for presentation, discussion and questions.
Tuesday, 15.30 – 17.30 h: All posters with odd numbers (e.g. XXP01, XXP03) will be presented on 15 March 2016 from 15.30 – 17.30 h. We kindly ask all poster authors with the corresponding poster IDs to be present at their poster during this time, for presentation, discussion and questions.

Poster Sessions are allocated in the following rooms:
Tent: Archaea and Extremophiles (AEP01 – AEP32); Biodiversity and Ecosystem Functions (BEFP01 – BEFP28); Biotechnology (BTP01 – BTP88); Microbial Communication (MCP01 – MCP86); Synthetic Microbiology (SnMP01 – SnMP16)
EG/ Hörsaal 4: Natural Products (NPP01 – NPP37)

1. OG
Foyer: Environmental Microbiology (EMP01 – EMP70); Systems Microbiology (SsMP01 – SsMP09)
SR 113: Biodegradation (BDP01 – BDP28); Fungal Biology (FBP01 – FBP46)
SR 114: Infection Biology (IBP01 – IBP57); Microbial Evolution (MEP01 – MEP15)

2. OG
SR 208: Chemotaxis and Motility (CMP01 – CMP07); Signal Transduction (STP01 – STP35)
SR 207: Bioenergetics (BEP01 – BEP15); Membranes and Transport (MTP01 – MTP15); Open Topics (OTP01 – OTP08)
SR 206: Open Topics (OTP09 – OTP48)

Travel and City Map

Travel by Car
Address: Friedrich Schiller University Jena · Carl-Zeiss-Straße 3/Ernst-Abbe-Platz · 07743 Jena (DE)
Please note that there are no parking spaces available on the campus. We recommend using one of the following parking areas near the venue:

Car Park Goethe Galerie	Ernst-Abbe-Straße 15, 07743 Jena (distance to the venue: 200 m)
Fees	1 EUR per hour
Opening Hours:	Mo-Fr: 07.00 – 23.00 h, Sa: 08.00 – 23.00 h
Car Park Neue Mitte	Leutragraben 1, 07743 Jena (distance to the venue: 200 m)
Fees:	1 EUR per 30 min
Opening Hours:	Mo-Su: 07.00 – 24.00 h

Parking „Eichplatz“ (distance to the venue: 250m)
Please note: the maximum parking time is 2 hours.

Travel by Public Transport
The tramway station Ernst-Abbe-Platz (served by tramway 5, 33 and 35) is located directly next to the congress venue.

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** The booking line is available from Monday to Saturday 07.00 – 22.00 h. Calls will be charged at 0.40 EUR to per call, from mobiles 0.60 EUR to per call at maximum.

Connection Hamburg-Berlin-Munich, destination: Jena-Paradies
From station Jena-Paradies – 10 min by foot
- After leaving the train station, cross the main road in front of the station and walk straight ahead into the street Am Volksbad
- Pass the bus station and turn left into Grietgasse, follow the street for 150 m
- At the crossroads turn right and walk on Schillerstraße for about 120 m
- Then turn left, follow the same route as the tram runs, onto Ernst-Abbe-Platz

IC/ICE-Connection Frankfurt-Leipzig-Dresden, with change in Weimar, destination: Jena-West
From station Jena-West – 10 min by foot
- After leaving the train station turn left and continue walking to the main road Westbahnhofstraße
- Turn right and follow the road downhill for 300m
- At the crossroads turn left into Ernst-Häckel-Straße
- After 150 m, walk across the Carl-Zeiss-Platz (right hand side) and turn right into Carl-Zeiss-Straße
- Use the underpass at the right hand side to the Ernst-Abbe-Platz

General Terms and Conditions

You can find the general terms and conditions on our conference website www.vaam-kongress.de.

City Map

1. Tagungsort / Conference Venue
2. Mixer, Volkshaus
3. Westbahnhof
4. Paradiesbahnhof

© Stadt Jena/Stand: April 2015

Mikrobiologie – Studium, Promotion und dann?

Veranstaltungen zur Karriereplanung für den wissenschaftlichen Nachwuchs

JSMC-Podiumsdiskussion: **Montag, 14. März 2016, 12.45–13.45 Uhr, Hörsaal 3**
The secrets to scientific success: career advice from experienced scientists

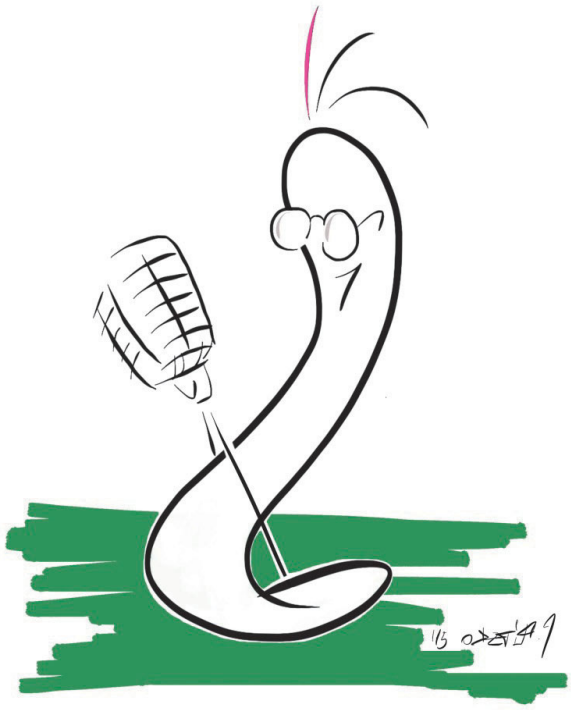
VAAM-Karrieresymposium: **Montag, 14. März 2016, 15.45–17.15 Uhr, Hörsaal 3**
Erfahrene Wissenschaftler berichten über ihre Berufswege und beantworten Fragen zur eigenen Karriereplanung
Andreas Strecker, DFG
Jan Krauss, Patentanwalt
Christine Beemelmanns, HKI Jena
Oskar Zelder, BASF

JSMC Lunch symposium*: **Dienstag, 15. März 2016, 13.00–13.55 Uhr, Hörsaal 3**
Microbiologists' communication: Networking and communicating your science

DFG-Antragsberatung:** **Montag, 14. März 2016, 09.00–12.00 Uhr, Seminarraum 130**

Andreas Strecker berät im Einzelgespräch zu den Förderprogrammen der Deutschen Forschungsgemeinschaft

*The number of participants is limited to 60. **Bitte registrieren Sie sich im Tagungsbüro.



1. Microbe Slam

15. März 2016 17.00 Uhr Hörsaal 1

RENÉ BENNDORF, Jena
The delightfulness of termites and their little helpers

OLGA SARENKOO, Berlin
Bakterielle Biofilme oder: Zusammen sind wir stark!

BENJAMIN KORTH, Leipzig
Doktorand Seltsam oder: Wie ich lernte mit Elektrochemie zu leben

BENJAMIN STEGMANN, Ulm
Phage my Clostridium

LISA SIEGMUND, Jena
A novel model for endosymbiosis in Protozoa

CORRADO NAI, Berlin
n. a.

Ihr wählt den Gewinner!

Moderator: Oliver Kling, Jena

Einladung zur Mitgliederversammlung der VAAM

■ Hiermit lade ich alle Mitglieder der VAAM zur Mitgliederversammlung ein. Sie wird am Dienstag, den 15. März, um 18.30 Uhr im Hörsaal 3 der Friedrich-Schiller-Universität in Jena stattfinden.

Vorläufige Tagesordnung:

1. Festlegung der Tagesordnung und Genehmigung der Niederschrift der Mitgliederversammlung vom 3. März 2015 in Marburg (siehe BIOspektrum 3/15, Seiten 326 und 327)
2. Bericht aus dem Präsidium und Vorstand, u. a. Haushalt 2015 und Haushaltsplan 2016, Öffentlichkeitsarbeit, Fachgruppen, BIOspektrum, VBIO, DGHM, Ort und Zeit der nächsten Jahrestagung
3. Bericht der Kassenprüfer
4. Entlastung des Vorstandes
5. Verschiedenes

Im Anschluss: Verleihung einer Ehrenmitgliedschaft

Reisekostenzuschüsse für studentische Mitglieder können bei fristgerecht eingegangenen Anträgen und bei Vorliegen der sonstigen Voraussetzungen **nur persönlich am Dienstag, den 15. März, von 13.00 Uhr bis 16.00 Uhr und Mittwoch, den 16. März 2016, von 9.30 Uhr bis 12.30 Uhr** im Tagungsbüro abgeholt werden. ■

Hubert Bahl
Schriftführer

Einladung zur Mitgliederversammlung der Sektion Mikrobiologie der Leopoldina

■ Hiermit lade ich alle Mitglieder der Sektion Mikrobiologie der Leopoldina zur Mitgliederversammlung ein. Sie findet am Montag, den 14. März, um 12.45 Uhr im Hörsaal 9 im Tagungszentrum Ernst-Abbe-Campus der Friedrich-Schiller-Universität Jena statt. ■

Michael Hecker
Senator

Leopoldina
Nationale Akademie
der Wissenschaften

Einladung zu den Mitgliederversammlungen der VAAM-Fachgruppen

Funktionelle Genomanalyse	Montag, 14.03.	12.45 Uhr	Hörsaal 8
Umweltmikrobiologie	Montag, 14.03.	17.30 Uhr	Hörsaal 1
Regulation und Signaltransduktion	Montag, 14.03.	19.30 Uhr	Hörsaal 2
Mikrobielle Pathogenität	Montag, 14.03.	19.30 Uhr	Hörsaal 3
Wasser / Abwasser	Montag, 14.03.	19.30 Uhr	Hörsaal 6
Hefen	Montag, 14.03.	19.30 Uhr	Hörsaal 9

SAVE THE DATE

MICROBIOLOGY AND INFECTION 2017

5. GEMEINSAME KONFERENZ VON DGHM UND VAAM

VAAM-Jahrestagung 2017

69. Jahrestagung DGHM

5.–8. MÄRZ 2017
WÜRZBURG

www.dghm-vaam.de

SAVE THE DATE

68.

Jahrestagung der Deutschen Gesellschaft für Hygiene und Mikrobiologie (DGHM) e. V.

11.–14. September 2016 • Messe Ulm • Ulm

Wissenschaftliche Leitung

Prof. Dr. med. Steffen Stenger
Universitätsklinikum Ulm

ulm university | universität ulm

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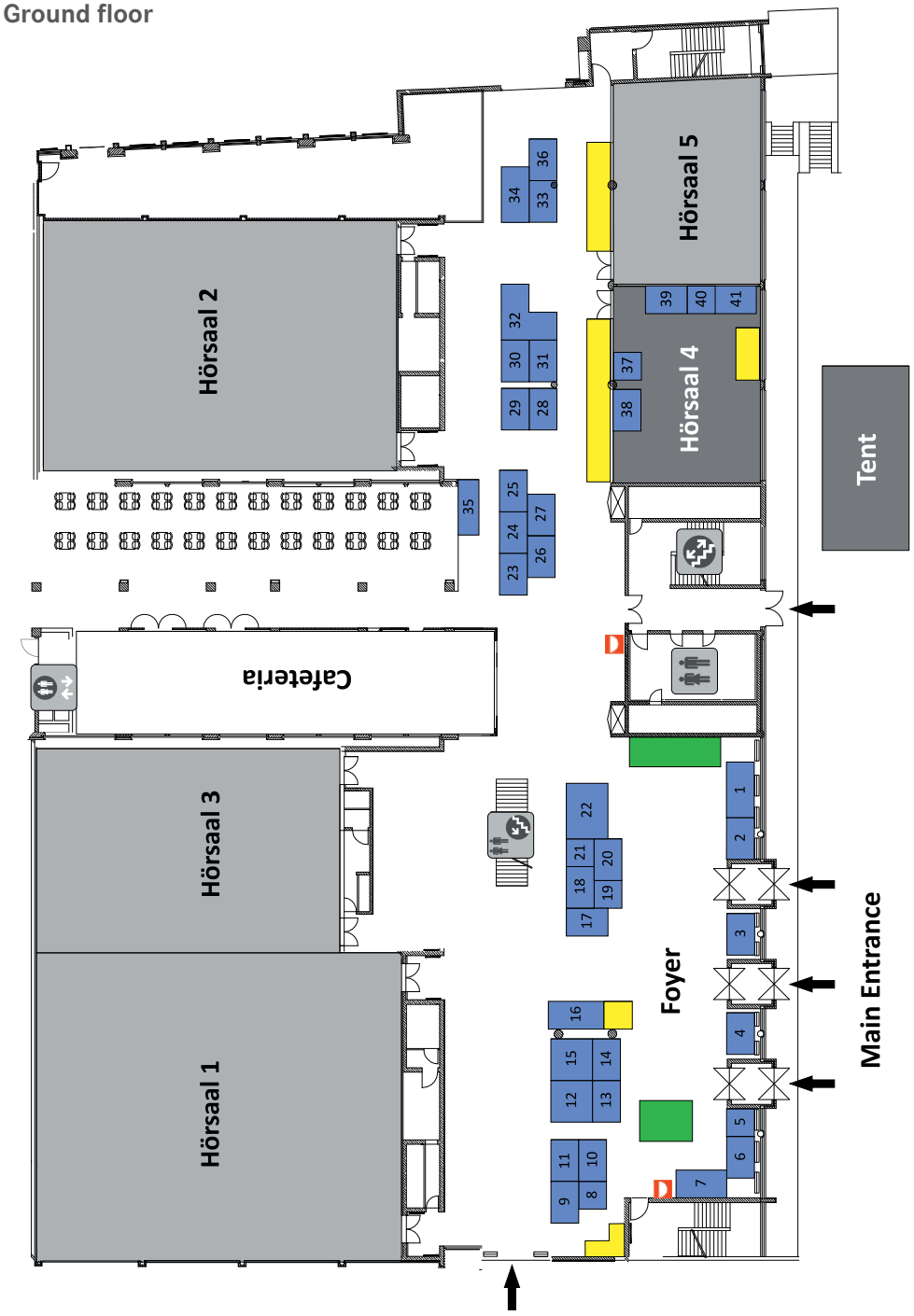


Poster Session

Tent
- Archaea and Extremophiles (AEP01 – AEP32)
- Biodiversity and Ecosystem Functions (BEFP01 – BEFP28)
- Biotechnology (BTP01 – BTP88)
- Microbial Communication (MCP01 – MCP86)
- Synthetic Microbiology (SnMP01 – SnMP16)

Hörsaal 4
- Natural Products (NPP01 – NPP37)

Industrial Exhibition
Poster Exhibition
Check-In & Quick Check-In
Lecture Hall/Seminar Room
Catering

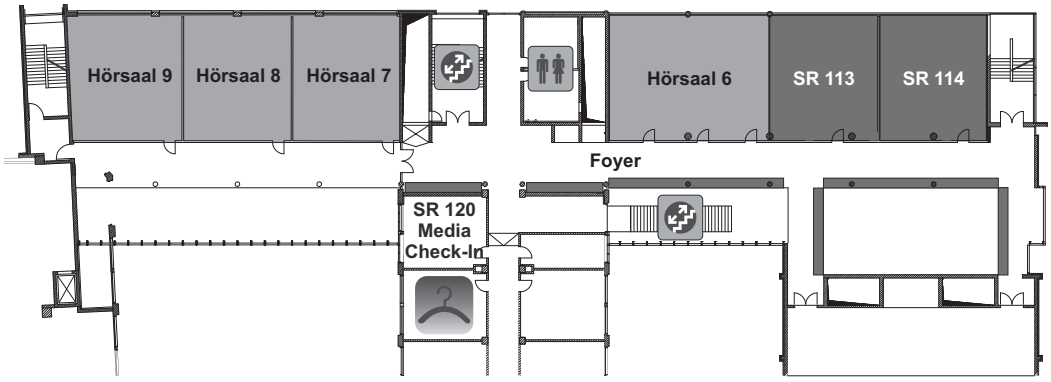
1st floor

Poster Session

Foyer
- Environmental Microbiology (EMP01 – EMP70)
- Systems Microbiology (SsMP01 – SsMP09)

SR 113
- Biodegradation (BDP01 – BDP28)
- Fungal Biology (FBP01 – FBP46)

SR 114
- Infection Biology (IBP01 – IBP57)
- Microbial Evolution (MEP01 – MEP15)



Poster Exhibition Lecture Hall/Seminar Room

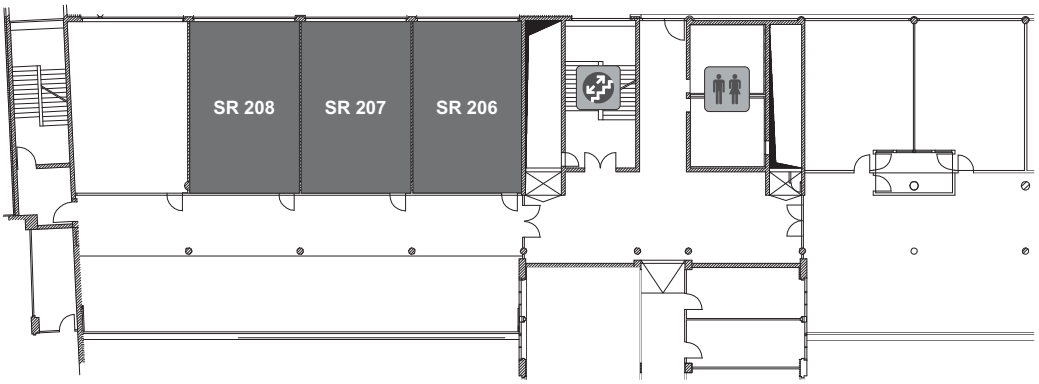
2nd floor

Poster Session

SR 208
- Chemotaxis and Motility (CMP01 – CMP07)
- Signal Transduction (STP01 – STP35)

SR 207
- Bioenergetics (BEP01 – BEP15)
- Membranes and Transport (MTP01 – MTP15)
- Open Topics (OTP01 – OTP08)

SR 206
- Open Topics (OTP09 – OTP48)



Poster Exhibition

Please remember to bring your copy of the BIOspektrum Sonderausgabe 2016; at the meeting it will cost 20 EUR

Mikrobiologie - Aktuelle Neuerscheinungen & Highlights

Joan L. Slonczewski, John W. Foster

Mikrobiologie

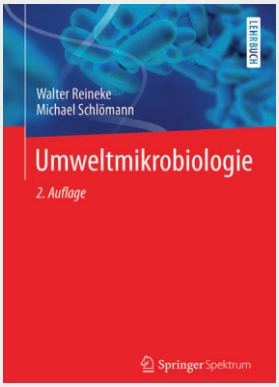
Eine Wissenschaft mit Zukunft

2. Aufl. 2012. XXVI, 1425 S. 940 Abb. in Farbe. Geb.

€ (D) 29,99 | € (A) 30,83 | *sFr 32.00

ISBN 978-3-8274-2909-4

- Neues großes Lehrbuch zur Mikrobiologie, übersetzt und adaptiert von der 2. Auflage des amerikanischen Bestsellers.
- Neue didaktische Aufbereitung des Lernstoffes: Grundlagenwissen der Mikrobiologie erklärt mit Beispielen aus der aktuellen Forschung
- Stilistisch einheitliche besonders anschauliche Farbbebilderung mit Erklärungen und korrekten Maßangabene



Walter Reineke, Michael Schlömann

Umweltmikrobiologie

2., überarb. u. aktualisierte Aufl. 2015. XIV, 494 S. 293 Abb. Brosch.

€ (D) 39,99 | € (A) 41,11 | *sFr 50.00

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- Das Werk fußt auf der erfolgreichen Umwelt-Mikrobiologie von Wolfgang Fritsche (Gustav Fischer Verlag, 1998) und setzt dessen Tradition fort

Gerhart Drews

Bakterien – ihre Entdeckung und Bedeutung für Natur und Mensch

2., überarb. u. aktualisierte Aufl. 2015. XIII, 255 S. 47 Abb. 22 Abb. in Farbe. Brosch.

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€ 29,99 | *sFr 40.00

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- Die bedeutendsten Entdeckungen der Mikrobiologie
- Fundgrube von Details und Zusammenhängen
- Verzicht auf unnötige Fachbegriffe

Eckhard Bast

Mikrobiologische Methoden

Eine Einführung in grundlegende Arbeitstechniken

3., überarb. u. erg. Aufl. 2014, XVIII, 472 S. 31 Abb. Sonderbindung

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ISBN 978-3-8274-1813-5

- 3. überarbeitete Auflage des bewährten Laborhandbuch zu den Routinemethoden der Mikrobiologie
- Der Autor hat 30 Jahre Erfahrung mit dem mikrobiologischen Praktikum
- Neu hinzugekommen sind Regeln der Biostoffverordnung, Schnelltests zur Gramfärbung und die Epifluoreszenzmikroskopie mit zahlreichen Färbeverfahren

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Social Programme

Sunday, 13 March 2016 · Welcome Reception
The organisers welcome all participants of the conference at the industrial exhibition area. Meet your colleagues and other participants by fresh drinks and snacks.

Time 19:30 h
Place Industrial Exhibition

Tuesday, 15 March 2016 · Mixer
We like to invite you to the MIXER for speakers, participants and exhibitors. The marvellous music group "AnnRed" will play for your entertainment. An extensive buffet will conduce to your well-being. Please note that the maximum number of participants to the Mixer is limited to 1.000 simultaneously due to the safety regulations of the Volkshaus. Please make sure to wear your name badge as this serves as entrance ticket to the Mixer.

Time 20:00 h
Place Volkshaus Jena, Carl-Zeiss-Platz 15

Monday, 14 March 2016 · Young scientists get-together
The Jena School for Microbial Communication (JSMC) invites all postdocs, PhD, master and bachelor students to a get-together on Monday, 14 March 2016. The get-together focuses on networking and encourages scientific discussions with other students from different countries in a harmonious and relaxed atmosphere. Sharing experiences and ideas can be synergistic with having fun! Free admission for everybody – please bring your name tag with you.

Time 20.00 h – open end
Place Rosenkeller
Johannisstrasse 13 (meet at Venue entrance and walk 5 min to Rosenkeller)

Fachgruppe Archaea

Die Fachgruppe Archaea wurde 2005 gegründet und hat derzeit etwa 100 Mitglieder. Sie soll den an Archaea Interessierten ein Diskussionsforum bieten und den wissenschaftlichen Austausch erleichtern.

Archaea bilden eine kohärente, aber physiologisch und ökologisch sehr diverse Gruppe von Mikroorganismen. Sie wurden 1977 von Carl Woese als 3. Domäne des Lebens neben den Bakterien und Eukaryonten erkannt. In Deutschland waren Otto Kandler, Karl Otto Stetter und Wolfram Zillig an der Entwicklung dieses Konzeptes maßgeblich beteiligt. Viele Archaea leben in extremen Biotopen. Studien zu ihren spezifischen Eigenschaften haben unser Wissen über das Leben unter Extrembedingungen revolutioniert. Archaea kommen aber auch in moderaten Biotopen vor und spielen eine wichtige Rolle bei der Methanbildung bzw. im Kreislauf des Stickstoffs. Die Erforschung ihrer Ökologie, Physiologie, Energetik, aber auch ihrer Evolution und Phylogenie ist nach wie vor lohnend, wie die jüngste Entdeckung der Lokiarchaeota, die ein Bindeglied zwischen Archaeen und Eukaryoten darstellen, zeigt. Sie bieten Eigenschaften, die auch für anwendungsorientierte Fragestellungen interessant sind. Neue Zellwandmaterialien, neue Stoffwechselwege und neuartige Zellstrukturen wurden hier entdeckt, und die Enzyme aus hyperthermophilen Archaea finden Anwendung vor allem in der Molekularbiologie.

Regelmäßige Treffen der molekular orientierten Archaea-Forscher im deutschsprachigen Raum finden im jährlichen Turnus statt und werden rechtzeitig über die Webseite angekündigt. Geplant sind zudem regelmäßige Workshops und Mini-Symposien bei den VAAM-Tagungen. Für 2016 weisen wir außerdem auf folgende Veranstaltung hin:

1.–3. August 2016, Molecular Biology of Archaea, London, UK (http://www.microbiologysociety.org/conferences/focused-meetings.cfm/focused-meeting-2016-molecular-biology-of-archaea-5)

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Stellvertretende Sprecherin: Ruth Schmitz-Streit, Universität Kiel
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Fachgruppe Biologie bakterieller Naturstoffproduzenten

Die Fachgruppe Biologie bakterieller Naturstoffproduzenten beschäftigt sich mit vielfältigen Aspekten der Naturstoffbiosynthese. Dazu gehören genetische Grundlagen der Sekundärmetabolitproduktion ebenso wie deren Regulation und Biochemie. Die Fachgruppe war ursprünglich auf Streptomyceten fokussiert, die eine bedeutende Rolle als mikrobielle Antibiotikaproduzenten spielen. Neben den Naturstoffbiosynthesen waren von Beginn an auch charakteristische biologische Aspekte dieser Bakteriengruppe wie Zelldifferenzierung, Synthese von Exoenzymen und Genomstruktur Schwerpunktthemen. In den letzten Jahren haben sich die Entwicklung von Genom- bzw. Bioinformatik-basierten Techniken zur rationalen Naturstoffsuche und die Aktivierung stiller Naturstoff-Gencluster zu zentralen Themen der Fachgruppe entwickelt.

Da sich aber sowohl bei den Biosyntheseleistungen als auch bei der Zelldifferenzierung und Biologie von Actinomyceten einige Parallelen zu anderen Naturstoffproduzenten wie Myxobakterien und Cyanobakterien zeigten, wurde das Konzept der Fachgruppe erweitert. Die Fachgruppe bietet nun allen VAAM-Mitgliedern, die ein Interesse an Naturstoffbiosynthesen sowie der Biologie mikrobieller Naturstoffproduzenten haben, einen fachlichen Rahmen. Es sind auch Kolleg/inn/en willkommen, die verwandte Themen an Pilzen bearbeiten. Die Fachgruppe ist nicht zuletzt deshalb auch stark interdisziplinär ausgerichtet und vereint neben Mikrobiolog/inn/en auch Kolleg/inn/en der Chemie und Pharmazie. Die insgesamt ca. 200 Mitglieder widmen sich Themen der Grundlagenforschung genauso wie angewandten Aspekten. Traditionell gibt es eine enge Verbindung zur Pharmazeutischen Industrie, wo vor allem das therapeutische Potenzial der Naturstoffproduzenten von Interesse ist.

Die Fachgruppe beteiligt sich an der Organisation internationaler Tagungen wie der „2. European Conference on Natural Products“ im September 2015 in Frankfurt und veranstaltet seit 1985 jährlich einen Workshop, auf dem vor allem jungen Mitgliedern die Gelegenheit gegeben wird, ihre Forschungsergebnisse zu präsentieren. Das nächste Treffen wird voraussichtlich im September 2016 in Freiburg stattfinden. Die Fachgruppe hat 2015 Wahlen durchgeführt. Der langjährige Sprecher und Vizesprecher der Fachgruppe, Prof. Wolfgang Wohlleben (Universität Tübingen) wurde dabei durch den neuen Vizesprecher Helge Bode (Universität Frankfurt) abgelöst.

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Stellvertretender Sprecher: Helge B. Bode, Universität Frankfurt
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Fachgruppe Biologie und Biotechnologie der Pilze

■ Themen der Biologie und Biotechnologie der Pilze werden seit Jahrzehnten aktiv in Deutschland beforscht. Die weitreichende Bedeutung von Pilzen für Mensch und Umwelt ist unstrittig, sei es, weil sie gefürchtete Krankheitserreger sind, als effiziente biotechnologische Produktionsplattformen genutzt werden, als Saprophyten oder Symbionten in Mykorrhiza und Flechten zum Stoffkreislauf innerhalb von Ökosystemen beitragen oder als leicht handhabbare und genetisch zugängliche Modelsysteme für Eukaryonten genutzt werden.

Die Entwicklung und Anwendung neuer systembiologischer Technologien erlaubt einen tieferen und ganzheitlichen Einblick in die Molekulargenetik und die Stoffwechselleistungen pilzlicher Systeme. Dies wird in erheblichem Maße zu einem verbesserten Verständnis ihrer Biologie sowie ihrer Anwendungen führen.

Die mehr als 150 Mitglieder der Fachgruppe Biologie und Biotechnologie der Pilze widmen sich in ihren Forschungs- und Lehraktivitäten diesen Themen. Zu den Aktivitäten der Fachgruppe zählt das jährliche Mini-Symposium im Rahmen der VAAM-Jahrestagung (Seite 44) sowie eine alle zwei Jahre stattfindende Fachtagung „Molecular Biology of Fungi“, die über große internationale Strahlkraft verfügt. Die 12. MBF-Tagung wird 2017 in Jena stattfinden. ■

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Fachgruppe Cyanobakterien

■ Während des 15. Internationalen Symposiums phototropher Prokaryoten im August 2015 in Tübingen trafen sich 34 Wissenschaftler/innen zur ersten konstituierenden Sitzung der neu gegründeten Fachgruppe Cyanobakterien. Karl Forchhammer wurde als Fachgruppensprecher, Annegret Wilde als Stellvertreterin gewählt. Die Fachgruppe will die Bedeutung der Cyanobakterien im wissenschaftlichen und öffentlichen Raum vertreten und forcieren sowie die wissenschaftliche Vernetzung und den Nachwuchs fördern. Ziel ist ein jährliches Symposium speziell für Doktorand/inn/en, ein Fachgruppen-Symposium im Rahmen der VAAM-Jahrestagung oder einer Summer School. Es soll ein Forum für Doktoranden eingerichtet werden und eventuell ein jährliches Meeting, in denen Methodenkompetenzen ausgetauscht werden können. Im Rahmen der VAAM-Jahrestagung in Jena gibt es ein Symposium der Fachgruppe zum Thema „Natural Products from Cyanobacteria“ (Seite 41). ■

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Fachgruppe Qualitätssicherung und Diagnostik

■ Die von der Fachgruppe Qualitätssicherung und Diagnostik vertretenen Themen sind eng an die industrielle Praxis ausgerichtet: Desinfektion und Sterilisation von Reinräumen etwa für die Wirkstoffproduktion sowie die Nutzung der Diagnostik mit dem Ziel einer Bewertung, ob eine relevantes mikrobiologisches Risiko vorliegt. Zudem verfolgen wir den rascheren Nachweis von Mikroorganismen und Antibiotika-Resistenzen und unterstützen schnellere Nachweisverfahren in der Mikrobiologie (*rapid microbiological methods*).

Die Fachgruppe veranstaltete zwei Treffen 2015: während der Jahrestagung in Marburg sowie Mitte September in Düsseldorf. Spannende und vielseitige Vorträge kennzeichneten diese beiden Treffen – in Marburg beispielsweise zu Bioaerosolen, Reinräumen und Biofilm-Detektion. In Düsseldorf ging es um aseptische Weltraumsonden und den Nachweis von Krankheitserregern.

Auch in 2016 werden wieder zwei Fachgruppentreffen stattfinden, in denen über unterschiedlichen Themen berichtet wird: während der Jahrestagung in Jena (siehe Programm Seite 45) sowie am 18.11.16 in Braunschweig auf Einladung der DSMZ und mit Beteiligung des HZI. Für dieses Treffen können gerne Vorschläge für Präsentationen eingereicht werden. ■

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Stellvertretender Sprecher: Andreas Seiffert-Störiko, Sanofi, Frankfurt am Main
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Fachgruppe Identifizierung und Systematik

Die Bakterientaxonomie und die damit verbundene Beschreibung von neuen Taxa (Arten, Gattungen, Familien, etc.) ist die Basis, auf der neue Bakterienisolate identifiziert werden können. Dabei sollte die Nomenklatur die Bakteriensystematik widerspiegeln. In der Bakteriensystematik spielt die 16S-rRNA-Gensequenz eine essenzielle Rolle, da sie eine phylogenetische Klassifizierung neuer Arten erlaubt. Weiterhin ist eine möglichst umfassende phänotypische Charakterisierung (Morphologie, Physiologie, Chemotaxonomie) dieser neuen Arten erforderlich, um eine spätere Identifizierung neuer Isolate zu erlauben. Mit dem schnellen Anwachsen der Artneubeschreibungen gelangt die 16S-rRNA-Gensequenz häufig an die Grenzen ihrer Auflösung. Daher werden in den letzten Jahren immer öfter die Sequenzen der *house-keeping*- Gene analysiert, die in vielen Fällen dann auch die erwünschten Erkenntnisse geben. Um diesen Entwicklungen Rechnung zu tragen beschäftigten sich die Minisymposien im Rahmen der VAAM/DGHM-Jahrestagung in Dresden (2014) und der VAAM-Jahrestagung in Marburg (2015) auf Analysen von Genen und Genomen. Dabei lag der Fokus der letztjährigen Tagung auf Genomen und ihrer Bedeutung für die Bakterientaxonomie. Dieses Minisymposium fand regen Zuspruch, was auch durch eine intensive Diskussion nach den Vorträgen deutlich wurde.

Bei der diesjährigen Tagung wird kein Minisymposium stattfinden; dies ist erst wieder für die gemeinsame Tagung mit der DGHM in 2017 geplant. Dort sollen, während einer Mitgliederversammlung Sprecher und stellvertretender Sprecher der Fachgruppe gewählt werden, alternativ über Email.

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VAAM-DGHM-Fachgruppe Lebensmittelmikrobiologie und -hygiene

Vom 15. bis 17. April 2015 fand das 15. Fachsymposium Lebensmittelmikrobiologie in Freising statt, das traditionell von den beiden Fachgruppen Lebensmittelmikrobiologie der VAAM und Lebensmittelmikrobiologie und -hygiene der DGHM (Deutsche Gesellschaft für Hygiene und Mikrobiologie) veranstaltet wird. Diesmal organisierte turnusgemäß die FG der VAAM die Veranstaltung mit 126 Teilnehmern aus Wissenschaft, Lebensmittelüberwachung und -industrie.

Auf der Mitgliederversammlung, die im Rahmen des Symposiums stattfand, wurde einstimmig die Fusion der beiden Fachgruppen der DGHM und VAAM zu einer neuen gemeinsamen Fachgruppe Lebensmittelmikrobiologie und -hygiene beschlossen. Die beiden Fachgruppen kooperieren seit vielen Jahren erfolgreich und bringen dies nun durch einen gemeinsamen Fachgruppenvorstand zum Ausdruck. Zur Sprecherin wurde Mareike Wenning vom Lehrstuhl für mikrobielle Ökologie der TU München gewählt; ihre Stellvertreterin ist Agnes Weiß vom Institut für Lebensmittelwissenschaft und Biotechnologie, Fachgebiet Lebensmittelmikrobiologie und -hygiene der Universität Hohenheim in Stuttgart. Horst Neve vom Institut für Mikrobiologie und Biotechnologie am Max Rubner-Institut in Kiel wird die Aufgaben des Schriftführers im neuen Vorstand wahrnehmen.

Sprecherin:
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Das nächste Symposium wird vom 30. März bis 1. April 2016 in Hohenheim stattfinden. Informationen hierzu finden Sie unter: www.lebensmittelmikrobiologie.org.

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VAAM-DGHM-Fachgruppe Mikrobielle Pathogenität

■ Die Fachgruppe Mikrobielle Pathogenität der DGHM und VAAM richtet auf der VAAM-Jahrestagung in Jena ein Mini-Symposium mit dem Schwerpunkt „Virulence and immune evasion strategies of pathogens“ mit sieben Vorträgen aus. Nach den erfolgreichen Workshops auf der DGHM-Tagung in Münster 2015 mit 18 Vorträgen und 48 Postern ist der Schwerpunkt bei der VAAM die Modulation der Immunantwort und neue Virulenzfaktoren von pathogenen Bakterien und Pilzen (Seite 43).

Von zentraler Bedeutung in 2016 ist das dreitägige Minisymposium „Mikrobielle Pathogenität“, das im zweijährigen Turnus stattfindet und eine erneute Auflage im Juni 2016 (20.–22.6.2016) erfährt. An dieser in Bad Urach im Haus auf der Alb durchgeführten Fachgruppentagung nehmen regelmäßig über 60 Mitglieder teil. Hier steht die interaktive Diskussion mit den jüngeren Wissenschaftlern im Vordergrund. Weiterhin sind im Jahr 2016 neben der Ausrichtung von Workshops auf der DGHM-Jahrestagung in Ulm (11.–14.9.2016) der Chlamydien-Workshop in Freiburg (16.–18.3.2016), das 3. Treffen der Pneumokokken/Streptokokken Forscher (3rd German Pneumococcal and Streptococcal Symposium) in Braunschweig am HZI (8.–10.9.2016) und die vom Transregio 34 organisierte 3. Internationale Konferenz „Pathophysiologie of Staphylococci“ in Tübingen (14.–17.9.2016) unter Beteiligung der Fachgruppe geplant. Im diesem Jahr werden auch erneut die Wahlen zum Vorstand der Fachgruppe durchgeführt. Mitglieder mit Interesse an einer aktiven Mitarbeit im Vorstand möchten sich bitte beim derzeitigen Vorstand melden. ■

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Fachgruppe Mikrobielle Zellbiologie

■ Im vergangenen Jahr hat sich die Fachgruppe weiter erfreulich entwickelt und seit ihrer Neuausrichtung 2012 interessierte Mikrobiolog/inn/en hinzugewinnen können. Wir sind nun eine Gruppe mit rund 200 Mitgliedern. Das Interesse an der Fachgruppe spiegelt den zunehmenden Stellenwert des Wissenschaftsgebiets wider.

Das Minisymposium während der Jahrestagung 2015 in Marburg war gut besucht. Es hatte die Bildung von Membrandomänen in Bakterien zum Thema, ein Beispiel für die differenzierte funktionelle Kompartimentierung in Mikroorganismen. In diesem Jahr wird die Fachgruppe eine Diskussionstagung *Microbial Cell Biology 2016* auf Schloss Rauischholzhausen bei Marburg abhalten. Die *MCB 2016* findet vom 10. bis 12. Oktober statt und wird dieses Mal von Peter Graumann und seinem Marburger Team organisiert. Das erste Treffen 2014 war ein gelungener Start für diese alle zwei Jahre geplante Tagung zur mikrobiellen Zellbiologie.

Bis 2017 steht die alle vier Jahre erforderliche Verlängerung unserer Fachgruppe an, für die wir Ihre befürwortende Unterschrift benötigen. Wir werden uns dafür per Rundschreiben an Sie wenden. Bitte unterstützen Sie mit Ihrer Unterschrift und Ihrem Engagement die Fortführung und Entwicklung unserer Fachgruppe auch für die nächsten Jahre. Bei dieser Gelegenheit werde ich mich nach langer Zeit als aktiver Sprecher unserer Fachgruppe verabschieden. Für das Vertrauen, das Sie uns Sprechern in den vergangenen Jahren geschenkt haben, bedanken wir uns herzlich. Die Wahl der neuen Sprecher werden wir im Online-Verfahren durchführen; bitte nehmen Sie daran teil!

Für die Jahrestagung in Jena wünschen wir Ihnen allen wissenserweiternde Beiträge, erkenntnisreiche Gespräche und nette Begegnungen! ■

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Fachgruppe Hefen

■ Mitglieder der Fachgruppe Hefen trafen sich am Rande der 27. Conference on Yeast Molecular Genetics in Trevico Therme, Italien, vom 6. bis 12. September 2015 und tauschten sich über ihre aktuellen Forschungsprojekte aus.

Auf Antrag der Mitglieder der Fachgruppe Hefe wurde die Fachgruppe in der VAAM-Vorstandsitzung um weitere vier Jahre verlängert.

Bei der VAAM-Tagung in Jena beteiligt sich die Fachgruppe mit einem Fachgruppensymposium mit sechs Beiträgen (Seite 45).

Nach dem Symposium wird eine Mitgliederversammlung stattfinden, bei der auch ein Stellvertreter des FG-Sprechers gewählt wird. Hierzu werden die FG-Mitglieder gesondert eingeladen. ■

Sprecher: Karl-Dieter Entian, Universität Frankfurt
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Fachgruppe Regulation und Signaltransduktion in Prokaryoten

■ Die außergewöhnliche Anpassungsfähigkeit prokaryotischer Mikroorganismen unterliegt der Regulation durch ein komplexes regulatorisches Netzwerk, das die Wahrnehmung und Weiterleitung verschiedenster Umweltreize sowie intrazellulärer Prozesse ermöglicht und diese über die Steuerung der Genexpression in eine physiologische Antwort übersetzt. Die Fachgruppe Regulation und Signaltransduktion in Prokaryoten bildet eine Interessensgemeinschaft, die verschiedene Themenbereiche in diesem spannenden Forschungsfeld bündelt und ihren Mitgliedern eine Plattform für Interaktionen und Symposien bietet. Im Rahmen der VAAM-Tagung veranstaltet die Fachgruppe jährlich ein Symposium zu einem aktuellen Forschungsthema, in Jena mit dem Titel „Synthetic Regulatory Circuits in Metabolic Engineering“ (Seite 43). Für diese Veranstaltung konnten wir renommierte internationale Wissenschaftler gewinnen, die mit ihren Arbeiten zeigen, wie das grundlegende Verständnis regulatorischer Schaltkreise gewinnbringend in der biotechnologischen Stammentwicklung zum Einsatz gebracht werden kann. Als Plenarsprecher konnten wir Fuzhong Zhang von Washington University in St. Louis gewinnen, der mit seinen Arbeiten zur dynamischen Kontrolle synthetischer Stoffwechselwege einen interessanten Beitrag zu dieser Thematik leistet. Als Sprecher der Fachgruppe laden wir die Mitglieder außerdem herzlich dazu ein, sich mit Themenvorschlägen für zukünftige Symposien an uns zu richten.

Darüber hinaus möchten wir das kommende 31th Symposium „Mechanisms of Gene Regulation“ (früher geführt unter dem Namen „Plasmid-Meeting“) ankündigen, 28.–30.09.2016 in Bad Bergzabern. Wir möchten uns an dieser Stelle bereits bei Rheinhold Brückner (TU Kaiserslautern) für die Organisation dieser Veranstaltung bedanken. Mit üblicherweise ca. 80–100 Teilnehmern, bietet diese Symposiums-Serie insbesondere Nachwuchswissenschaftler/inne/n eine exzellente Plattform für Diskussionen und Kontakt zu eingeladenen internationalen Sprecher/inne/n. ■

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Stellvertretender Sprecher: Thorsten Mascher, TU Dresden
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Fachgruppe Umweltmikrobiologie

■ Der fällig Antrag für das Weiterbestehen der Fachgruppe Umweltmikrobiologie wurde 2015 mit der erforderlichen Anzahl von Unterschriften eingereicht und vom Vorstand einstimmig befürwortet. Damit können wir auch weiterhin die Umweltmikrobiologie in der VAAM unterstützen. Auf der Jahrestagung in Marburg hatten wir ein Mini-Symposium zu Methoden der Einzellzellanalytik veranstaltet, wobei die hohe Teilnehmerzahl deutlich das konstante Interesse an der Umweltmikrobiologie zeigt.

Die Jahrestagung in Jena hat einen starken Umweltschwerpunkt, den wir auch hier wieder mit einem Beitrag unterstützen. Heribert Cypionka hat ein schönes Mini-Symposium zu mikroskopischen Methoden und damit verbundenen Entdeckungen in der Umweltmikrobiologie zusammengestellt (Seite 42). In diesem Rahmen werden wir auch die Fachgruppensitzung abhalten. Wir möchten besonders die jungen Mitglieder der Fachgruppe auf die Möglichkeit hinweisen, mit Hilfe der VAAM Workshops oder kleine Meetings zu Umweltthemen organisieren zu können, die auch gerne außerhalb der VAAM-Jahrestagung stattfinden können. Interessenten können sich jederzeit an den Sprecher der Fachgruppe wenden. ■

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Stellvertetender Sprecher: Karl-Heinrich Engesser, Universität Stuttgart
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Fachgruppe Symbiotische Interaktionen

■ Ziel der VAAM-Fachgruppe Symbiotische Interaktionen ist es, den regelmäßigen Kontakt und Austausch zwischen den Arbeitsgruppen im deutschsprachigen Raum zu fördern, die Arbeiten der Fachgruppe international sichtbar zu machen sowie gemeinsame Fortbildungsveranstaltungen für den wissenschaftlichen Nachwuchs durchzuführen. Die Forschungsaktivitäten sind, ebenso wie die Fachgruppe selbst, stark interdisziplinär ausgerichtet. Im Vordergrund stehen die vielfältigen Interaktionen von Mikroorganismen mit tierischen, menschlichen oder pflanzlichen Wirten.

Im vergangenen Jahr veranstaltete unsere Fachgruppe unter der Leitung von Andreas Brune (MPI Marburg) ein Minisymposium zum Thema "*Insect Microbe Symbioses*". Zwei internationale Gastredner, Angela E. Douglas (Cornell University, USA) und Yuichi Hongoh (Tokyo Institute of Technology, Japan), referierten über Funktion und metabolische Koevolution zwischen Insekten und Mikroorganismen. Die renommierte Evolutionsbiologin Nancy A. Moran (University of Texas, USA) schilderte die Rolle von Darmbakterien in der Symbiose mit Honigbienen. Weitere Kurzvorträge aus deutschen und niederländischen Arbeitsgruppen über die vielfältigen Interaktionen von Mikroorganismen und Insekten machten das Minisymposium zu einem vollen Erfolg. Im Rahmen der Jahrestagung 2015 wurden auf derMitgliederversammlung Ute Hentschel Humeida (Universität Kiel/GEOMAR) und Martin Kaltenpoth (Universität Mainz) einstimmig als Sprecherin und Vertreter der Fachgruppe gewählt. Martin Kaltenpoth löst somit Andreas Schwiertz (Institut für Mikroökologie, Herborn) ab. Martin Kaltenpoth hat im Jahr 2015 eine W3-Professur am Lehrstuhl für Ökologie an der Universität Mainz angetreten und erforscht die Symbiose von Insekten und Bakterien. Die Fachgruppe dankt Andreas Schwiertz herzlich für sein Engagement in der Gründung der Fachgruppe und für seine vielen konstruktiven Beiträge.

Auf der VAAM-Jahrestagung Jena veranstaltet die Fachgruppe ein Minisymposium mit dem Titel "*The Chemical Language of Symbioses*", geleitet von Christian Kost (MPI Jena) (Seite 45). Mit der Stadt Jena verbindet sich ein langjähriges wissenschaftliches Interesse daran, die chemische Struktur von Naturstoffen aufzuklären, sowie deren Rolle in ökologischen bzw. angewandten Zusammenhängen, wie beispielsweise als Kommunikationssignal, Verteidigungsmetabolit oder Antibiotikum aufzuklären. Vertreten wird dieser Forschungsschwerpunkt unter anderem von dem hier ansässigen Max Planck Institut für chemische Ökologie sowie dem Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie – Hans-Knöll-Institut. Daneben wird in Forschungsverbünden wie der Graduiertenschule Jena School for Microbial Communication sowie dem SFB 1127 Chemische Mediatoren in komplexen Biosystemen die Bedeutung chemischer Moleküle bei Interaktionen von Mikroorganismen mit Pflanzen, Tieren oder anderen Mikroorganismen in einem fächerübergreifenden Ansatz erforscht. Inspiriert von dieser reichen Tradition wird sich das diesjährige Minisymposium ebenfalls diesem wissenschaftlichen Schwerpunkt widmen. Wir freuen uns, dass wir mehrere herausragende Redner/innen für dieses Symposium gewinnen konnten, unter anderen Christian Hertweck (HKI Jena) und Paul Johnston (FU Berlin), die das Thema der chemischen Kommunikation in Wirts-Symbionten-Beziehungen aus verschiedenen Blinkwinkeln beleuchten werden. ■

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Fachgruppe Wasser/Abwasser

■ Die Fachgruppe Wasser/Abwasser war an der Organisation der erfolgreichen internationalen Konferenz *How dead is dead IV* beteiligt. Die Tagung fand an der eawag, Dübendorf in der Schweiz im Mai 2015 statt und wurde von Frederik Hammes und seiner engagierten Arbeitsgruppe Trinkwasser-Mikrobiologie organisiert (http://www.hdid-conference.de). 100 Teilnehmer aus 15 Ländern diskutierten über die Relevanz und Nachweismöglichkeiten von VBNC (viable but non-culturable)-Stadien von Bakterien in der Umwelt, technischen Systemen und der Medizin. Die nächste *How dead is dead*-Tagung wird 2017 in Österreich stattfinden.

Während der VAAM-Jahrestagung in Jena lädt die Fachgruppe Wasser/Abwasser alle Interessierten am 14.3.2016 zum Mini-Symposium mit dem Titel „*Legionella* in water and air: legislation, occurrence and new detection methods" ein (Seite 44). In sechs Beiträgen referieren Expert/inn/en über aktuelle gesetzliche Rahmenbedingungen zur Legionellenproblematik, Veränderung der Artenzusammensetzung bei der Trinkwasserversorgung vom Rohwasser bis zum Zapfhahn des Verbrauchers, die Kontaminationssituation in großen Gebäuden und neue Verfahren zum Nachweis und zur Typisierung von Legionellen in Wasser und Luft.

Im Anschluss findet ab 19:30 Uhr die Fachgruppensitzung statt. Hier werden u. a. die geplanten Aktivitäten in 2016 besprochen, darunter die Beteiligung an der Organisation der HDID V-Konferenz in Österreich. ■

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VAAM-DECHEMA-Fachgruppe Biotransformationen

In dem Bestreben nach ökonomischen, öko- und ressourceneffizienten Prozessen in der Chemie-, Pharma-, Energie- und Lebensmittelindustrie haben sich Biotransformationen und biokatalytische Verfahren als Schlüsseltechnologien hervorgetan und bilden eine Grundlage der Industriellen Biotechnologie. Dieses sehr interdisziplinäre Forschungsgebiet erlebt zurzeit ein rasantes Wachstum, dem auch moderne Methoden wie Metagenomanalyse und Protein-Engineering Impulse verleihen. Biotransfomationen mit Ganzzellsystemen oder isolierten Enzymen bieten einen substanziellen Beitrag zur Herstellung von Produkten der Bioökonomie.

Die Fachgruppe Biotransformationen der VAAM wurde 1996 gegründet. Nicht zuletzt um Deutschlands gute Position in Forschung und Anwendung auszubauen, beschlossen im Jahr 2009 die beiden großen Fachgesellschaften DECHEMA und VAAM, ihre Kräfte in einer gemeinsamen Fachgruppe zu bündeln. Derzeit hat die Fachgruppe ca. 320 registrierte Mitglieder.

Die Mitglieder der Gruppe, die in der akademischen und industriellen Forschung tätig sind, vertreten verschiedene Fachgebiete von Mikro- und Molekularbiologie über die Chemie bis hin zur Bioverfahrenstechnik. Neben regelmäßigen eigenständigen meist ein- bis zweitägigen Veranstaltungen zu ausgewählten Themen, auch mit ausländischen Fachgruppen und/oder anderen VAAM- oder DECHEMA-Fachgruppen, der Organisation von DECHEMA-Kolloquien sowie in geraden Jahren der Beteiligung an der Process-Net-Tagung der DECHEMA und in ungeraden Jahren an der Frühjahrstagung der VAAM jeweils mit eigenen halb- bis ganztägigen Vortragsslots ist angestrebt, alle drei Jahre eine internationale interdisziplinär ausgerichtete Sommerschule zum Thema „Biotransformationen“ für Promovierende und junge Wissenschaftler/innen aus der Industrie zu organisieren.

Für das Jahr 2015 sind rückblickend die Veranstaltungen „Paving the way from Protein expression to Protein production“ im Rahmen der VAAM-Jahrestagung in Marburg, „New Reactions with Enzymes and Microorganisms“ in Stuttgart sowie die Unterstützung der „BioFlavor 2015“ in Frankfurt zu nennen.

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Aufruf zur Neugründung

VAAM-Fachgruppe Weltraummikrobiologie und Astrobiologie

Die Forschungsgebiete der Weltraummikrobiologie und Astrobiologie haben in den letzten Jahrzehnten Erstaunliches über die Eigenschaften und Fähigkeiten von Mikroorganismen ans Tageslicht gebracht. So sind beispielsweise bakterielle Sporen etwa 500 Mal strahlungsresistenter als Menschen und können sechs Jahre unter realen Weltraumbedingungen überleben. Doch Weltraummikrobiologie ist nicht nur spannend und interessant – sie kann auch wichtige Erkenntnisse für angewandte Forschung und Probleme auf der Erde liefern. So ist zum Beispiel die Untersuchung von bakteriellen Sporen, ihrer Resistenz und ihres Keimungsverhaltens sowohl von großem Interesse für die Astrobiologie als auch für industrielle Anwendungen (z. B. Bioindikatoren, Pharma- und Lebensmittelindustrie). Auch Plasmasterilisation, die zukünftig zur Dekontamination von Raumschiffen eingesetzt werden soll, um die Verbreitung terrestrischer Organismen auf andere Planeten und Monde zu vermeiden (*Planetary Protection*), kann gleichzeitig als effiziente Sterilisationsstrategie im medizinischen und lebensmittelbiologischen Bereich eingesetzt werden. Außerdem beeinflusst die Schwerelosigkeit sowohl die Pathogenität als auch die Antibiotikaresistenzentwicklung von Bakterien, sodass Weltraumforschung in diesem Bereich wichtige neue Erkenntnisse auf zugrunde liegende Mechanismen liefern kann, von denen wiederum die medizinische Forschung profitieren kann. Abgesehen von den vielfältigen Anwendungsgebieten auf der Erde sind Weltraumikrobiologie und Astrobiologie von grundlegender Bedeutung für die Erforschung unseres Sonnensystems, zum Beispiel mit Hinblick auf Astronautengesundheit, *Planetary Protection*, Lebenserhaltungssysteme und natürlich die Suche nach extraterrestrischem Leben.

Für die Gründung einer VAAM-Fachgruppe Weltraummikrobiologie und Astrobiologie werden 25 interessierte ordentliche VAAM-Mitglieder mit Interesse und Faszination an Raumfahrt, Weltraumexperimenten und extremen Mikroorganismen gesucht.

Bei Interesse kontaktieren Sie bitte:

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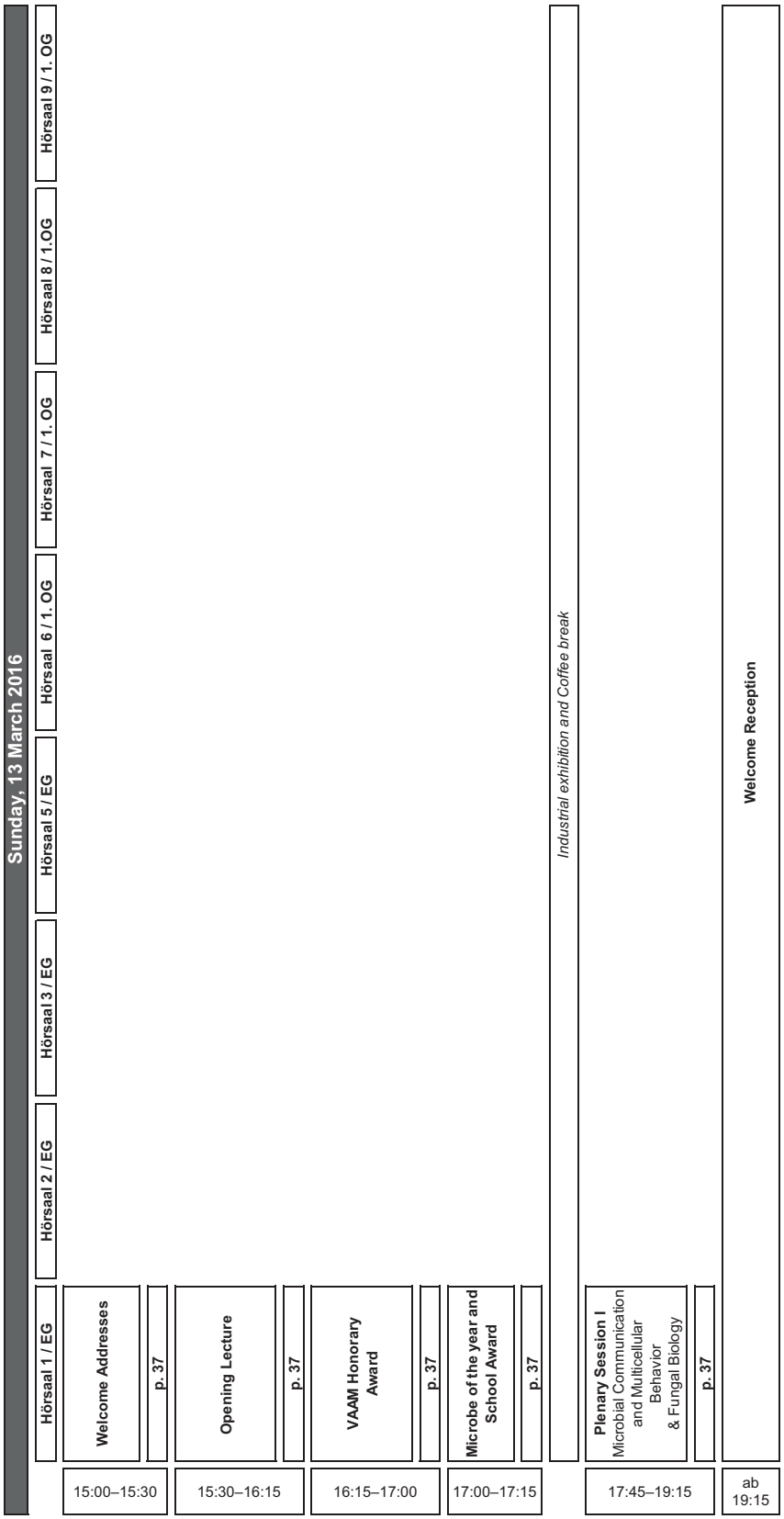
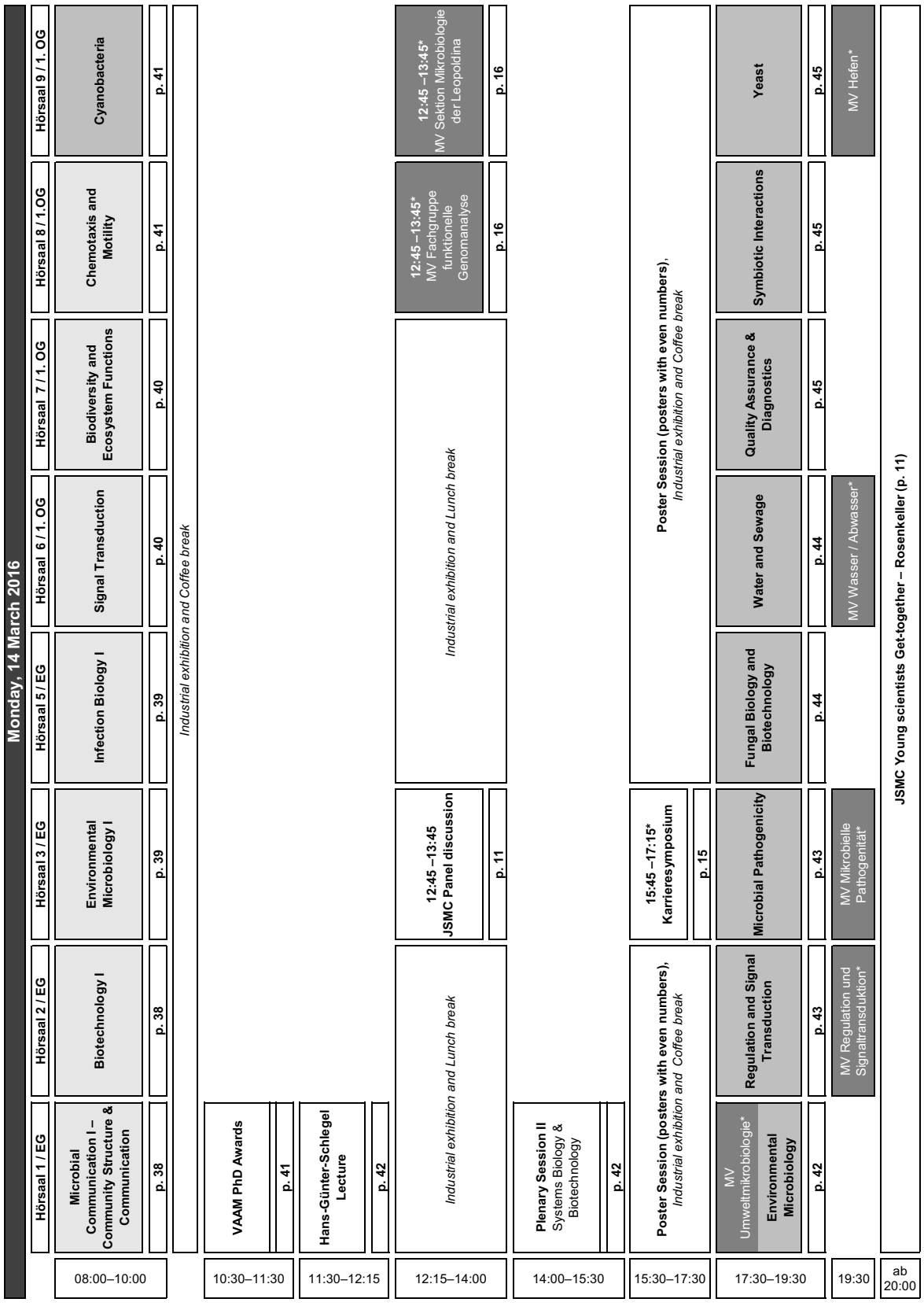
Programme Overview · Sunday · March 13, 2016



Programme Overview · Monday · March 14, 2016



Programme Overview · Tuesday · March 15, 2016

Tuesday, 15 March 2016

	Hörsaal 1 / EG	Hörsaal 2 / EG	Hörsaal 3 / EG	Hörsaal 5 / EG	Hörsaal 6 / 1. OG	Hörsaal 7 / 1. OG	Hörsaal 8 / 1.OG	Hörsaal 9 / 1. OG
08:30–10:30	**Microbial Communication II – Biofilms & Bio-Geo-Interactions**	**Biotechnology II**	**Environmental Microbiology II**	**Infection Biology & Systems Microbiology**	**Fungal Biology I**	**Natural Products**	**Synthetic Microbiology**	**Bioenergetics & Membranes and Transport**
	p. 46	p. 46	p. 47	p. 47	p. 48	p. 48	p. 49	p. 49
	Industrial exhibition and Coffee break							
11:00–11:30	**How the Nagoya-Protocol challenges microbiological research in Germany**							
	p. 50							
11:30–13:00	**Plenary Session III** Infection & Natural Products							
	p. 50							
13:00–14:00	*Industrial exhibition and Lunch break*		**13:00-13:55 JSMC Lunch symposium**	*Industrial exhibition and Lunch break*				
			p. 11					
14:00–15:30	**Plenary Session IV** Bio-Geo-Interactions & Biodegradation							
	p. 50							
15:30–17:30	**Poster Session (posters with odd numbers)**/ *Industrial exhibition and Coffee break*		**15:40 –16:55* InfectControl 2020 Panel discussion**	**Poster Session (posters with odd numbers)**/ *Industrial exhibition and Coffee break*				
			p. 11					
17:00–18:30	**Microbe Slam***							
	p. 15							
18:30–20:00			**VAAM Annual General Meeting***					
			p. 16					
ab 20:00	**Mixer – Volkhaus Jena (p. 16)**							

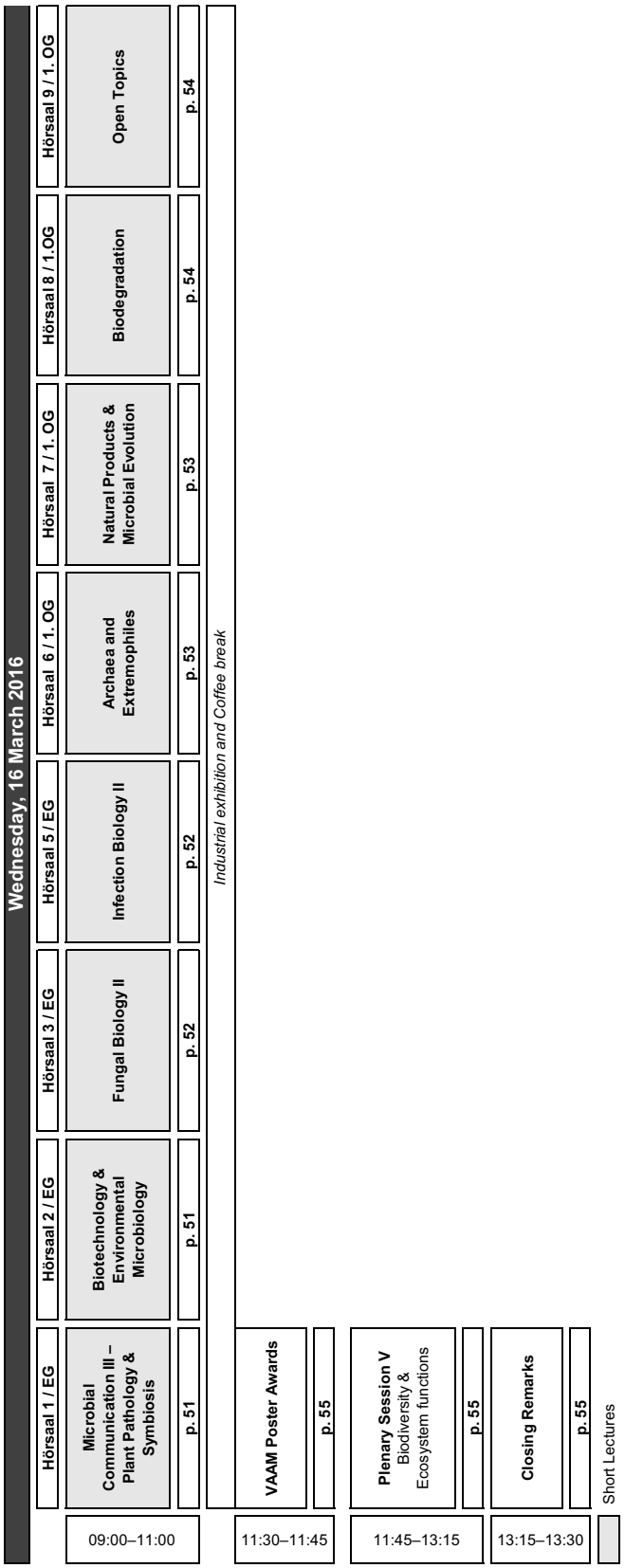
Short Lectures

Special Group Mini-Symposium

Mitgliederversammlung

* in German

Programme Overview · Wednesday · March 16, 2016



CONFERENCE PROGRAMME

Annual Conference 2016 of the VAAM

Sunday, 13 March 2016

Room	Hörsaal 1, live broadcast in Hörsaal 3
15:00–15:30	**Welcome Addresses** Axel A. Brakhage (Conference Chair) Wolfgang Tiefensee (Thuringian Minister of Economy, Science and the Digital Society) Walter Rosenthal (President of the Friedrich Schiller University Jena)
15:30–16:15 ISV01	**Opening Lecture** Science and society – infectious diseases as an example Jörg Hacker (Berlin/DE)
16:15–17:00 ISV02	**VAAM Honorary Award** Harnessing nature's sensory devices for metabolic engineering and single-cell analysis Julia Frunzke (Jülich/DE)
17:00–17:15	**Microbe of the year and School Award**
17:15–17:45	*Coffee break/Industrial exhibition*
17:45–19:15	**Plenary Session I – Microbial Communication and Multicellular & Behavior Fungal Biology**
17:45 ISV03	Exploring the pole – cellular asymmetry and adhesin localization drive biofilm formation in *Agrobacterium tumefaciens* Clay Fuqua (Bloomington, IN/US)
18:30 ISV04	Dissecting the biology and pathology of the Irish potato famine pathogen *Phytophthora infestans* Francine Govers (Wageningen/NL)
19:15–21:00 Room	**Welcome Reception** Industrial Exhibition

Monday, 14 March 2016

08:00–10:00	**Short lectures** and **Mini-Symposia Special Group** (see page 38–41)
10:00–10:30	*Coffee break/Industrial exhibition*
10:30–11:30 Room	**VAAM PhD Awards** Hörsaal 1, live broadcast in Hörsaal 3
11:30–12:15 Room ISV05	**Hans-Günter-Schlegel-Lecture** Hörsaal 1, live broadcast in Hörsaal 3 Physiological proteomics of Gram-positive model bacteria Michael Hecker (Greifswald/DE)
12:15–14:00	*Lunch break/Industrial exhibition*
12:45–13:45 Room	**JSMC Panel discussion** (see page 11) Hörsaal 3
14:00–15:30 Room	**Plenary Session II – Systems Biology & Biotechnology** Hörsaal 1, live broadcast in Hörsaal 3
14:00 ISV06	Systems biology of yeast metabolism Jens Nielsen (Göteborg, Lyngby, Stockholm/SE)
14:45 ISV07	Engineering microbial metabolism for the production of fuels and chemicals Greg Stephanopoulos (Cambridge, MA/US)
15:30–17:30	**Poster Session** (see page 13), *Coffee break/Industrial exhibition*
15:45–17:15 Room	**Karrieresymposium** (see page 15) Hörsaal 3
17:30–19:30	**Mini-Symposia Special Groups** (see page 42–45)
20:00	**JSMC Young scientists get-together** (see page 11)

CONFERENCE PROGRAMME

Annual Conference 2016 of the VAAM

Tuesday, 15 March 2016

08:30–10:30	**Short lectures** (see page 46–49)
10:30–11:00	*Coffee break/Industrial exhibition*
11:00–11:30 Room ISV08	**How the Nagoya-Protocol challenges microbiological research in Germany** Hörsaal 1, live broadcast in Hörsaal 3 Jörg Overmann (Braunschweig/DE)
11:30–13:00 Room	**Plenary Session III – Infection & Natural Products** Hörsaal 1, live broadcast in Hörsaal 3
11:30 ISV09	*Legionella pneumophila*, a unique model to study host pathogen interactions and the evolution of virulence Carmen Buchrieser (Paris/FR)
12:15 ISV10	Natural products from entomopathogenic bacteria – from chemical ecology to synthetic biology Helge B. Bode (Frankfurt a. M./DE)
13:00–14:00	*Lunch break/Industrial exhibition*
13:00–13:55 Room	**JSMC Lunch symposium** (see page 11) Hörsaal 3
14:00–15:30 Room	**Plenary Session IV – Bio-Geo-Interactions & Biodegradation** Hörsaal 1, live broadcast in Hörsaal 3
14:00 ISV11	Geomycology – metals, minerals and mycota Geoffrey M. Gadd (Dundee/UK)
14:45 ISV12	They can't do it on their own – community control over organohalide-respiring Chloroflexi Frank Loeffler (Knoxville, TN/US)
15:30–17:30 Room	**Poster Session** (see page 13), *Coffee break/Industrial exhibition* Foyer
15:40–16:55 Room	**InfectControl 2020 Panel discussion** (see page 11) Hörsaal 3
17:00–18:30 Room	**Microbe Slam** (see page 15) Hörsaal 1
18:30–20:00 Room	**VAAM Annual General Meeting** Hörsaal 3
20:00	**Mixer**

Wednesday, 16 March 2016

09:00–11:00	**Short Lectures** (see page 51–54)
11:00–11:30	*Coffee break/Industrial exhibition*
11:30–11:45 Romm	**VAAM Poster Awards** Hörsaal 1
11:45–13:15 Room	**Plenary Session V – Biodiversity & Ecosystem functions** Hörsaal 1
11:45 ISV13	Methane oxidation in Lake Constance Bernhard Schink (Konstanz/DE)
12:30 ISV14	Environmental change, niche specialisation and microbial communities – What can we learn from ammonia oxidisers? James Prosser (Aberdeen/UK)
13:15–13:30 Room	**Closing Remarks** Hörsaal 1

SCIENTIFIC PROGRAMME, SUNDAY, 13 MARCH 2016

Annual Conference 2016 of the VAAM

13:00–15:00 **Registration & Industrial Exhibition**

Room Industrial Exhibition

15:00–15:30 **Welcome Addresses**

Room Hörsaal 1, live broadcast in Hörsaal 3

Axel A. Brakhage (Conference Chair)
Wolfgang Tiefensee (Thuringian Minister of Economy, Science and the Digital Society)
Walter Rosenthal (President of the Friedrich Schiller University Jena)

15:30–16:15 **Opening Lecture**

Room Hörsaal 1, live broadcast in Hörsaal 3
Chair Axel Brakhage (Jena/DE)

ISV01 Science and society – infectious diseases as an example
Jörg Hacker (Berlin/DE)

16:15–17:00 **VAAM Honorary Award**

Room Hörsaal 1, live broadcast in Hörsaal 3
Chair Oskar Zelder (Ludwigshafen/DE)

ISV02 Harnessing nature's sensory devices for metabolic engineering and single-cell analysis
Julia Frunzke (Jülich/DE)

17:00–17:15 **Microbe of the year and School Award**
Room Hörsaal 1, live broadcast in Hörsaal 3

17:45–19:15 **Plenary Session I – Microbial Communication and Multicellular Behavior & Fungal Biology**

Room Hörsaal 1, live broadcast in Hörsaal 3
Chairs Wilhelm Boland, Erika Kothe (Jena/DE)

17:45–18:30 ISV03 Exploring the pole – cellular asymmetry and adhesin localization drive biofilm formation in *Agrobacterium tumefaciens*
Clay Fuqua (Bloomington, IN/US)

18:30–19:15 ISV04 Dissecting the biology and pathology of the Irish potato famine pathogen *Phytophthora infestans*
Francine Govers (Wageningen/NL)

19:15–21:00 **Welcome Reception**

Room Industrial Exhibition

SCIENTIFIC PROGRAMME, MONDAY, 14 MARCH 2016

Annual Conference 2016 of the VAAM

08:00–10:00 **Short Lectures – Microbial Communication: Community Structure & Microbial Communication**

Room	Hörsaal 1
Chairs	Ulrich Kück (Bochum/DE), Miguel Tovar (Jena/DE)
08:00–08:15 MCV09	The social amoeba and its opponents – a source of novel small molecules Pierre Stallforth (Jena/DE)
08:15–08:30 MCV10	Microbial invasion into drinking water-related bacterial communities Nicole Hahn (Ghent/BE)
08:30–08:45 MCV11	*Staphylococcus schleiferi* volatiles inhibit quorum sensing controlled phenotypes in Gram-negative bacteria Marie Chantal Lemfack (Rostock/DE)
08:45–09:00 MCV12	Ultrafast alignment and analysis of metagenomic DNA sequence data from the Tyrolean Iceman using MALT Alexander Herbig (Jena/DE)
09:00–09:15 MCV13	Auxotrophy and intrapopulation complementary in the 'interactome' of a cultivated freshwater model community Sarahi L. Garcia (Uppsala/SE)
09:15–09:30 MCV14	Genome-wide mapping of *Aspergillus nidulans* and *Streptomyces* interaction Juliane Fischer (Jena/DE)
09:30–09:45 MCV15	Molecular basis of the symbiotic interaction between prokaryotes in phototrophic consortia Petra Henke (Braunschweig/DE)
09:45–10:00 MCV16	Global and local patterns of bacterial communities associated with peatland bryophytes Andrea Kiss (Potsdam/DE)

08:00–10:00 **Short Lectures – Biotechnology I**

Room	Hörsaal 2
Chairs	Uwe Horn (Jena/DE), Vera Meyer (Berlin/DE)
08:00–08:15 BTV01	Model-based metabolic engineering of *Escherichia coli* for high yield itaconic acid production Björn-Johannes Harder (Magdeburg/DE)
08:15–08:30 BTV02	Construction of plasmid-free bacterial strains for the synthesis of human milk oligosaccharides Florian Baumgärtner (Stuttgart/DE)
08:30–08:45 BTV03	The 2-C-methyl-D-erythritol 4-phosphate pathway as a platform for isoprenoid formation – metabolic regulation and engineering of isoprenoid production in microbes Daniel Volke (Aachen/DE)
08:45–09:00 BTV04	Enzymatic hydrolysis of macroalgae for the production of biobased chemicals Christin Burkhardt (Hamburg/DE)
09:00–09:15 BTV05	Acetoin production via unbalanced fermentation in *S. oneidensis* Thea Bursac (Karlsruhe/DE)
09:15–09:30 BTV06	Microbial synthesis of butadienes – a look into patent literature Jens Harder (Bremen/DE)
09:30–09:45 BTV07	Engineering industrial acetogenic biocatalysts – a comparative metabolic and genomic analysis Frank R. Bengelsdorf (Ulm/DE)
09:45–10:00 BTV08	α-Ketoglutarate production from pentose *in vitro* – one of the bedstones for hydroxyl amino acids production *in vivo* Lu Shen (Essen/DE)

Annual Conference 2016 of the VAAM

08:00–10:00 Short Lectures – Environmental Microbiology I

Room: Hörsaal 3
Chairs: Christiane Dahl (Bonn/DE), Georg Pohnert (Jena/DE)

08:00–08:15 EMV01	The sRNA NsiR4 is involved in nitrogen assimilation control in cyanobacteria by targeting glutamine synthetase inactivating factor IF7 Stephan Klähn (Freiburg/DE)
08:15–08:30 EMV02	Unprecedented hydrogen production of free-living Epsilonproteobacteria (*Sulfurospirillum* spp.) Stefan Kruse (Jena/DE)
08:30–08:45 EMV03	Where to dig for exoelectrogens or is there any ecological niche of electroactive microorganisms? Christin Koch (Leipzig/DE)
08:45–09:00 EMV04	Environmental distribution and enrichment of anaerobic methanotrophs from Italian paddy fields Claudia Lüke (Nijmegen/NL)
09:00–09:15 EMV05	*Methylomagnum ishizawai* gen. nov., sp. nov., a mesophilic type I methanotroph isolated from rice rhizosphere Ashraf Khalifa (Hofuof/SA)
09:15–09:30 EMV06	Nutrient and increasing temperature effects on the microbial community structure and function in streambed sediments Elisabeth Pohlon (Gießen/DE)
09:30–09:45 EMV07	The dark side of the Mushroom Spring microbial mat – life in the shadow of chlorophototrophs Vera Thiel (Tokyo/JP, University Park, PA/US)
09:45–10:00 EMV08	Bacteria dominate the short-term assimilation of plant-derived N in soil Robert Starke (Leipzig, Hohenheim/DE)

08:00–10:00 Short Lectures – Infection Biology I

Room: Hörsaal 5
Chairs: Bettina Löffler (Jena/DE), Ilse Jacobsen (Jena/DE)

08:00–08:15 IBV01	CRASP11 recruits soluble human complement regulators and mediates complement evasion of *Candida albicans* Justus Linden (Jena/DE)
08:15–08:30 IBV02	*Candida albicans* modulates the immune response of human blood monocytes Emeraldo Jo (Jena/DE)
08:30–08:45 IBV03	The killing of macrophages by *Corynebacterium ulcerans* Elena Hacker (Erlangen/DE)
08:45–09:00 IBV04	Identification of Biomarkers for Invasive Aspergillosis in the Urine Silke Silva (Jena/DE)
09:00–09:15 IBV05	The extracellular adherence protein (Eap) of *Staphylococcus aureus* affects proliferation and migration of eukaryotic cells by altering the adhesive and morphological properties of the host cell Janina Eisenbeis, Markus Bischoff (Homburg/DE)
09:15–09:30 IBV06	Quantitative proteomics reveals the dynamics of protein phosphorylation in human bronchial epithelial cells during internalization, phagosomal escape and intracellular replication of *Staphylococcus aureus* Erik Richter (Greifswald/DE)
09:30–09:45 IBV07	The cystic fibrosis lower airways microbial metagenome Patricia Moran Losada (Hannover/DE)
09:45–10:00 IBV08	Clinical *Streptococcus pneumoniae* isolates from patients with pneumococcal hemolytic uremic syndrome efficiently control host innate immune attack Christian Meinel (Jena/DE)

SCIENTIFIC PROGRAMME, MONDAY, 14 MARCH 2016

Annual Conference 2016 of the VAAM

08:00–10:00 Short Lectures – Signal Transduction

Room: Hörsaal 6
Chairs: Sabine Brantl (Jena/DE), Gerhard Braus (Göttingen/DE)

08:00–08:15 STV01 Cross-talk between the Kdp and Pho two-component systems interconnects K^+ and PO_4^{3-} homeostasis in *Escherichia coli*
Hannah Schramke (Martinsried, München/DE)

08:15–08:30 STV02 Coping with stress – convergence of cell cycle and stress signaling pathways by a bifunctional histidine kinase
Kristina Heinrich (Marburg/DE)

08:30–08:45 STV03 Genetic analysis of competence development in *Micrococcus luteus*
Angel Angelov (Freising/DE)

08:45–09:00 STV04 Regulation of phenotypically heterogeneous anthraquinone production in *Photorhabdus luminescens* via the novel transcriptional activator AntJ
Angela Glaeser (Martinsried, München/DE)

09:00–09:15 STV05 The *Aspergillus fumigatus* DHN-melanin production is regulated by MEF2-like (RlmA) and bHLH (DevR) transcription factors
Vito Valiante (Jena/DE)

09:15–09:30 STV06 Regulation by the nitrogen PTS^{Ntr} in *Pseudomonas putida* – metabolism rules
Katharina Pflüger-Grau (Garching/DE)

09:30–09:45 STV07 Phosphorylation and thiol-redox modifications as molecular switches in host-microbe interactions
Falko Hochgräfe (Greifswald/DE)

09:45–10:00 STV08 Regulation of the C_4-Dicarboxylate sensor kinase DcuS by the transporters DcuB and DctA
Sebastian Wörner (Mainz/DE)

08:00–10:00 Short Lectures – Biodiversity and Ecosystem Functions

Room: Hörsaal 7
Chairs: Martina Herrmann (Jena/DE), Jörg Overmann (Braunschweig/DE)

08:00–08:15 BEV01 Effects of micro-predators of different specialization on the adaptation of three different prey species
Julia Johnke (Leipzig/DE)

08:15–08:30 BEV02 The intestinal microbiome of root fly larvae – a source of isothiocyanate degrading enzymes
Tijs van den Bosch (Nijmegen/NL)

08:30–08:45 BEV03 Elucidation of the structural and functional diversity of the rumen microbiota
Simon Deusch (Stuttgart/DE)

08:45–09:00 BEV04 Contribution of uncultured *Planctomycetaceae* to the degradation of 4-chloro-2-methylphenoxyacetic acid in the drilosphere
Marcus A. Horn (Bayreuth/DE)

09:00–09:15 BEV05 Genomic signatures of plant growth promoting *Bacillus*
Oleg Reva (Pretoria/ZA)

09:15–09:30 BEV06 Distribution pattern of arbuscular mycorrhizal fungi in a tropical dry forest
Natalia M.F. Sousa (Dahlem/DE, Recife/BR)

09:30–09:45 BEV07 Diversity and species recognition of the *Mucor circinelloides* complex
Lysett Wagner (Jena/DE)

09:45–10:00 BEV08 The troublesome life of microbes in leached slag
Carl-Eric Wegner (Marburg/DE)

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08:00–10:00 Short Lectures – Chemotaxis and Motility

Room: Hörsaal 8
Chairs: Sonja-Verena Albers (Freiburg/DE), Markus Nett (Jena/DE)

08:00–08:15 CMV01	Laws of attraction and repulsion – a novel family of bacterial chemosensors Anna Roujeinikova (Clayton/AU)
08:15–08:30 CMV02	How a bacterial cell detects the direction of light? Annegret Wilde (Freiburg/DE)
08:30–08:45 CMV03	Better together – a simultaneous tactic and kinetic response of the diatom *Seminavis robusta* in response to nutrient and pheromone gradients Karen Grace Bondoc (Jena/DE)
08:45–09:00 CMV04	How to analyse motility, chemoreceptors and chemotaxis in hyperthermophilic Archaea Annett Bellack (Regensburg/DE)
09:00–09:15 CMV05	*In situ* structure of the archaellar assembly and motor complex Bertram Daum (Frankfurt a. M./DE)
09:15–09:30 CMV06	The nucleotide-dependent interaction of FlaH and FlaI is essential for assembly and function of the archaellum motor Paushali Chaudhury (Freiburg/DE)
09:30–09:45 CMV07	The bacterial flagellum of *Salmonella* – length control and type-III protein export mechanisms of a macromolecular machine Marc Erhardt (Braunschweig/DE)
09:45–10:00 CMV08	Identification and characterization of minor pilins and *PilY1* proteins involved in type IV pili-dependent motility in *Myxococcus xanthus* Anke Treuner-Lange (Marburg/DE)

08:00–10:00 Mini-Symposium Special Group – FG Cyanobacteria
Natural Products from Cyanobacteria – from secondary metabolites to biopolymers

Room: Hörsaal 9
Chairs: Karl Forchhammer, Julia Kleinteich (Tübingen/DE)

08:00–08:40 CBV-FG01	Orthogonal natural product studies of the jamaican marine cyanobacterium *Moorea producens* JBH William H. Gerwick (La Jolla/US)
08:40–08:55 CBV-FG02	The role of carbon-polymer biosyntheses of both glycogen and poly-β-hydroxybutyrate in non-diazotrophic cyanobacteria Yvonne Zilliges (Berlin/DE)
08:55–09:10 CBV-FG03	Current and potential exploitation of cyanobacterial natural products in health care and biotechnology industry Wolfram Lorenzen (Berlin/DE)
09:10–09:25 CBV-FG04	Metabolic pathway engineering using the central signal processor P_{II} Björn Watzer (Tübingen/DE)
09:25–09:40 CBV-FG05	GC-MS based profiling of primary metabolism in Cyanobacteria Joachim Kopka (Potsdam-Golm/DE)
09:40–09:55 CBV-FG06	Physiological aspects of microcystin production in *Microcystis aeruginosa* PCC 7806 Sven Meissner (Potsdam-Golm/DE)

10:30–11:30 VAAM PhD Awards

Room: Hörsaal 1, live broadcast in Hörsaal 3
Chair: Wolfgang Buckel (Marburg/DE)
Sponsored by BASF SE, Bayer Healthcare AG, Evonik Degussa, New England Biolabs GmbH and Sanofi-Aventis Deutschland GmbH

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11:30–12:15 **Hans-Günter-Schlegel-Lecture**

Room Hörsaal 1, live broadcast in Hörsaal 3
Chair Dieter Jahn (Braunschweig/DE)

11:30–12:15 Physiological proteomics of Gram-positive model bacteria
ISV05 Michael Hecker (Greifswald/DE)

12:45–13:45 **JSMC Panel discussion** (see page 11)

Room Hörsaal 3
Chairs Natalie Töpfer, Carolin Dewald, Daniel Leichnitz, Miguel Tovar (Jena/DE)

14:00–15:30 **Plenary Session II – Systems Biology & Biotechnology**

Room Hörsaal 1, live broadcast in Hörsaal 3
Chairs Christian Hertweck, Thomas Munder (Jena/DE)

14:00–14:45 Systems biology of yeast metabolism
ISV06 Jens Nielsen (Göteborg/SE)

14:45–15:30 Engineering microbial metabolism for the production of fuels and chemicals
ISV07 Greg Stephanopoulos (Cambridge, MA/US)

15:30–17:30 **Poster Session** (see page 13)

15:45–17:15 **Karrieresymposium**

Room Hörsaal 3
Vorstellung verschiedener Berufsbilder in den Biowissenschaften (siehe Seite 15).

17:30–19:30 **Mini-Symposium Special Group – FG Environmental Microbiology**
Microscopy-supported discoveries in environmental microbiology

Room Hörsaal 1
Chairs Heribert Cypionka (Oldenburg/DE), Rainer Meckenstock (Essen/DE)

17:30–18:00 How do *Anabaena* cells communicate?
EMV-FG01 Amin Omairi-Nasser (Chicago/US)

18:00–18:15 Determinants of heterogeneous cell development of *Dinoroseobacter shibae*
EMV-FG02 Jürgen Tomasch (Braunschweig/DE)

18:15–18:30 A microscopic perspective on the planctomycetal ecology
EMV-FG03 Christian Jogler (Braunschweig/DE)

18:30–18:45 Towards applications of superresolution microscopy in environmental microbiology
EMV-FG04 Christina Moraru (Oldenburg/DE)

18:45–19:00 Simple generation of stereoscopic 3D images with any light- or scanning electron microscope
EMV-FG05 Heribert Cypionka (Oldenburg/DE)

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17:30–19:30 **Mini-Symposium Special Group – FG Regulation and Signal Transduction in Procaryotes**
Synthetic regulatory circuits in metabolic engineering

Room	Hörsaal 2
Chairs	Thorsten Mascher (Dresden/DE), Julia Frunzke (Jülich/DE)
17:30–17:40	Welcome Julia Frunzke (Jülich/DE)
17:40–18:20 RSV-FG01	Engineering synthetic regulatory systems for enhanced chemical production Fuzhong Zhang (St. Louis/US)
18:20–18:45 RSV-FG02	Hunting for new genetic targets – biosensor-based FACS screening of microorganisms Jan Marienhagen (Jülich/DE)
18:45–19:10 RSV-FG03	Engineered riboswitches – convenient building blocks for the construction of synthetic genetic circuits Beatrix Suess (Darmstadt/DE)
19:10–19:30 RSV-FG04	Synthetic RNA-based control units for balanced triterpene biosynthesis in cyanobacteria Ilka Maria Axmann (Düsseldorf/DE)

17:30–19:30 **Mini-Symposium Special Group – FG Microbial Pathogenicity**
Virulence and immune evasion strategies of pathogens

Room	Hörsaal 3
Chairs	Sven Hammerschmidt (Greifswald/DE), Peter F. Zipfel (Jena/DE)
17:30–17:47 MPV-FG01	Phase-Locked Mutants elucidate novel functions and differential virulence of variable surface lipoproteins encoded by mycoplasma multigene families Rohini Chopra Dewasthaly (Wien/AT)
17:47–18:04 MPV-FG02	Skin-specific unsaturated fatty acids were taken by *Staphyloccocus aureus* and their incorporation into lipoprotein boosts innate immune response Minh Thu Nguyen (Tübingen/DE)
18:04–18:21 MPV-FG03	Immunogenicity of lipoproteins and other classical pneumococcal surface proteins Franziska Voß (Greifswald/DE)
18:21–18:38 MPV-FG04	Plasminogen interaction to *Helicobacter pylori* confers serum resistance Birendra Singh (Malmö/SE)
18:38–18:55 MPV-FG05	Pra1, the *Candida* immune evasion protein is a protease that cleaves complement C3 and also blocks the effector components C3a and C3b Prasad Dasari (Jena/DE)
18:55–19:12 MPV-FG06	Stoichiometry of the bacterial type III secretion export apparatus Susann Zilkenat (Tübingen/DE)
19:12–19:29 MPV-FG07	Essential role of the SepF mycobacterial cell division protein Susanne Gola (Madrid/ES)

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17:30–19:30 **Mini-Symposium Special Group – FG Fungal Biology and Biotechnology**
Gene regulatory networks

Room: Hörsaal 5
Chairs: Philipp Benz (München/DE), Julia Schumacher (Münster/DE)

17:30–18:00 FBV-FG01 — The novel Zn_2Cys_6 transcription factor BcGaaR regulates D-galacturonic acid utilization in *Botrytis cinerea*
Jan A.L. van Kan (Wageningen/NL)

18:00–18:15 FBV-FG02 — Characterization of novel regulators for pectin degradation in *Neurospora crassa*
Nils Thieme (Freising/DE)

18:15–18:30 FBV-FG03 — Regulatory networks of the gibberellin cluster in *Fusarium fujikuroi*
Eva-Maria Niehaus (Münster/DE)

18:30–18:45 FBV-FG04 — Aspects in microbial interactions and intracellular regulation of *Schizophyllum commune*
Elke-Martina Jung (Jena, Berlin/DE)

18:45–19:00 FBV-FG05 — Comparative genomics and transcriptomics to study fruiting body development in ascomycetes
Minou Nowrousian (Bochum/DE)

19:00–19:15 FBV-FG06 — Regulation dynamics in the HOG signaling pathway in filamentous fungi
Stefan Bohnert (Kaiserslautern/DE)

19:15–19:30 FBV-FG07 — A gene co-expression network as a tool to predict functional modules in *Aspergillus niger*
Vera Meyer (Berlin/DE)

17:30–19:30 **Mini-Symposium Special Group – FG Water and Sewage**
***Legionella* in water and air – legislation, occurrence and new detection methods**

Room: Hörsaal 6
Chairs: Bernd Bendinger (Hamburg/DE), Ulrich Szewzyk (Berlin/DE)

17:30–17:55 WAV-FG01 — *Legionella* in evaporative cooling systems – new federal immission control act
Regine Szewzyk (Berlin/DE)

17:55–18:20 WAV-FG02 — Occurrence, frequency and distribution of *Legionella pneumophila* strains isolated from environmental sources in Germany
Christian Lück (Dresden/DE)

18:20–18:45 WAV-FG03 — *Legionella* species diversity and dynamics from surface reservoirs to cold and hot tap water – from a cold adapted to a thermophilic community
Ingrid Brettar (Braunschweig/DE)

18:45–19:00 WAV-FG04 — Detection of system-wide *Legionella* contaminations in drinking water plumbing systems – risk factors, temporal-spatial variability, strategies
Christiane Schreiber (Bonn/DE)

19:00–19:15 WAV-FG05 — New detection methods for *Legionella* in water and air
Michael Seidel (München/DE)

19:15–19:30 WAV-FG06 — Detection of *Legionella* in aerosols from cooling towers
Martin Strathmann (Mühlheim a. d. Ruhr/DE)

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17:30–19:30 Mini-Symposium Special Group – FG Quality Assurance and Diagnostics
New aspects of applied quality assurance and diagnostics

Room	Hörsaal 7
Chairs	Andreas Seiffert-Störiko (Frankfurt a. M./DE), Steffen Prowe (Berlin/DE)
17:30–18:00 QDV-FG01	Molecular diagnostic in the era of MRGN bacteria Oliwia Makarewicz (Jena/DE)
18:00–18:20 QDV-FG02	Acceleration of microbiological diagnostics of sepsis Evgeny A. Idelevich (Münster/DE)
18:20–18:40 QDV-FG03	DiAL-FISH for the rapid detection and identification of bacterial agents Karin Aistleitner (München/DE)
18:40–19:00 QDV-FG04	Effect of nisin on the survival of *Listeria monocytogenes* in sour curd cheese after artificial contamination Maik Szendy (Coburg/DE)
19:00–19:20 QDV-FG05	A view to a kill? – ambient bacterial load of frames and lenses of spectacles and evaluation of different cleaning methods Markus Egert (Villingen-Schwenningen/DE)

17:30–19:30 Mini-Symposium Special Group – FG Symbiotic Interactions
The chemical language of symbiosis

Room	Hörsaal 8
Chairs	Christian Kost (Jena/DE), Ute Hentschel Humeida (Kiel/DE)
17:30–18:00 SIV-FG01	Cryptic pathways at the host-microbe interface Christian Hertweck (Jena/DE)
18:00–18:15 SIV-FG02	Does indole-3-acetic acid modulate *Tricholoma vaccinum* ectomycorrhiza? Katrin Krause (Jena/DE)
18:15–18:30 SIV-FG03	Bacterial-macroalgal interactions – the symbiotic tripartite community of *Ulva* (Chlorophyta) Thomas Wichard (Jena/DE)
18:30–19:00 SIV-FG04	Host and symbiont jointly control gut microbiota during complete metamorphosis Paul R. Johnston (Berlin/DE)
19:00–19:15 SIV-FG05	Nitric oxide is an ambivalent mediator of microbial interactions in beewolves Tobias Engl (Mainz/DE)
19:15–19:30 SIV-FG06	Pleasant guests restrain – Can selective advantages explain the AT-bias of endosymbiotic genomes? Anne-Kathrin Dietel (Jena/DE)

17:30–19:30 Mini-Symposium Special Group – FG Yeast

Room	Hörsaal 9
Chair	Karl-Dieter Entian (Frankfurt a. M./DE)
17:30–17:50 YEV-FG01	Two novel yeast species from the gut of two different termite species Steffen Handel (Mainz/DE)
17:50–18:10 YEV-FG02	Three alcohol dehydrogenase genes are responsible for ethanol degradation in *Y. lipolytica* Michael Gatter (Dresden/DE)
18:10–18:30 YEV-FG03	Triterpenoids from *Saccharomyces cerevisiae* Thomas Polakowski (Berlin/DE)
18:30–18:50 YEV-FG04	Urm1 – a unique ubiquitin-like protein that functions in protein and tRNA modification André Jüdes (Kassel, Mainz/DE)
18:50–19:10 YEV-FG05	Hypermodification of eukaryotic 18S rRNAs Britta Meyer (Frankfurt a. M./DE)
19:10–19:30 YEV-FG06	Analysis of 25S rRNA Base modifications David Hartmann (Frankfurt a. M./DE)

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08:30–10:30 Short Lectures – Microbial Communication: Biofilms & Bio-Geo-Interactions

Room: Hörsaal 1
Chairs: Ákos Kovács (Jena/DE), Michael Schlömann (Freiberg/DE)

08:30–08:45 MCV01 — Architectural transitions in *Vibrio cholerae* biofilms at single-cell resolution
Knut Drescher (Marburg/DE)

08:45–09:00 MCV02 — Spatial segregation in *Bacillus subtilis* biofilm allows the emergence of growth yield strategists
Eisha Mhatre (Jena/DE)

09:00–09:15 MCV03 — SiaABCD coordinates cellular aggregation and virulence of *Pseudomonas aeruginosa* in response to environmental conditions
Janosch Klebensberger (Stuttgart/DE)

09:15–09:30 MCV04 — Formation and integrity of multicellular aggregates in *Staphylococcus aureus*
Charlotte Wermser (Würzburg/DE)

09:30–09:45 MCV05 — Viability of *Deinococcus geothermalis* in biofilms during desiccation
Jan Frösler (Essen/DE)

09:45–10:00 MCV06 — Biofilms as a protective niche for non-halophilic sulfur cycling bacteria at groundwater springs in the Dead Sea
Dheeraj Kanaparthi (Neuherberg, Halle a. d. Saale/DE)

10:00–10:15 MCV07 — Omics in metal resistant *Streptomyces*
Thomas Krauße (Jena/DE)

10:15–10:30 MCV08 — Metagenomic analysis of an acidophilic (pH 3.5) and microaerophilic enrichment culture dominated by iron oxidising strains of the genus *Sideroxydans*
Martin Mühling (Freiberg/DE)

08:30–10:30 Short Lectures – Biotechnology II

Room: Hörsaal 2
Chairs: Johannes Gescher (Karlsruhe/DE), Volker F. Wendisch (Bielefeld/DE)

08:30–08:45 BTV09 — Metabolic engineering of *Corynebacterium glutamicum* for production of astaxanthin
Nadja A. Henke (Bielefeld/DE)

08:45–09:00 BTV10 — Systems metabolic engineering of *Corynebacterium glutamicum* for the production of bio-based nylon
Judith Becker (Saarbrücken/DE)

09:00–09:15 BTV11 — Extracellular targeting of an active endoxylanase by a TolB negative mutant of *Gluconobacter oxydans*
Konrad Kosciow (Bonn/DE)

09:15–09:30 BTV12 — A surprising diversity of solventogenic clostridia
Anja Poehlein (Göttingen/DE)

09:30–09:45 BTV13 — Proteotyping of biogas plant microbioms
Robert Heyer (Magdeburg/DE)

09:45–10:00 BTV14 — Microbial electron uptake during biocorrosion and electrosynthesis
Joerg Deutzmann (Stanford/US)

10:00–10:15 BTV15 — Production of biobased fuels and plastics from CO_2 and light in defined mixed cultures
Hannes Löwe (Garching/DE)

10:15–10:30 BTV16 — Enoate reductase whole cell biocatalysis in *Synechocystis* sp. PCC 6803
Katharina Köninger (Bochum/DE)

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08:30–10:30 Short Lectures – Environmental Microbiology II

Room: Hörsaal 3
Chairs: Matthias Boll (Freiburg/DE), Rainer Meckenstock (Essen/DE)

08:30–08:45 EMV09	A novel central carbon assimilation pathway in the marine Alphaproteobacterium *Erythrobacter* sp. NAP-1 Iria Bernhardsgrütter (Marburg/DE)
08:45–09:00 EMV10	Biological significance of glucosinolate break-down products on soil microbiome Meike Siebers (Bonn/DE)
09:00–09:15 EMV11	Metabolic labor united – complete nitrification by a single microorganism Sebastian Lücker (Nijmegen/NL)
09:15–09:30 EMV12	Microbial communities in extremely oligotrophic sediments of the South Pacific Gyre – Do they eat phages? Franziska Preuss (Oldenburg/DE)
09:30–09:45 EMV13	Regulatory network of *Dinoroseobacter shibae* DFL 12^T for the adaptation to low oxygen tension Matthias Ebert (Braunschweig/DE)
09:45–10:00 EMV14	Diversity and function of bacterial communities in sublittoral marine surface sediments David Probandt (Bremen/DE)
10:00–10:15 EMV15	A targeted mutation system is active in the filamentous N_2-fixing cyanobacteria *Trichodesmium erythraeum* Ulrike Pfreundt (Freiburg/DE)
10:15–10:30 EMV16	Low wind speed induces strong bacterial community changes in the sea surface microlayer of a wind-wave system Janina Rahlff (Wilhelmshaven/DE)

08:30–10:30 Short Lectures – Infection Biology and Systems Microbiology

Room: Hörsaal 5
Chairs: Thilo Figge (Jena/DE), Katharina Pflüger-Grau (Garching/DE)

08:30–08:45 IbSV01	Dual proteome analysis towards understanding neutrophil interaction with *Aspergillus fumigatus* Iordana Shopova (Jena/DE)
08:45–09:00 IbSV02	Real-time imaging of the bacillithiol redox potential in the human pathogen *Staphylococcus aureus* using a novel genetically encoded redox biosensor Van Loi Vu (Berlin/DE)
09:00–09:15 IbSV03	Prognostic model of urinary tract infections Ivana Blazenovic (Braunschweig/DE)
09:15–09:30 IbSV04	Sorting of Vancomycin BODIPY FL labeled *Staphylococcus aureus* from infection experiments – fast and easy enrichment of *S. aureus* isolates for analysis by mass spectrometry Kristin Surmann (Greifswald/DE)
09:30–09:45 IbSV05	Predicting compositions of microbial communities from stoichiometric models with applications for the biogas process Sabine Koch (Marburg/DE)
09:45–10:00 IbSV06	Anatomy of the bacitracin resistance network in *Bacillus subtilis* Georg Fritz (Marburg/DE)
10:00–10:15 IbSV07	Bacterial phase diagrams – using engineering concepts to predict cell-to-cell heterogeneity of microbial gene expression Alexander Grünberger (Jülich/DE)
10:15–10:30 IbSV08	Transcriptional, proteomic and metabolic networks of the Fur regulated iron metabolism of *Clostridium difficile* Mareike Berges (Braunschweig/DE)

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08:30–10:30 Short Lectures – Fungal Biology I

Room: Hörsaal 6
Chairs: Ursel Kües (Göttingen/DE), Johannes Wöstemeyer (Jena/DE)

08:30–08:45 FBV01	Intracellular and potential extracellular roles of the *Ustilago maydis* Acyl-CoA-binding protein Acb1 Joachim Jungmann (Marburg/DE)
08:45–09:00 FBV02	Soil amoeba impose predatory selection pressure on environmentally acquired pathogenic fungi Silvia Novohradska (Jena/DE)
09:00–09:15 FBV03	Molecular background of virulence in human pathogenic Mucoralean fungi Gábor Nagy (Szeged/HU)
09:15–09:30 FBV04	*Parasitella parasitica*, an experimental laboratory system for studying horizontal gene transfer Johannes Wöstemeyer (Jena/DE)
09:30–09:45 FBV05	Redox regulation of hypoxic response in *Aspergillus fumigatus* Elena Shekhova (Jena/DE)
09:45–10:00 FBV06	Post-transcriptional regulation impacts on iron metabolism regulation in *Candida glabrata* Franziska Gerwien (Jena/DE)
10:00–10:15 FBV07	A glimpse into the role of the fungal rhodopsins CarO and OpsA in *Fusarium fujikuroi* Ulrich Terpitz (Würzburg/DE)
10:15–10:30 FBV08	Regulatory networks of the gibberellin cluster in *Fusarium fujikuroi* Eva-Maria Niehaus (Münster/DE)

08:30–10:30 Short Lectures – Natural Products

Room: Hörsaal 7
Chairs: Elke Dittmann (Potsdam/DE), Pierre Stallforth (Jena/DE)

08:30–08:45 NPV01	Biosynthesis of the 6-pentylsalicylate building block in the antibiotic micacocidin Hirokazu Kage (Jena/DE)
08:45–09:00 NPV02	NPS2 of *Ceriporiopsis subvermispora* exemplifies the model for the most conserved basidiomycete peptide synthetase Eileen Brandenburger (Jena/DE)
09:00–09:15 NPV03	SimC7 is an unusual angucyclinone ketoreductase essential for antibiotic activity of simocyclinone D8 Martin Schäfer (Norwich/GB)
09:15–09:30 NPV04	Elucidation of the biosynthetic gene cluster involved in the biosyntehsis of the natural compound sodorifen in *S. plymuthica* 4Rx13 Dajana Domik (Rostock/DE)
09:30–09:45 NPV05	The role of short-lived intermediates in the *Pseudomonas aeruginosa* alkylquinolone biosynthesis pathway Steffen Lorenz Drees (Münster/DE)
09:45–10:00 NPV06	The cyclochlorotine mycotoxin is produced by the nonribosomal peptide synthetase CctN in *Talaromyces islandicus* (“*Penicillium islandicum*”) Thomas Schafhauser (Tübingen/DE)
10:00–10:15 NPV07	A yersiniabactin-like siderophore/virulence factor of entomopathogenic bacteria Merle Hirschmann (Frankfurt a. M./DE)
10:15–10:30 NPV08	Construction of a *Corynebacterium glutamicum* platform strain for the production of high-value plant secondary metabolites Nicolai Kallscheuer (Jülich/DE)

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08:30–10:30 Short Lectures – Synthetic Microbiology

Room: Hörsaal 8
Chairs: Tobias Erb (Marburg/DE), Vito Valiante (Jena/DE)

08:30–08:45 SMV01	The *Bacillus* BioBrick Box 2.0 – generation and evaluation of new essential genetic building blocks for standardized work with *Bacillus subtilis* Philipp Popp (Dresden/DE)
08:45–09:00 SMV02	CRISPy-web – design sgRNAs for CRISPR applications in microbes using an easy online tool Kai Blin (Hørsholm/DK)
09:00–09:15 SMV03	Synthetic secondary chromosomes to study chromosome maintenance in *Escherichia coli* Torsten Waldminghaus (Marburg/DE)
09:15–09:30 SMV04	Employing photocaged carbohydrates in light-controlled cell factories for synthetic bio(techno)logy and single cell applications Dennis Binder (Jülich/DE)
09:30–09:45 SMV05	Investigation of the anaerobic propionate metabolism in *Escherichia coli* K12 Francesca Simonte (Karlsruhe/DE)
09:45–10:00 SMV06	Metabolic engineering of *Escherichia coli* for the biosynthesis of *para*-amino-L-phenylalanine Jung-Won Youn (Stuttgart/DE)
10:00–10:15 SMV07	Introduction of the Calvin-Benson-Bassham cycle to create synthetic autotrophy in *Methylobacterium extorquens*, a heterotrophic Alphaproteobacterium Lennart Schada von Borzyskowski (Marburg/DE, Zürich/CH)
10:15–10:30 SMV08	Metabolic engineering of syngas fermenting *Clostridium ljungdahlii* for jet fuel production using an efficient genomic delivery system Gabriele Philipps (Aachen/DE)

08:30–10:30 Short Lectures – Bioenergetics and Membranes and Transport

Room: Hörsaal 9
Chairs: Volker Müller (Frankfurt a. M./DE), Torsten Schubert (Jena/DE)

08:30–08:45 BMV01	The tetrathionate/thiosulfate reduction potential determined by catalytic protein film electrochemistry Julia Kurth (Bonn/DE)
08:45–09:00 BMV02	A class C radical S-adenosylmethionine methyltransferase synthesizes 8-methylmenaquinone Oliver Klimmek (Darmstadt/DE)
09:00–09:15 BMV03	Occurrence and function of the Rnf complex in bacteria Martin Kuhns (Frankfurt a. M./DE)
09:15–09:30 BMV04	Microbes with identity issues – the cell wall and energy-conserving prokaryotic organelle of anaerobic ammonium-oxidizing bacteria Laura van Niftrik (Nijmegen/NL)
09:30–09:45 BMV05	Molecular model of the pIP501 type IV secretion system from *Enterococcus faecalis* Ines Probst (Freiburg/DE)
09:45–10:00 BMV06	From substrate specificity to promiscuity – molecular analysis of a hybrid ABC transporter Laura Teichmann (Marburg/DE)
10:00–10:15 BMV07	The DNA translocator of *Thermus thermophilus* – *In situ* structure and structure/function correlation of a dynamic channel for DNA uptake and pilus extrusion Ralf Salzer (Frankfurt a. M./DE)
10:15–10:30 BMV08	The h-region of the TMAO reductase signal peptide – a major determinant for Tat-dependent protein translocation Agnes Ulfig (Jülich/DE)

SCIENTIFIC PROGRAMME, TUESDAY, 15 MARCH 2016

Annual Conference 2016 of the VAAM

11:00–11:30 **How the Nagoya-Protocol challenges microbiological research in Germany**

Room Hörsaal 1, broadcast in Hörsaal 3
Chair Kerstin Voigt (Jena/DE)

11:00–11:30 How the Nagoya-Protocol challenges microbiological research in Germany
ISV08 Jörg Overmann (Braunschweig/DE)

11:30–13:00 **Plenary Session III – Infection & Natural Products**

Room Hörsaal 1, broadcast in Hörsaal 3
Chairs Ilse Jacobsen, Stefan Schuster (Jena/DE)

11:30–12:15 *Legionella pneumophila*, a unique model to study host pathogen interactions and the evolution of virulence
ISV09 Carmen Buchrieser (Paris/FR)

12:15–13:00 Natural products from entomopathogenic bacteria – from chemical ecology to synthetic biology
ISV10 Helge B. Bode (Frankfurt a. M./DE)

13:00–13:55 **JSMC Lunch symposium** (see page 11)

Room Hörsaal 1, broadcast in Hörsaal 3
Chairs Rene Benndorf, Maja Rischer (Jena/DE)

14:00–15:30 **Plenary Session IV – Bio-Geo-Interactions & Biodegradation**

Room Hörsaal 1, broadcast in Hörsaal 3
Chairs Georg Büchel, Gabriele Diekert (Jena/DE)

14:00–14:45 Geomycology – metals, minerals and mycota
ISV11 Geoffrey Gadd (Dundee/GB)

14:45–15:30 They can't do it on their own – community control over organohalide-respiring Chloroflexi
ISV12 Frank Loeffler (Knoxville, TN/US)

15:40–16:55 **InfectControl 2020 Panel discussion**

Room Hörsaal 3
Chair Axel Brakhage (Jena/DE)

15:30–17:30 **Poster Session** (see page 13)

17:00–18:30 **Microbe Slam** (see page 15)

Room Hörsaal 1

18:30–19:45 **Annual Meeting VAAM**

Room Hörsaal 3

20:00 **Mixer** (see page 15)

Room Volkshaus

Annual Conference 2016 of the VAAM

09:00–11:00 Short Lectures – Microbial Communication: Plant Pathology & Symbiosis

Room	Hörsaal 1
Chairs	Holger Deising (Halle a. d. Saale/DE), Falk Hillmann (Jena/DE)
09:00–09:15 MCV17	Microbial hub taxa link host and abiotic factors to plant microbiome variation Matthew Agler (Köln/DE)
09:15–09:30 MCV18	Transcriptional profiling during *in planta* development of the corn smut fungus Daniel Lanver (Marburg/DE)
09:30–09:45 MCV19	Acclimatization of arbuscular mycorrhizal fungi leads to increased stress tolerance of their host plants Philipp Franken (Erfurt/DE)
09:45–10:00 MCV20	The tripartite symbiosis of *Piriformospora indica*, its endofungal bacterium, and plants Stefanie P. Glaeser (Gießen/DE)
10:00–10:15 MCV21	What it takes to be a giant gut bacterium – metabolic flexibility and diel lifestyle of *Epulopiscium* David Kamanda Ngugi (Thuwal/SA)
10:15–10:30 MCV22	Surface modifications of *Escherichia coli* influence ingestion and digestion of the ciliate *Tetrahymena pyriformis* Lisa Siegmund (Jena/DE)
10:30–10:45 MCV23	Evidence of terpene degradation by pine weevil (*Hylobius abietis*) microbiota and its effect on host fitness Aileen Berasategui (Jena/DE)
10:45–11:00 MCV24	Exploiting *Streptomyces* in agro-ecosystems for biological control and plant growth promotion Xiaoyulong Chen (Milan/IT)

09:00–11:00 Short Lectures – Biotechnology and Environmental Microbiology

Room	Hörsaal 2
Chairs	Erhard Bremer (Marburg/DE), Christian Jogler (Braunschweig/DE)
09:00–09:15 BEmV01	Mining the treasures of microbial diversity for industrial biotechnology with an optimized droplet-microfluidic screening platform Miguel Tovar (Jena/DE)
09:15–09:30 BEmV02	Development of a self-cleavable protein linker for the purification of fusion proteins Susanne Zehner (Dresden/DE)
09:30–09:45 BEmV03	Upgrading the toolbox for fermentation of (crude) syngas Florian Oswald (Karlsruhe/DE)
09:45–10:00 BEmV04	A super competent *Bacillus subtilis* 168 strain enables the genome manipulation without using plasmid DNA Regine Rahmer (Stuttgart/DE)
10:00–10:15 BEmV05	A grass associated microbial diversity and structure across the two successional stages of arctic inland dunes Anbu Poosakkannu (Jyväskylä/FI)
10:15–10:30 BEmV06	Evolution of ecological diversity in *Acidobacteria* in German grassland soils Johannes Sikorski (Braunschweig/DE)
10:30–10:45 BEmV07	Eco-systems biology of a rare biosphere member active in cryptic sulfur cycling of a model peatland Michael Pester (Konstanz/DE)
10:45–11:00 BEmV08	Microbiological assessment and prevalence of food borne pathogens from aprons of meat vendors in an abattoir, Awka Anambra Nigeria Malachy Ugw (Awka/NG)

SCIENTIFIC PROGRAMME, WEDNESDAY, 16 MARCH 2016

Annual Conference 2016 of the VAAM

09:00–11:00 Short Lectures – Fungal Biology II

Room: Hörsaal 3
Chairs: Bernhard Hube (Jena/DE), Stefanie Pöggeler (Götingen/DE)

09:00–09:15 FBV09 — Coordinated process in polarized growth of the filamentous fungus *Aspergillus nidulans*
Norio Takeshita (Karlsruhe/DE)

09:15–09:30 FBV10 — Role of the autophagy-related gene *Smatg12* in fruiting-body development of the filamentous ascomycete *Sordaria macrospora*
Stefanie Pöggeler (Göttingen/DE)

09:30–09:45 FBV11 — In-depth characterization of the *Aspergillus fumigatus* mating-type system
Yidong Yu (Erlangen/DE)

09:45–10:00 FBV12 — Impact of light on differentiation and virulence of the plant pathogen *Botrytis cinerea*
Julia Schumacher (Münster/DE)

10:00–10:15 FBV13 — Why some like it on the rocks – recurring stresses select for organisms with manifold protective pigments
Nicole Knabe (Berlin/DE)

10:15–10:30 FBV14 — Identification of novel factors involved in dimorphism and pathogenicity of *Zymoseptoria tritici*
Alexander Yemelin (Kaiserslautern/DE)

10:30–10:45 FBV15 — Environmental decisions in early steps of fruiting of *Coprinopsis cinerea*
Shanta Subba (Göttingen/DE)

10:45–11:00 FBV16 — Towards making *Agrocybe aegerita* a modern model basidiomycete for mushroom formation
Florian Hennicke (Frankfurt a. M./DE)

09:00–11:00 Short Lectures – Infection Biology II

Room: Hörsaal 5
Chairs: Friedrich Götz (Tübingen/DE), Melanie Blokesch (Lausanne/CH)

09:00–09:15 IBV09 — The biochemical RNA landscape of a cell revealed by Grad-seq
Jörg Vogel (Würzburg/DE)

09:15–09:30 IBV10 — The RNA chaperone Hfq mediates post-transcriptional regulation of adhesins in the enteropathogen *Yersinia enterocolitica*
Ombeline Rossier (München/DE)

09:30–09:45 IBV11 — tRNA modifications – a novel virulence factor in pathogenic *Candida* species
Bettina Böttcher (Jena/DE)

09:45–10:00 IBV12 — The extracellular adherence protein (Eap) of *Staphylococcus aureus* exhibits DNase activity
Henrik Peisker, Markus Bischoff (Homburg/DE)

10:00–10:15 IBV13 — The phospholipases of *A. baumannii* – role in interbacterial competition and pathogenicity
Julia Stahl (Frankfurt a. M./DE)

10:15–10:30 IBV14 — Flotillin controls the assembly of protein complexes related to staphylococcal virulence
Benjamin Mielich-Süß (Würzburg/DE)

10:30–10:45 IBV15 — Structure of the bacterial cell division determinant GpsB and its interaction with penicillin binding proteins
Sven Halbedel (Wernigerode/DE)

10:45–11:00 IBV16 — Identification of a pneumococcal enzyme essential for anchoring of lipoteichoic acid to the bacterial cell surface
Nathalie Heß (Greifswald/DE)

Annual Conference 2016 of the VAAM

09:00–11:00 Short Lectures – Archaea and Extremophiles

Room	Hörsaal 6
Chairs	Peter Schönheit (Kiel/DE), Michael Thomm (Regensburg/DE)
09:00–09:15 AEV01	How to stay in hell – how hyperthermophiles colonize black smokers Reinhard Wirth (Regensburg/DE)
09:15–09:30 AEV02	Structure of the formylmethanofuran dehydrogenase-polyferredoxin complex – methanogens' chemical trick to fix CO_2 without ATP consumption Tristan Wagner (Marburg/DE)
09:30–09:45 AEV03	Methanogenesis upside down – metabolic reconstruction of an archaeon performing nitrate-dependent anaerobic oxidation of methane Cornelia Welte (Nijmegen/NL)
09:45–10:00 AEV04	Calvin-Cycle reinvented – autotrophic CO_2 fixation in *Ammonifex degensii* Achim Mall (Freiburg/DE)
10:00–10:15 AEV05	Ced – a DNA import system conserved in Crenarchaea Alexander Wagner (Freiburg/DE)
10:15–10:30 AEV06	New insights into the functions of TrmB proteins in *Pyrococcus furiosus* Robert Reichelt (Regensburg/DE)
10:30–10:45 AEV07	Small RNA_{41} involved in carbon metabolism in *Methanosarcina mazei* Gö1 Anne Buddeweg (Kiel/DE)
10:45–11:00 AEV08	Circularization restores signal recognition particle RNA functionality in *Thermoproteus* Michael Daume (Marburg/DE)

09:00–11:00 Short Lectures – Natural Products and Microbial Evolution

Room	Hörsaal 7
Chairs	Christine Beemelmanns, Christian Kost (Jena/DE)
09:00–09:15 NMV01	Plant pathogenic anaerobic bacteria use aromatic polyketides to access aerobic territory Gulimila Shabuer (Jena/DE)
09:15–09:30 NMV02	Treasures of the submarine rain forests – Kelp-associated Planctomycetes as a novel source for powerful bioactive compounds Patrick Rast (Braunschweig/DE)
09:30–09:45 NMV03	Diversity and metabolite profiles of Actinobacteria from the Atacama Desert Álvaro Villalobos (Kiel/DE)
09:45–10:00 NMV04	Discovery of the trypacidin gene cluster in the human-pathogenic fungus *Aspergillus fumigatus* Derek J. Mattern, Jakob Weber (Jena/DE)
10:00–10:15 NMV05	Experimental evolution of metabolic dependency in bacteria Glen D'Souza (Jena/DE)
10:15–10:30 NMV06	Virulence in smut fungi – insights from evolutionary comparative genomics Gabriel Schweizer (Marburg/DE)
10:30–10:45 NMV07	Ancient *Yersinia pestis* genome from a post-Black Death outbreak in Southwestern Germany Maria Alexandra Spyrou (Jena/DE)
10:45–11:00 NMV08	Impact of the extent of pyoverdine production in *Pseudomonas* populations on the development of cooperation Felix Becker (Martinsried, München/DE)

SCIENTIFIC PROGRAMME, WEDNESDAY, 16 MARCH 2016

Annual Conference 2016 of the VAAM

09:00–11:00 Short Lectures – Biodegradation

Room: Hörsaal 8
Chairs: Susanne Fetzner (Münster/DE), Sandra Studenik (Jena/DE)

09:00–09:15 BDV01
Soil – sediment microbial community adaptation due to a long history of oil contamination
Antonios Michas (Neuherberg/DE)

09:15–09:30 BDV02
Biodegradation of aromatic compounds in a coal tar polluted aquifer studied by denitrifying BTEX-degrading enrichment cultures and *in-situ* community analysis
Martin Sperfeld (Jena/DE)

09:30–09:45 BDV03
Conversion of *cis*-2-carboxycyclohexylacetic acid in the down-stream pathway of anaerobic naphthalene degradation
Philip Weyrauch (München, Essen/DE)

09:45–10:00 BDV04
Anoxic degradation of auxin in denitrifying Betaproteobacteria
Karola Schühle (Marburg/DE)

10:00–10:15 BDV05
Identification of glyphosate degradation pathways in soil and water-sediment systems – a stable isotope co-labeling approach
Karolina Nowak (Leipzig, Aachen/DE)

10:15–10:30 BDV06
Characterization of Latex Clearing Protein (Lcp) from *Rhodococcus rhodochrous*
Wolf Röther (Stuttgart/DE)

10:30–10:45 BDV07
Formation of bisphenol-phosphate conjugates by *Bacillus amyloliquefaciens* – a novel mechanism to reduce toxicity and estrogenicity of bisphenols
Marie-Katherin Zühlke (Greifswald/DE)

10:45–11:00 BDV08
Identification of an unusual decarboxylase crucial for norcobamide biosynthesis in the tetrachloroethene-respiring bacterium *Sulfurospirillum multivorans*
Sebastian Keller (Jena/DE)

09:00–11:00 Short Lectures – Open Topics

Room: Hörsaal 9
Chairs: Reinhard Fischer (Karlsruhe/DE), Oliver Kurzai (Jena/DE)

09:00–09:15 OTV01
Two regulatory RNA elements affect toxin-driven depolarization and persister formation in *Escherichia coli*
Bork Berghoff (Gießen, Uppsala/DE)

09:15–09:30 OTV02
IscR of *Rhodobacter sphaeroides* functions as repressor of genes for iron-sulfur metabolism and represents a new type of iron-sulfur-binding protein
Bernhard Remes (Gießen/DE)

09:30–09:45 OTV03
Formation of polyphosphate by polyphosphate kinases and its relationship to Poly(3-Hydroxybutyrate) accumulation in *Ralstonia eutropha*
Tony Tumlirsch (Stuttgart/DE)

09:45–10:00 OTV04
Identification of the key enzyme of roseoflavin biosynthesis
Valentino Konjik (Mannheim/DE)

10:00–10:15 OTV05
The cell cycle of *Corynebacterium glutamicum*
Kati Böhm (München/DE)

10:15–10:30 OTV06
The heterododecameric, membrane-associated bacterioferritin of *Magnetospirillum gryphiswaldense* is not involved in magnetite biosynthesis
René Uebe (Bayreuth/DE)

10:30–10:45 OTV07
The early immune response by human monocytes to *Candida albicans*
Luke Donald Halder (Jena/DE)

10:45–11:00 OTV08
Biological agents in sight – danger avoided the GESTIS-database on biological agents combines expertise
Matthias Rastetter (Heidelberg/DE)

Annual Conference 2016 of the VAAM

11:30–11:45 **VAAM Poster Awards**

Room Hörsaal 1
Chairs Georg Fuchs (Freiburg/DE), Felicitas Pfeifer (Darmstadt/DE)

11:45–13:15 **Plenary Session V – Biodiversity & Ecosystem Functions**

Room Hörsaal 1
Chairs Dirk Hoffmeister, Kirsten Küsel (Jena/DE)

11:45–12:30 Methane oxidation in Lake Constance
ISV13 Bernhard Schink (Konstanz/DE)

12:30–13:15 Environmental change, niche specialisation and microbial communities – What can we learn from ammonia oxidisers?
ISV14 James Prosser (Aberdeen/GB)

13:15–13:30 **Closing Remarks**

Room Hörsaal 1

Oral Presentations

Poster Presentations

ISV01
Science and society – infectious diseases as an example
*J. Hacker[1]
[1]*Deutsche Akademie der Naturforscher Leopoldina, Nationale Akademie der Wissenschaften, Berlin, Germany*

U.S. Surgeon General W.H. Smith declared in 1967 that „it was time to close the book on infectious diseases". People were confident that the war against infectious diseases was all but won since tuberculosis, polio and other threatening infectious diseases were on decline. But since then several new infectious pathogens and diseases like HIV, MRSA or EHEC have been established. The recent Ebola outbreak in 2014 and 2015 showed that emerging and re-emerging infectious diseases continue to represent a serious international threat despite major research advances in recent years. Concerning to WHO-data, infectious diseases cause nowadays 12 % of global deaths and pathogenic microbes and infections still pose a continuing threat to human health and play a prominent role in health politics worldwide.
The spread of infectious diseases is on the one hand due to changes in human behavior, as increased trade and travel, globalized food distribution and inappropriate use of antibiotics. On the other hand mutations, gene transfer and recombination are responsible for pathogen variability. Continuing progress in the treatment of many infections is threatened by the increasing number and expanded distribution of antibiotic resistant pathogens. In 2013, the German National Academy of Sciences Leopoldina together with the Academy of Science in Hamburg published the report "Antibiotics research: problems and perspectives". It declares that the policy makers in the fields of science, politics, society and industry need to cooperate and act on a national and international level and that special emphasis should be placed on research and development. And in 2015, the G7 science academies prepared statements on resistance to antibiotics and neglected tropical diseases to advise heads of the state and government at their annual summit.
The challenges facing the control of infectious diseases today are tremendous and make a more global approach necessary to improve health of populations. In 2015, the UN General Assembly adopted the 2030 Development Agenda titled *Transforming our world: the 2030 Agenda for Sustainable Development*. The agenda includes 17 sustainable development goals. Goal 3 Ensure healthy lives and promote well-being for all at all ages has put health at front and declares that by 2023, the epidemics of AIDS, tuberculosis, malaria and neglected tropical diseases should end.
In conclusion, it can be stated that: the fight against infectious diseases stands as an example for the globalization of science and that policy and science need to establish new models of collaboration and interaction.

ISV02
Harnessing nature's sensory devices for metabolic engineering and single-cell analysis
*J. Frunzke[1]
[1]*Forschungszentrum Jülich, Institute of Bio- and Geosciences, IBG-1: Biotechnology, Jülich, Germany*

Nature has evolved a plethora of different sensor devices to sense and respond to a variety of intra- and extracellular stimuli, including physical parameters, nutrient availability and the production of small molecules. In this talk, I will illustrate how we can harness nature's toolbox for the analysis of microbial population dynamics and for accelerating biotechnological strain development. Recently, we have developed genetically-encoded biosensors based on bacterial transcriptional regulators to report on intracellular amino acid accumulation in the biotechnological platform organisms *Corynebacterium glutamicum* and *Escherichia coli.* In ongoing studies, we apply these sensors in FACS high-throughput (HT) screening approaches for the isolation of amino acid producing mutants as well as for biosensor-driven adaptive evolution of production strains. Furthermore, the design of synthetic regulatory circuits translating a certain intra- or extracellular stimulus into a measurable reporter output enables the visualization of microbial population dynamics at the single-cell level and spatiotemporal resolution. This is exemplified by our recent studies of spontaneous prophage induction representing a common phenomenon of lysogenic bacterial cultures.

ISV03
Exploring the pole – cellular asymmetry and adhesin localization drive biofilm formation in *Agrobacterium tumefaciens*
*C. Fuqua[1]
[1]*Indiana University, Department of Biology, Bloomington, USA*

The bacterial pathogen *Agrobacterium tumefaciens* is well known for its ability to genetically modify plants through interkingdom gene transfer and integration of a segment of DNA (T-DNA) into the plant genome, causing the disease called crown gall. Despite extensive studies of T-DNA transfer, little is known regarding the initial processes and interactions at the plant surface. *A. tumefaciens* can assemble dense biofilms on both abiotic and biotic surfaces which on plants may increase the gene transfer efficiency while affording protection against host defense responses. One of the primary factors mediating surface attachment and biofilm formation in *A. tumefaciens* is the unipolar polysaccharide (UPP), an adhesin produced at a single cellular pole following surface contact. The UPP is only one of several polarly-localized *A. tumefaciens* cellular structures, including a tuft of unipolar flagella, and components of the Type IV secretion system (T4SS) that exports T-DNA and several associated virulence proteins. The process of cell division for *A. tumefaciens* is also decidedly polar, with a profound asymmetry and highly localized, or a zonal pattern of cell biogenesis. *A. tumefaciens* cells attached by their poles to surfaces produce daughter cells from their unattached pole, and flagella emerge from the old pole of the daughter cell. This cellular asymmetry is orchestrated at least in part through a complex phosphorelay cascade designated the Control of Division and Development (CDD) pathway. However, distinct from the CDD pathway, UPP targeting to the pole is dependent on the PodJ protein, thought to function as a distinct localization factor. Regulation of surface-contact dependent polar extrusion of the UPP is strictly mediated through the bacterial second messenger cyclic diguanosine monophosphate (c-di-GMP). Over 30 separate putative diguanylate cyclases (DGCs) could drive synthesis of c-di-GMP in *A. tumefaciens*, but several specific DGCs have been found to have a pronounced impact on UPP production and other attachment processes. One of the dominant regulators in this respect is a dual function enzyme called DcpA with DGC activity, and a separable phosphodiesterase (PDE) activity, responsible for c-di-GMP inactivation. DcpA is controlled by a complex pathway that involves several other partner proteins, and small metabolites called monapterins. Production of a monapterin is required to foster the PDE activity of DcpA under laboratory conditions, maintaining low levels of c-di-GMP and preventing inappropriate deployment of the UPP. Mutants in this pterin-DcpA response pathway are uncoupled from surface contact dependence, and produce the UPP in planktonic phase.

ISV04
Dissecting the biology and pathology of the Irish potato famine pathogen *Phytophthora infestans*
*F. Govers[1]
[1]*Wageningen University, Laboratory of Phytopathology, Wageningen, Netherlands*

The oomycete *Phytophthora infestans* is the causal agent of late blight in potato and tomato. This plant pathogen has a hemibiotrophic life style and exploits a variable repertoire of effector proteins for manipulating plant defence and facilitating colonization. It has a 'two-speed' genome of ~ 240 Mb with 74 % repetitive DNA and composed of gene-dense and gene-poor regions. Comparative genomics revealed features illuminating the success of *Phytophthora* as a pathogen, such as a massive expansion of families encoding effectors, and peculiar gene innovations resulting in proteins with oomycete-specific domain combinations. Examples of novel proteins are the GPCR-PIPKs (GKs) that have a N-terminal 7-transmembrane domain typical for G-protein coupled receptors (GPCRs) combined with a phosphatidylinositol phosphate kinase (PIPK) domain at the C-terminus. This domain structure suggests that GKs use GPCRs to directly feed extracellular signals into phospholipid signalling pathways. For one GK we could demonstrate a role in asexual development, including spore germination, hyphal elongation and sporangia cleavage, whereas inactivation of another GK disturbed sexual development. We have also indications for a role of GK in the dynamics of the actin cytoskeleton, an organized intracellular framework that is indispensable for the viability of eukaryotic cells and functions in e.g. intracellular transport, formation of contractile rings, nuclear segregation, and endocytosis. Microscopic analysis of *P. infestans* transformants expressing the actin binding peptide Lifeact-eGFP revealed actin filament cables and plaques. The latter are nearly immobile structures with average lifetimes exceeding one hour;

much longer (over 500-fold) than the lifetimes of actin patches in fungi. Moreover, in contrast to actin patches in yeast, plaque disassembly is not accompanied with formation and internalization of endocytic vesicles. The oomycete specific features of GKs and actin plaques justify more in depth research to evaluate their potential as target for novel oomicides.

ISV05
Physiological proteomics of Gram-positive model bacteria
*M. Hecker[1]
[1]*University of Greifswald, Institute for Microbiology, Greifswald, Germany*

The genome sequence of an organism, the blueprint of life, does not explain life. Because proteins are the main workhorses of cell physiology, it is the proteome that largely translates the genome sequence into cell function. The knowledge on physiology and molecular genetics of model bacteria such as *Bacillus subtilis* and *Staphylococcus aureus* combined with the new perspective of functional genomics/proteomics should bring a new quality in understanding the life style of these model organisms.
Complex mechanisms of global gene expression control guarantee that each single protein is provided in sufficient amounts, at the right time window and at the right place to organize cellular life. For our model bacteria we identified almost 70 to 80 % of the proteome, absolute quantitative data included, shown in the first part of the talk. The main challenge that follows is to understand how hundreds of different proteins leaving the ribosome tunnel organize the main processes of life. Proteomics is a great toolbox to follow the fate of the single proteins from birth at the ribosome via aggregate formation, modification, damage, repair and finally to death in the Clp maschine.
In the following part the question will be addressed: How can we use physiological proteomics to understand cell physiology and pathophysiology of both model organisms. This will be demonstrated for the SigB-dependent general stress response of *B. subtilis*. New results on the function of the general stress proteins will be discussed. In the final part a proteomic view of pathogenicity of *S. aureus* will be presented.

ISV06
Systems biology of yeast metabolism
*J. Nielsen[1,2,3]
[1]*Chalmers University of Technology, Department of Biology and Biological Engineering, Gothenburg, Sweden*
[2]*Technical University of Denmark, Novo Nordisk Foundation Center for Biosustainability, Lyngby, Sweden*
[3]*Royal Institute of Technology, Science for Life Laboratory, Stockholm, Sweden*

Metabolism is highly complex involving a large number of chemical reactions. These reactions are traditionally grouped into pathways with dedicated functions, but recent analysis of metabolism has shown that there is a high degree of connectivity between these pathways due to common sharing of co-factors and key metabolites. Also regulation of metabolism is complex due to the requirements for maintaining cellular homeostasis. In this talk there will be given illustrations of how different parts of cellular metabolism are connected, i.e. central carbon metabolism, lipid metabolism and protein secretion. The role of key regulatory components, e.g. Sir2 and Snf1, on controlling the central carbon metabolism will be discussed together with presentation of a new hypothesis for how these two regulators interact in controling shifts between fermentation and respiration. Snf1 regulation of lipid metabolism will also be discussed, and finally it will be discussed how lipid metabolism interacts with protein secretion. All the pathways discussed are conserved between yeast and human and the findings are therefore having direct impact on our understanding of different human diseases.

ISV07
Engineering microbial metabolism for the production of fuels and chemicals
*G. Stephanopoulos[1]
[1]*MIT, Department of Chemical Engineering, Cambridge, USA*

Biotechnology is rapidly coming of age as enabling technology for the production of biobased chemicals and biofuels. In this task, it is aided by critical advancements in Metabolic Engineering that transforms microbes into little chemical factories capable of converting renewable feedstocks to a variety of products. While in earlier years this application was limited to specialized chemicals and pharmaceutical products, recent advances in metabolic engineering have expanded the portfolio of biotechnological applications beyond the space of specialty products and into the domain of commodity chemicals that have been traditionally the realm of chemical process industry using fossil fuels as feedstocks. As such, we are witnessing a process of creative destruction whereby one manufacturing technology based on chemistry is gradually being replaced by another based on biology. To be sure, chemical technologies have some definite advantages such as very high space times due to high temperatures of operation. These benefits can be counterbalanced by the superb specificity of biotechnological processes and their unique ability to convert with high efficiency renewable feed stocks.
In this talk, I will present the origin and basic technologies of metabolic engineering and illustrate its applications with examples from the engineering of microbes for lipid synthesis for biodiesel production, as well as synthesis of mono ethylene glycol and fermentation of gases for biofuel production. I will expand on the parameters that will define the winners and losers of the antagonism between chemistry and biotechnology whose ultimate result will be efficient processes for the *sustainable* manufacturing of the products needed by current society.

ISV08
How the Nagoya-Protocol challenges microbiological research in Germany
*J. Overmann[1]
[1]*Leibniz-Institut DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany*

The Convention on Biological Diversity (CBD) acknowledges the right of each state to exploit the biological resources under its jurisdiction. The *Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization* (short, "Nagoya Protocol") to the CBD has been adopted to provide legal certainty for the actors involved in the use and international exchange of genetic resources and specifies means to share benefits gained from the use of genetic materials. Motives of the Nagoya Protocol were to (i) prevent misappropriation of biological resources and traditional knowledge that fall under the sovereignty of a provider state, (ii) contribute to the conservation and the sustainable use of biological diversity, and (iii) develop scientific capabilities in developing countries through international scientific cooperation in biodiversity research. The Nagoya Protocol entered into force on October 12, 2014, and within the EU is implemented through regulation 511/2014; the national law for implementation was passed on October 15, 2015.
According to the Nagoya Protocol "use" of biological resources also includes non-commercial, basic research activities. The definition of "genetic resources" not only extends to microbial strains as such, but also their biochemical compounds, DNA/RNA, and even the information on nucleic or protein sequences. Compliance with the new legislation requires (1) proof of legal acquisition of any microbial resource, (2) documentation of this proof and (3) inspections of users by the national authorities. Particular features of microorganisms that are relevant for the complicance with the Nagoya Protocol are the frequent lack of biogeography and of latitudinal diversity gradients.
Against this background, the presentation will focus on the far-reaching implications of the Nagoya Protocol for basic microbiological research. Real world examples will be used to illustrate the new obligations for the individual scientist and practical recommendations for future work with microbial cultures and genetic materials will be provided.

ISV09
***Legionella pneumophila*, a unique model to study host pathogen interactions and the evolution of virulence**
*C. Buchrieser[1]
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Legionella pneumophila is the etiological agent of Legionnaire's disease, a Gram-negative bacterium present in fresh and artificial water environments that replicates in protozoan hosts. When aerosolized bacteria are inhaled, they are able to colonize the respiratory tract, invade alveolar macrophages and replicate therein causing the disease. Replication within protozoa is essential for the survival of the bacterium. Genome sequencing and analyses led to a giant step forward in our understanding of how *L. pneumophila* replicates intracellularly by suggesting new ways by which this bacterium might subvert host functions. Our analyses revealed that the *L. pneumophila* genome encodes a high number and great diversity of eukaryotic-like proteins. We suggested that these are mimicking host proteins to subvert host-signalling pathways. Indeed, we and others have shown that *L. pneumophila* employs its F-box encoding proteins to exploit

the ubiquitin signalling pathways of the host or a sphingosine-1-phosphate lyase to restrain autophagy. Recently we characterized a SET-domain encoding protein and showed that it is secreted in the host cell where it induces epigenetic regulations to down regulate the host transcriptional response to infection. Comparative and evolutionary genomics analyses of the eukaryotic like proteins demonstrated that lateral gene transfer from eukaryotic hosts contributed to the evolution of these proteins within *Legionella*. Collectively these data shed new light on the virulence strategies of *L. pneumophila*, a major aspect of which is molecular mimicry.

ISV10
Natural products from entomopathogenic bacteria – from chemical ecology to synthetic biology
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Most antibiotics and several other therapeutics used in the clinic are derived from natural products produced by bacteria and fungi. Despite the importance of such compounds their natural function is often unknown but clearly they are not made originally to cure diseases.
Using entomopathogenic bacteria of the genera *Xenorhabdus* and *Photorhabdus* we are trying to answer the following questions: What is the natural function of these natural products? How have these compounds been optimized for what mode of action? How is their biosynthesis regulated and what triggers their production?
These questions typical for chemical ecology can be addressed since we can study the function of the bacterial natural products in the bacteria alone, together with their nematode host or the insect prey that is infected and killed by the nematodes carrying the bacteria in their gut and we can maintain all levels of this complex life cycle in the lab.
Once the basic mechanisms have been identified they can be applied to manipulate the regulatory mechanisms for the production of specific natural products. Moreover, the detailed analysis of several bacterial genomes with their encoded biosynthetic capacity allows the identification of rules for synthetic biology enabling the modification of biosynthesis pathways and even the *de novo* design of "non-natural" natural products.

ISV11
Geomycology – metals, minerals and mycota
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"Geomycology" is a part of "geomicrobiology" and can be defined as the impact of fungi on geological processes, including the bioweathering of rocks and minerals, metal accumulation and transformations, and element and nutrient cycling. There is growing appreciation of fungi as geoactive agents, particularly in soil, rock and mineral surface layers, and the built environment, as well as in symbioses with phototrophs. Fungal activities are dependent on hyphal growth form and chemoorganotrophic metabolism and, as with bacteria, many geomycological processes are of relevance to pollutant fate in the environment. Metal mobility can be altered through such processes as mineral dissolution, metal accumulation and biomineral formation. This presentation will emphasise some important activities of fungal systems in the transformation of metal(loid)s such as Pb, U, Mn, Se, Te, Ca and Co where the formation of insoluble phosphate, oxide, carbonate or oxalate minerals can provide a means of metal immobilization and biorecovery. Furthermore, some biominerals are formed at the micro- and nanoscale providing further interest for the development of novel biomaterials. Finally, mention will be made of the biodeteriorative properties of fungi regarding the destruction of mineral-based building materials, including concrete, which may have positive or negative consequences for nuclear decommissioning and radionuclide containment, and cultural heritage such as stone and mineral-based artefacts.

ISV12
They can't do it on their own – community control over organohalide-respiring Chloroflexi
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Hydrogen-driven reductive dechlorination is the only energy-conserving pathway in obligate organohalide-respiring Chloroflexi such as *Dehalococcoides mccartyi* (*Dhc*). The reductive dehalogenase (RDase) enzyme systems that serve as terminal oxidoreductases require a corrinoid cofactor for catalytic activity. Remarkably, *Dhc* strains lack the ability for *de novo* corrin ring biosynthesis and strictly depend on corrinoid scavenging. *Dhc* strain BAV1 cultures that received 25 µg vitamin B_{12} L^{-1} dechlorinated 1,2-dichloroethene (cDCE) to ethene, but incomplete dechlorination to vinyl chloride (VC) occurred in cultures amended with 1 µg vitamin B_{12} L^{-1}. Experiments with *Dhc* strain BAV1 and strain GT carrying the cDCE/VC reductive dehalogenase genes *bvcA* and *vcrA*, respectively, demonstrated that the lower bases of the cobamides affected dechlorination rates and extents. Amendment of 5',6'-dimethylbenzimidazolyl-cobamide (DMB-Cba) to *Dhc* strain BAV1 and strain GT supported high-rate cDCE-to-ethene reductive dechlorination. Significantly lower reductive dechlorination rates were observed with cobamides carrying 5'-methylbenzimidazole (MeBen), 5'-methoxybenzimidazole (MeOBen), or benzimidazole (Ben) as the lower base. Strain-specific responses were observed in cultures supplied with MeOBen-Cba or Ben-Cba, and only strain BAV1 harboring the BvcA VC RDase, but not strain GT expressing the VcrA VC RDase, produced ethene. Amendment with DMB restored the VC-to-ethene-dechlorinating phenotype in strain GT. The characterization of cobamides synthesized in mixed cultures enriched with nitrate, sulfate, ferric iron or carbon dioxide as electron acceptors revealed that the redox conditions affect the bioavailable corrinoid pool, and thus *Dhc* activity. Taken together, these findings reveal that site geochemical conditions determine cobamide quantity and quality (i.e., the type of lower base), which affect *Dhc* reductive dechlorination rates and extents (i.e., ethene formation) and thus impact bioremediation.

ISV13
Methane oxidation in Lake Constance
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Methane is produced in the deeper layers of the sediment of Lake Constance and is reoxidized nearly completely within the oxygen-supplied upper 3-5 mm of the sediment. The methane-oxidizing community is rather heterogeneous, including largely type I methanotrophs. Novel methane-oxidizing bacteria were isolated in oxygen-limited cultivation devices, and growth could be improved by addition of methanol-oxidizing partners. At methane seeps in the Eastern part of the lake, higher numbers of methanotrophs were detected, and the predominant methane oxidizers differed also qualitatively from those at reference sites. Besides aerobic methane oxidation, nitrite-dependent anaerobic methane oxidation by NC10-like bacteria appeared to be dominant, especially in sediments at greater water depths (> 40 m). Sulfate-dependent methane oxidation was not detected. Our results show that the diversity of methane oxidation in a freshwater lake is far greater than expected until only a few years ago.

ISV14
Environmental change, niche specialisation and microbial communities – What can we learn from ammonia oxidisers?
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Molecular techniques revolutionised both our ability to characterise natural communities of ammonia oxidisers and our views of their diversity and community structure. The first 16S rRNA gene-based studies demonstrated considerable diversity within soil ammonia oxidiser communities, phylogenetic groups with no cultivated representatives and environmental differences in community composition. Techniques that linked phylogeny and activity were then developed and the field was further revolutionised by the discovery of archaeal ammonia oxidisers. Ammonia oxidisers therefore provided excellent model organisms to address ecological questions, including the links between diversity, community composition and ecosystem function, the consequences of environmental change for microbial communities and the existence of niche specialisation and differentiation. These questions have been addressed using a combination of ecophysiological studies of cultivated organisms, genomics, correlation-based field studies and experimental microcosms. They have answered some long-standing questions in ammonia oxidiser ecology and have raised new ones, but have also highlighted many issues that plague studies of soil microbial diversity in general and ammonia oxidisers in particular. This presentation will therefore consider the influence of environmental change on ammonia oxidisers and niche specialisation in the context of broader studies of soil microbiology and the ways in which it is studied.

AEV01

How to stay in hell – how hyperthermophiles colonize black smokers

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Question: Black smokers are an extreme habitat (inside: 400 °C; outside: 2-4 °C) with a constant fluid stream, transporting potential colonizers within one second ca. 100,000 cell diameters off a hostile surrounding. How then, can hyperthermophiles colonize such chimney structures?
Methods: We have used various electron microscopic and light microscopic techniques, combined with swimming assays to study cell appendages of hyperthermophiles and their motility.
Results: We have shown that e.g. *Pyrococcus furiosus* uses its flagella not only for motility, but also for adhesion (e.g. to sand grains collected at the original biotope) and formation of cell-cell connections (1). Similar data have been obtained for *Methanocaldococcus villosus* (2), a hyperthermophile isolated from black smokers. This latter organism swims extremely fast (3); if speed is measured in relative units bps (= bodies per second) *M. villosus* is the fastest organism on earth. Hyperthermophiles exhibit two different swimming modes: a very fast one covering long distances in liquid surroundings, and a slower "seek mode" if encountering surfaces (3). We also have shown - by using a newly developed device (4) - that hyperthermophiles can react within < 3 seconds to high temperature by starting swimming, even if stored for 9 months at 4 °C.
Conclusions: Hyperthermophiles are able to survive for prolonged time at low temperature (e.g. 4 °C). If they are transported by chance to a hostile surrounding for them, they immediately can react to high temperatures by starting swimming. Thereby they are able to swim to a place having their optimal temperature; they can scan such a region for an optimal surface for colonization, and finally adhere there by using their flagella.

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AEV02

Structure of the formylmethanofuran dehydrogenase-polyferredoxin complex – methanogens' chemical trick to fix CO_2 without ATP consumption

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Doubling of the concentration of methane in the atmosphere within the last 100 years is of concern since methane is a potent green house gas which significantly contributes to global warming (1). This fact highlights the importance of the methane forming pathway only performed by methanogenic archaea. The first reaction step of hydrogenotrophic methanogenesis is catalyzed by tungsten- and molybdenum-containing formylmethanofuran dehydrogenases (Fwd/Fmd). CO_2 is fixed to methanofuran as a formyl group using ferredoxin as electron donor (2). In previous biochemical studies, it was proposed that, at first, a carboxy-methanofuran intermediate is formed which was subsequently reduced to a formyl group (3). Most of the carboxylation reaction requires ATP to activate CO_2. Detailed mechanistic information about this new type of reductive CO_2 fixation would provide an important step to understand methanogenic biochemistry.
We purified the native Fwd complex from a hydrogenotrophic methanogen, *Methanothermobacter wolfeii*, in an anoxic atmosphere. The enzyme complex was anaerobically crystallized and analyzed by X-ray crystallography. The structure was determined using the anomalous signal from the active site tungsten. The Fwd complex is composed of six different subunits FwdABCDFG organized in a Fwd(ABCDFG)$_2$ dodecameric or Fwd(ABCDFG)$_4$ 24-meric supercomplex. The FwdF subunit is the first polyferredoxin, which was structurally solved. A giant electron wire of 42[4Fe-4S] clusters in Fwd(ABCDFG)$_4$ electronically connects the four active sites. FwdB and FwdD form a formate dehydrogenase-like module, which uses the high-energy electrons from FwdF polyferredoxin to reduce CO_2 to formate on a tungstopterin cofactor. Formate is a stable intermediate of the reaction and is transferred from the tungstopterin active site in FwdB/FwdD to the metallohydrolase subunit FwdA most likely via a positively charged tunnel buried in the complex. The dinuclear metal center of FwdA condenses formate with the amino group of methanofuran forming formylmethanofuran. The accumulation of formate in the vicinity of the FwdA active site might increase the reactivity of formate to methanofuran.
Present structural results reinterpret the previous catalytic mechanism of the Fwd complex. Fwd first activates CO_2 to formate, which is funneled to a metallohydrolase site via an inner cavity for reacting with methanofuran. The arrangements of the polyferredoxins for forming a giant electron network might accumulate electron and thus build up an electron storage device that drives/enhances CO_2 reduction by an unknown mechanism.

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AEV03

Methanogenesis upside down – metabolic reconstruction of an archaeon performing nitrate-dependent anaerobic oxidation of methane

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Methane oxidation is an important process to prevent the emission of the greenhouse gas methane and further exacerbating of climate forcing. Both aerobic and anaerobic microorganisms have been reported to catalyze methane oxidation with only few substrates as electron acceptors.
Here, we investigated a methane oxidizing enrichment culture on physiological, biochemical and genomic level to establish a metabolic model of nitrate-driven anaerobic oxidation of methane (nitrate-AOM). Nitrate-AOM is catalyzed by an archaeon closely related to methanogens. Methane may be activated by methyl-CoM reductase and subsequently undergo full oxidation to carbon dioxide via reverse methanogenesis. All enzymes of this pathway were present and expressed in the investigated culture. The genome of the archaeal culture encoded a variety of proteins involved in an electron transport chain similar to those found in Methanosarcina species in addition to proteins not usually found in methanogenic archaea. Nitrate reduction seems to be located in the extracellular space and may be catalyzed by an unusual Nar-like protein complex. As membrane-integral electron carriers we identified quinones and not methanophenazines as in methanogens. The enrichment culture produced mainly nitrite but also some ammonium during nitrate reduction, presumably by the action of an extracellular Nrf enzyme. One of the key questions is how electrons from cytoplasmic reverse methanogenesis reach the nitrate reduction enzymes in the extracellular space. We will present a tentative model, based on genome and transcriptome studies, how electrons from reverse methanogenesis are transported to the nitrate and nitrite reductases, thereby suggesting how central energy metabolism and energy conservation of nitrate-driven anaerobic oxidation of methane could work.

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AEV04

Calvin-Cycle reinvented – autotrophic CO_2 fixation in *Ammonifex degensii*

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Question: A thermophilic, strictly anaerobic and autotrophic member of the low-GC, Gram-positive bacteria, *Ammonifex degensii*, harbours genes for two carbon fixation pathways, the Wood-Ljungdahl pathway (WJP) and the Calvin-Benson cycle (CBC). Almost all CBC genes, including rubisco and phosphoribulokinase are organised in an operon, whereas the gene for another characteristic CBC enzyme, sedoheptulose bisphosphatase (SBPase) is missing from the genome of *A. degensii.* The only candidate enzyme for this reaction in *A. degensii* is a bifunctional fructose-bisphosphate aldolase/phosphatase (FBPAP), which is encoded on the same operon as rubisco and phosphoribulokinase. Our aim was to investigate if both the CBC and the WJP are active *in vivo* and whether the missing SBPase can be replaced by FBPAP.
Methods: To test how the WJP and the CBC contribute to autotrophic carbon fixation in *A. degensii*, we (1) measured specific reactions of both pathways in cell extracts of autotrophically grown *A. degensii* cells; (2)

expressed rubisco, phosphoribulokinase and FBPAP in *E. coli* and purified and characterized them; (3) screened for characteristic phosphosugar intermediates of the CBC by LC-MS; (4) profiled amino acids, fatty acids and sugars from cells that were grown in the presence of ^{13}C-labelled carbon monoxide (a characteristic intermediate in the WJP) for their isotopologue composition by GC-MS.

Results & Conclusion: We have strong evidence that *A. degensii* fixes carbon via both the CBC and the WJP: (1)The key enzymes of both WJP and CBC were active in autotrophically grown *A. degensii* cells; (2) recombinant rubisco, phosphoribulokinase and FBPAP were active with their respective substrates; (3) large amounts of ribulose bisphosphate, the substrate for rubisco, could be detected in *A. degensii* cells; (4) ^{13}CO was incorporated specifically into the C_1-position of acetyl-CoA, which can only be explained by an active WJP. Incorporated label could be detected in all examined amino acids, but glucose and amino acids that derive from phosphosugars were labelled less heavily than fatty acids or amino acids that derive from acetyl-CoA or pyruvate. This can be explained by an influx of unlabelled carbon through the CBC. No SBPase activity could be detected in recombinant FBPAP; furthermore, no heptulose bisphosphate could be detected by metabolomics. This is why we propose an alternative route that proceeds via transaldolase, an enzyme that is also encoded on *A. degensii*'s CBC operon. Phylogenetic analysis revealed that rubisco, phosphoribulokinase and FBPAP show a high similarity to archaeal sequences, indicating that *A. degensii*'s CBC has evolved through lateral gene transfer.

AEV05

Ced – a DNA import system conserved in Crenarchaea

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Sulfolobales exchange DNA in a pili dependent manner upon treatment with UV-light or other DNA double strand break inducing agents like bleomycin. Previously we showed that the UV-inducible pili system (Ups) initiates cellular aggregation, which is essential for the exchange of chromosomal DNA within these aggregates. Yet, intriguingly so far no obvious homologs of bacterial DNA transporters could be found among Sulfolobales and Crenarchaea in general, the mode of DNA transport therefore remained a mystery. We identified *saci0568* and *saci0748,* two highly induced genes upon UV treatment encoding a transmembrane protein and an FtsK-/HerA-ATPase homolog. Upon UV-treatment, deletion mutants of *saci0568* and *saci0748* are able to form wild type-like cellular aggregates suggesting that the proteins are not involved in Ups-pili formation. Interestingly, with DNA transfer assays we were able to show that both proteins are essential for DNA transfer between *Sulfolobus* cells. By screening the genotype of recombinants of mixtures of Δ*ups* strains with Δ*saci0568*-or Δ*saci0748*-strains, we were able to show that Δ*saci0568* and Δ*saci0748* strains can only export DNA. We therefore propose that Saci0568 and Saci0748 are part of a novel DNA import system. In addition, we showed that two small neighboring genes (*saci_0567* and *saci_0569*) encode proteins that seem to be part of this DNA transfer complex. This transporter system is widely spread among Crenarchaea and therefore we propose to name it the Crenarchaeal system for exchange of DNA (Ced).

AEV06

New insights into the functions of TrmB proteins in *Pyrococcus furiosus*

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Question: Transcription factors of the TrmB family of transcriptional regulators are widely distributed in Archaea, but especially accumulated within the *Thermococcales* (1). TrmBL1 of *Pyrococcus furiosus* is one of the best studied regulators of this family. It is a global regulator, which controls as repressor or activator genes expressing proteins involved in sugar uptake, glycolysis or gluconeogenesis (2). The dual functionality of TrmBL1 relies on specific binding upstream or downstream of the corresponding promoters. TrmBL2 is also highly conserved within the *Thermococcales*, but recent data indicate that this protein is a DNA-architectural protein instead of a transcriptional regulator (3, 4, 5). These findings argue for a more non-sequence-specific DNA binding in the case of TrmBL2, although the DNA-binding domains of both proteins are highly conserved.

Methods: To get more information about the *in vivo* DNA binding specificity of these two proteins we performed Chromatin Immunoprecipitation experiments in combination with high-throughput sequencing (ChIP-seq). Identified binding sites were further assessed by bioinformatical and biochemical validation.

Results: ChIP-seq analysis of TrmBL1 confirmed known and predicted binding sites as well as revealed numerous novel binding sites of this transcription factor in the genome of *P. furiosus*, which are almost exclusively located in promoter regions. Moreover, these data verified the function of the TGM as cis regulatory DNA element for TrmBL1 binding *in vivo*. In contrast, TrmBL2 binding events are widely distributed over the whole genome and are located in coding as well as non-coding genomic regions. Further bioinformatical and biochemical analyses indicate that binding of TrmBL2 to the *P. furiosus* genome appears to be independent from a specific sequence feature or motif.

Conclusion: Combining ChIP-seq with detailed bioinformatical and biochemical analysis showed two different DNA binding modes by TrmBL1 and TrmBL2, which suggests differing functions of both proteins *in vivo*. Whereas TrmBL1 specifically binds promoter regions, where it acts as transcriptional repressor or activator, TrmBL2 binds the genomic DNA as architectural protein contributing to nucleoid organization and compaction.

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AEV07

Small RNA$_{41}$ involved in Carbon Metabolism in *Methanosarcina mazei* Gö1

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Question: In recent years, the versatility of roles associated to small non-coding RNAs in regulatory processes of Pro- and Eukaryotes has grown rapidly. In bacteria the majority of characterized sRNAs act as trans-encoded post-transcriptional regulators which target 5'-UTRs of mRNAs affecting the translation initiation e.g. by masking the ribosome binding site. In contrast relatively little is known about the regulatory mechanisms of sRNAs in archaea. *Methanosarcina mazei*, a methanogenic archaeon able to fix molecular nitrogen was studied concerning potential posttranscriptional regulation by sRNAs under nitrogen-stress. A genome wide RNA-sequencing approach identified 248 sRNAs of which 18 were regulated in response to different nitrogen availabilities [1]. One of those sRNAs, designated sRNA$_{41}$, is highly expressed under nitrogen sufficiency, whereas very low expression was detected under nitrogen limitation. This study aimed to elucidate the functional role and targets of this sRNA$_{41}$ in *M. mazei* Gö1.

Methods: First a computational target prediction using the tool IntaRNA [2] was performed, followed by a genome wide proteome analysis comparing wild type and a sRNA$_{41}$ chromosomal deletion mutant using LC/MS/MS. To confirm the results, Western blot analysis of the cell extracts were performed using polyclonal antibodies directed against different *M. mazei* proteins, as well as qRT-PCR to determine transcript levels.

Results: Computational target prediction, proteome and Western blot analysis identified the mRNA encoding the CO-dehydrogenase/Acetyl-CoA-synthase complex (ACDS) as target of the sRNA$_{41}$ in *M. mazei* Gö1. The target prediction further indicated that sRNA$_{41}$ is masking several ribosome binding sites of the polycistronic mRNA, all containing a highly conserved sequence upstream of their translational start site. Besides, no differences in transcript levels of ACDS genes were detected in a sRNA$_{41}$ deletion mutant versus wild type.

Conclusion: Computational and different experimental approaches revealed strong evidence that the mRNA encoding ACDS is a target of sRNA$_{41}$ in *M. mazei* Gö1. The physiological role will be discussed.

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AEV08
Circularization restores signal recognition particle RNA functionality in *Thermoproteus*
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All cells on earth generate proteins that are required to be transported to the cell membrane (in prokaryotes) or the endoplasmic reticulum (in eukaryotes) to allow their secretion. These proteins contain a signal peptide sequence that is recognized by the essential and evolutionarily conserved signal recognition particle (SRP) pathway. A SRP is always a ribonucleoprotein complex consisting of a single RNA molecule and few associated proteins. However, genome sequencing of archaeal species of the genus Thermoproteus did not identify standard SRP RNA genes. Thus, we aimed to solve the mystery of the "missing" component of this essential protein-targeting machine in these organisms using an RNA-Seq approach.
It was discovered that the "missing" universal SRP RNA gene was not yet identified due to its permutation; i. e. rearrangements of the genome resulted in a swap of the left and right portions of the SRP RNA gene. The correct sequence order is restored in mature SRP RNA molecules as their ends are permanently linked, resulting in circular SRP RNAs that can function in the cell. SRP assembly was confirmed *in vitro*. Furthermore, it was shown that the machinery that removes introns from transfer RNAs is responsible for the SRP RNA circularization reaction which highlights that moonlighting activities can evolve for ancient pathways (1).
We will discuss possible selective pressures that led to these drastic genome rearrangement events and propose that only the presence of an intron within an SRP RNA gene allows gene permutation. The resulting circular RNA molecules are highly stable which might be an advantage for Thermoproteus species growing at temperatures of up to 95 °C.

Plagens, A., *et al.* eLIFE (2015) doi:http://dx.doi.org/10.7554/eLife.11623).

BDV01
Soil – sediment microbial community adaptation due to a long history of oil contamination
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Oil contamination due to anthropogenic activity can persist in the environment for many years with severe environmental impact. Natural oil seeps offer a unique opportunity to study the evolution and adaptation of ecosystems to long-term contamination and the biodegradation potential of indigenous microorganisms. Most oil seeps investigated so far are located on the seabed, thus there is little data available about microbial communities in other environments.
Here, we investigate a natural coastal oil seep located in Zakynthos Island, western Greece, called Keri Lake. In this unique ecosystem, flow of tar and petroleum products to the surface has been observed for more than 2000 years. We hypothesize that a well-adapted microbial community has been shaped under the long presence of oil hydrocarbons in soil. In order to study its structure and functional potential related to hydrocarbon biodegradation, replicate soil cores up to 7 meters were collected in October 2013 from highly- and non-contaminated areas in Keri Lake. The cores were sampled *in situ* in various depths spanning from ~ 0.1 to 6.5 meters.
The influence of hydrocarbons and sampling depth on microbial communities was assessed using metagenomic shotgun sequencing. Our data revealed a significant influence of the contamination on community diversity. Highly- and non-contaminated sites were dominated by Bacteria, especially *Desulfobacteraceae* and *Dehalococcoidia* species. Evidence that the latter play an important role in the degradation of aromatics is presented. Additionally, a clear increase of archaeal sequences was observed in the highly contaminated samples together with a higher potential for methanogenesis. In contrast, processes like sulfate reduction and denitrification were slightly decreased compared to non-contaminated sites.
To confirm these observations and further characterize this unique ecosystem the obtained molecular data was further correlated to abiotic soil parameters and the chemistry of the oil.

BDV02
Biodegradation of aromatic compounds in a coal tar polluted aquifer studied by denitrifying BTEX-degrading enrichment cultures and *in-situ* community analysis
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Question: Petrochemical processing of fossil fuels is often accompanied by an unintentional release of pollutants into the groundwater. Contaminants such as monoaromatic BTEX are a serious risk to human health and their environment. Some microorganisms can utilize these compounds for energy conservation and growth. In this study, the capacity for natural attenuation was evaluated along a pollutant gradient in an anaerobic aquifer of a former gas works site.
Methods: Groundwater samples from three different wells were collected for DNA extraction and establishment of BTEX-degrading enrichment cultures. For enrichment, anoxic groundwater was incubated with additional nitrate and single BTEX (benzene, toluene, ethylbenzene, xylenes) and controlled for biotic substrate consumption. The community structure in the enrichment cultures as well as in the groundwater samples was analyzed by bacterial 16S rRNA next-generation sequencing and fingerprinting of a gene (*bam*A) involved in anaerobic degradation of monoaromatic compounds.
Results: Microorganisms that degrade toluene and ethylbenzene were enriched from all sampling wells, whereas *p*- and *m*-xylene degrading microorganisms were only enriched from two wells with high contaminant concentrations. Benzene and *o*-xylene were not degraded. Toluene and ethylbenzene degrading cultures were dominated by *Azoarcus* spp. In *p*-xylene degrading cultures, members of the genus *Georgfuchsia* were most frequent. Aromatic compound-degrading microorganisms were present in the groundwater well communities, including Fe(III)-reducing (*Geobacter*), sulfate-reducing (*Desulfosarcina*, *Desulfomonile*, *Desulfotomaculum*) and fermenting (*Syntrophus*, *Pelotomaculum*) genera. The highly conserved *bamA* gene was detected in all groundwater wells and in the enrichment cultures, supporting the applicability of *bamA* as a marker for monoaromatic compound degradation.
Conclusion: Enrichment cultures confirmed the presence of denitrifying BTEX-degrading microorganisms in the polluted aquifer. It was further shown that different genera (all belonging to the *Rhodocyclaceae*) were specialized for the degradation of different BTEX and that these organisms were unevenly distributed along the pollutant gradient. Besides the laborious application of enrichment cultures, new advances in 16S rRNA sequencing allowed an affordable and in-depth microbial community analysis, from which conclusions can be drawn about the prevailing metabolic processes. The combinatorial application of the methods described here is well suited for the evaluation of natural attenuation capacities; a prerequisite for the decision-making and monitoring of bioremediation projects.

BDV03
Conversion of *cis*-2-carboxycyclohexylacetic acid in the down-stream pathway of anaerobic naphthalene degradation
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Background: The ability of anaerobic naphthalene degradation has been demonstrated for the deltaproteobacterial strains N47 and NaphS2 under sulfate-reducing conditions (Galushko *et al.*, 1999, Meckenstock *et al.*, 2000). It was shown recently that after carboxylation to 2-naphthoic acid (Mouttaki *et al.*, 2012) and formation of the corresponding CoA-ester, the latter is stepwise reduced to a hexahydro-2-naphthoyl-CoA with unknown conformation of the remaining double bonds (Eberlein *et al.*, 2013). Furthermore, *cis*-2-carboxycyclohexylacetic acid could be identified as a metabolite of the down-stream pathway (Annweiler *et al.*, 2002).
Objectives: Starting from the known metabolite *cis*-2-carboxycyclohexylacetyl-CoA, our aim for this study was the elucidation of the next metabolites of the pathway and the identification of the enzyme reactions finally leading to the second ring-cleavage.

Methods: The metabolite *cis*-2-carboxycyclohexylacetic acid was chemically synthesised and converted to the corresponding coenzyme A thioester. The latter was tested for conversion in cell-free-extracts of N47 and NaphS2 in the presence of different potential co-factors and electron acceptors. Samples were analysed by HPLC and emerging metabolites were further characterised via GC-MS and high-resolution MS.
Conclusion: Conversion of *cis*-2-carboxycyclohexylacetyl-CoA was only observed in the presence of ferrocenium hexafluorophosphate, which can oxidise enzyme-bound co-factors like $FADH_2$. The first emerging metabolite had a double-bond introduced, indicating the activity of a FAD-dependent acyl-CoA dehydrogenase. Also the product of the next enzyme, a hydratase, could be detected. Combining these data, we can for the first time make a detailed proposal for the steps leading to the second ring-cleavage in the down-stream pathway of anaerobic naphthalene degradation.

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BDV04
Anoxic degradation of auxin in denitrifying Betaproteobacteria

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Indole-3-acetate occurs in nature as an intermediate of tryptophan degradation and is particularly important as the most common member of the auxin class of plant hormones. Auxins reach concentrations of 0.1 ppm in whole plants and are released into soil by secretion or upon the decomposition of plant tissue, representing a broadly available food source for soil microorganisms. Auxin synthesis and degradation by microorganisms also plays a role in many plant-microbe interactions. The processes leading to indoleacetate synthesis and degradation are therefore of profound interest for plant biology as well for the study of the corresponding microbes. Several bacterial species were described to catalyze degradation of indoleacetate under aerobic conditions, e.g. *Pseudomonas* or *Bradyrhizobium* species, but the metabolic pathways are still unclear. In addition, an oxygen-independent pathway of indoleacetate metabolism was discovered in the closely related denitrifying Betaproteobacteria *Azoarcus evansii* and *Aromatoleum aromaticum*, which are capable of anaerobic growth with indoleacetate and nitrate as sole substrates (Ebenau-Jehle *et al.* 2011). An hypothetical metabolic pathway was proposed based on the detection of ^{14}C-labeled intermediates in cell extracts, the identification of specifically induced proteins in indoleacetate-degrading cells and the bioinformatic analysis of an apparent operon coding for the involved proteins (*iaa* operon). From investigating the first enzymes, a modified version is of this pathway suggested which is initiated by activation of indoleacetate to the CoA-thioester, hydroxylation to oxoindoleacetyl-CoA by a molybdenum enzyme, followed by an ATP-dependent hydrolytic cleavage of the heterocycle to aminophenylsuccinyl-CoA. This intermediate is then rearranged by an intramolecular CoA-transfer reaction and further converted to aminobenzylmalonyl-CoA via a coenzyme B12-dependent mutase. Finally, a specific beta-oxidation pathway converts the latter intermediate to acetyl-CoA, CO_2 and aminobenzoyl-CoA. We present recent biochemical data on some of the enzyme of this pathway from overproduced Iaa proteins affirming the proposed pathway as well as indications on the regulation of the *iaa* gene cluster.

BDV05
Identification of glyphosate degradation pathways in soil and water-sediment systems – a stable isotope co-labeling approach

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Question: Glyphosate and its metabolite aminomethylphosphonic acid (AMPA) are frequently detected in soil and freshwater sedimentary environments, but there are no comprehensive studies on glyphosate behavior in these systems. Microorganisms can use C and N from a pesticide to synthesize their biomass compounds, e.g. amino acids (AA) and fatty acids (FA). The extraction of known microbial biomarkers from soil or sediment after addition of C and N isotope tracer allows an estimation of microbial activity in the transformation of pesticide. We investigated the degradation pathways of glyphosate with the particular focus on the metabolic incorporation of the isotope label into AA, FA and their fate over time.
Methods: An agricultural soil and water-sediment were incubated with co-labeled glyphosate ($^{13}C_3{}^{15}N$-glyphosate) in the dark and at constant temperature (20 °C). $^{13}C_3{}^{15}N$-glyphosate was used to determine its turnover mass balance over a period of 80 days. Soil and sediment samples at the respective sampling date were analyzed for the amount and the isotopic composition of AA, FA, CO_2, solvent-extractable parent compound and metabolites and total NER [1, 2].
Results: In the water-sediment system, 55.7 % of ^{13}C of glyphosate was ultimately mineralized, whereas the mineralization in the water system (without sediment) was low, reaching only 2.4 % of ^{13}C of glyphosate equivalents. This finding demonstrates the key role of sediments in its degradation. Glyphosate was mineralized in the soil more rapidly and at the end labelled CO_2 constituted about 73 % of $^{13}C_3$-glyphosate equivalents. A rapid increase in $^{13}C^{15}N$-AMPA after 10 days was noted in water-sediment system and these transformation products ultimately constituted 26.2 % of the $^{13}C_3$-glyphosate and 78.5 % of the ^{15}N-glyphosate equivalents. In contrast, in the soil, $^{13}C^{15}N$-AMPA increased initially but after 20 days decreased slowly reaching ultimately 12.3 % of the $^{13}C_3$-glyphosate and 39.6 % of the ^{15}N-glyphosate equivalents. Initially, glyphosate was biodegraded via the sarcosine pathway related to microbial growth, as shown by co-labeled $^{13}C^{15}N$-glycine and biogenic residue formation. Later, degradation via AMPA dominated under starvation conditions, as shown by the contents of ^{13}C-glycine.
Conclusion: The presented data provide the first evidence of the utilization of glyphosate as a C and N source and highlight the relevance of both the sarcosine and the AMPA pathways in the water-sediment and soil system.

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[2] Girardi C, Nowak KM, Lewkow B, Miltner A, Gehre M, Schäffer A, Kästner M. 2013. Comparison Microbial degradation of the C-isotope-labelled pharmaceutical ibuprofen and the herbicide 2,4-D in water and soil. Sci. Total Environ. 444C: 32-42.

BDV06
Characterization of Latex Clearing Protein (Lcp) from *Rhodococcus rhodochrous*

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Question: Rubber is an everyday product and with its use come vast amounts of waste that have to be dealt with. The human way is to dispose it by combustion, whereas numerous microorganisms developed a more sophisticated way to utilize this valuable resource as a source of carbon and energy by biodegradation. In Gram-positive bacteria, the ability to grow on polyisoprene correlates with the presence of Lcp (1). The heterologous expression of Lcp was achieved in 2014 (2,3) and recently Lcp_{K30} was biochemically characterized and identified as a *b*-type cytochrome (4). In this study, a newly isolated potent rubber degrading strain was described and its Lcp was biochemically characterized.
Methods: Enrichment cultivation, chromatographic purification, UV/Vis spectroscopy, HPLC, Oxygen consumption assay, ICP-MS, MALDI-TOF.
Results: A rubber-degrading bacterium isolated from an enrichment culture with latex gloves as carbon source was identified as *Rhodococcus rhodochrous.* Vulcanized rubber pieces in liquid culture were visibly disintegrated within 4 weeks by *R. rhodochrous* strain RPK1 indicating the presence of a potent rubber degrading enzyme. A gene of *R. rhodococcus* PRK1 coding for a polyisoprene-cleaving protein (lcp_{Rr}) was cloned, expressed in *Escherichia coli* and purified. Lcp_{Rr} degrades polyisoprene to a mixture of oligoisoprene molecules with terminal keto and aldehyde groups. The pH optimum (pH 8) of Lcp_{Rr} was substantially higher than that of other rubber-cleaving enzymes. UV/Vis spectroscopical analysis of Lcp_{Rr} revealed a heme-specific absorption spectrum. The presence of one *b*-type heme in Lcp_{Rr} as a co-factor was confirmed (i) by metal analysis, (ii) by extraction of heme with acidic acetone and (iii) by determination of heme-*b* specific *m/z* values via mass-spectrometry. Remarkably, the UV/Vis spectrum of Lcp_{Rr} revealed an additional band around 645 nm that was not present in Lcp_{K30}. As a result, the color of

concentrated Lcp_{Rr} was brown in contrast to the red color of Lcp_{K30} and of most other cytochromes. Evidence for an open conformation of Lcp_{Rr} and for a closed conformation of Lcp_{K30} was obtained by spectral analysis of both Lcp proteins in the presence of imidazole as a small heme ligand. Our data indicates substantial differences in the active sites of Latex clearing proteins from different rubber degrading bacteria.
Conclusion: Lcp_{Rr} from the newly isolated potent rubber degrading strain *Rhodococcus rhodochrous* RPK1 was purified, biochemically characterized and differs from Lcp_{K30} in the stated characteristics.

(1) M. Yikmis and A. Steinbüchel; AEM 78 (2012), p. 4543
(2) S. Hiessl, *et al.*; AEM 80 (2014), p. 5231
(3) J. Birke and D. Jendrossek; AEM 80 (2014), p. 5012
(4) J. Birke, *et al.*; AEM 81 (2015), p. 3793

BDV07
Formation of bisphenol-phosphate conjugates by *Bacillus amyloliquefaciens* – a novel mechanism to reduce toxicity and estrogenicity of bisphenols
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Question: Bisphenol A (BPA) and structurally related bisphenols are used as a basic monomer in the manufacture of polycarbonate plastics and epoxy resins. Among other sources sewage treatment plants (STP) represent an important entry path of bisphenols into the environment. Their distribution is of great concern since these compounds are poorly biodegradable and considered to be toxic as well as endocrine disruptors that interact with several hormone receptors. Whereas these interactions are well described for BPA, little is known about other bisphenols regarding risks for human health and the environment. Therefore we focused on the transformation of BPA and four structurally related bisphenols by *Bacillus amyloliquefaciens* SBUG 1837 isolated from sewage sludge to investigate the ability of bacteria in STP to biodegrade environmental pollutants.
Methods: The microbial transformation of bisphenols was analyzed by high performance liquid chromatography (HPLC). For structure elucidation of the products formed mass spectrometric (LC-MS, GC-MS) and nuclear magnetic resonance spectroscopic analyses (NMR) as well as deconjugation experiments were carried out. Estrogenic activity was determined by a yeast-estrogen-screen-assay. Morphological effects of bisphenols on bacterial cells were studied using electron microscopic examinations.
Results: *B. amyloliquefaciens* SBUG 1837 transformed each bisphenol into one major product but its formation was partly reversible. Transformation rates up to 77 % of the initial bisphenol concentration of 60 µg ml^{-1} were achieved. Structure elucidation proofed the formation of bisphenol-phosphate conjugates. In contrast to the transformation reaction, toxicity and estrogenicity strongly depended on structure of bisphenols correlating with their hydrophobicity. However, conjugates formed were non-toxic and considerably less estrogenic than their parent bisphenols.
Conclusion: The formation of phosphate conjugates by *B. amyloliquefaciens* SBUG 1837 eliminated toxicity and significantly reduced estrogenicity of bisphenols. There is hardly any information about the linkage of environmental pollutants with phosphate residues (Kurozumi *et al.*, 1978). Thus, we describe a new mechanism of conjugate formation with bisphenols. This demonstrates the potential of bacteria to transform pollutants in STP. However, it should be considered that the formation of bisphenol-phosphate conjugates by bacteria from wastewater can lead to a diminished analytical determination of bisphenols in STP and consequently to an underestimation of the potential risks to humans and animals due to the reversibility of this reaction.

Kurozumi, A., Kurokawa, T., Yamaguchi, I., Misato, T., 1978. Phosphorylation of 3,3'-dimethyl-4-hydroxybenzophenone by soil microorganism (*Bacillus*-sp). J. Pestic. Sci. 3, 59-60.

BDV08
Identification of an unusual decarboxylase crucial for norcobamide biosynthesis in the tetrachloroethene-respiring bacterium *Sulfurospirillum multivorans*
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Question: *Sulfurospirillum multivorans* is an epsilon-proteobacterium able to reductively dehalogenate tetrachloroethene (PCE) to *cis*-dichloroethene. This environmentally important process is catalyzed by the PCE reductive dehalogenase (PceA). The iron-sulfur protein harbors a unique cobamide cofactor termed norpseudo-B_{12}. PceA from *S. multivorans* showed an exceptionally high reactivity towards PCE in comparison to homologues found in other bacteria that utilize standard cobamides. Norpseudo-B_{12} is synthesized *de novo* in a multistep pathway in *S. multivorans* and this norcobamide varies in one structural property from other natural cobamides, such as pseudo-B_{12}. While pseudo-B_{12} harbors an (*R*)-1-aminopropan-2-ol *O*-2-phosphate (AP-P) in its nucleotide loop, norpseudo-B_{12} possesses an ethanolamine *O*-phosphate (EA-P) moiety lacking a methyl group. The biosynthesis of AP-P was studied in *Salmonella enterica* and L-threonine was found as precursor. After phosphorylation, *O*-phospho-L-threonine (L-Thr-P) is decarboxylated by the CobD enzyme yielding AP-P. Based on these findings, it was hypothesized that EA-P biosynthesis in *S. multivorans* originates from L-serine. The aim of the study was to unravel the modification of the cobamide biosynthesis pathway in this organism, which leads to the formation of norpseudo-B_{12} rather than pseudo-B_{12}. Structural data, obtained for *S. multivorans* PceA, pointed towards a specific binding of norcobamides by the enzyme. Thus, the compatibility of PceA to standard cobamides was studied in addition.
Methods: Guided cobamide biosynthesis experiments with exogenous L-Thr-P were performed with *S. multivorans*. The formation of pseudo-B_{12} and its influence on PCE-dependent growth and PceA enzyme activity was tested. The gene SMUL_1544 (*cobD* homologue) of *S. multivorans* was heterologously expressed in *Sa. enterica* Δ*cobD* and the phenotype complementation was analyzed by growth monitoring and cobamide analysis. The SMUL_1544 protein was purified and characterized by an HPLC-coupled decarboxylation assay.
Results: Exogenously applied L-Thr-P led to the biosynthesis of pseudo-B_{12} in *S. multivorans*. The amount of PceA in the crude extract and the enzyme activity were reduced under these conditions. Heterologous production of SMUL_1544 in a CobD-deficient *Sa. enterica* strain resulted in a complementation of the *cobD*-negative phenotype. The major cobamide produced was norpseudo-B_{12}. Purified SMUL_1544 converted *O*-phospho-L-serine to EA-P with 4 nkat/mg, while the conversion of L-Thr-P to AP-P had a significant lower rate.
Conclusion: The production of pseudo-B_{12} showed a negative effect on PceA formation in *S. multivorans*, but the modified cofactor seems to be functional in the enzyme. The protein SMUL_1544 is a novel *O*-phospho-L-serine decarboxylase determining the production of norcobamides in the organism.

BEV01
Effects of micro-predators of different specialization on the adaptation of three different prey species
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Water shortage is a growing concern worldwide and a main issue in the Middle East. Wastewater purification and the use of reclaimed sewage water save freshwater resources in these regions. Physical, chemical, and biological factors are involved in the pathogen removal during the purification steps. Biological removal processes include the predation by bacterivorous organisms and phages (by lysis). A better exploitation of predator-prey interactions may thus result in a more efficient reduction of pathogens in wastewater.
We investigated the direct and indirect, as well as positive and negative interactions between predator and prey model organisms in a miniature membrane bioreactor (mMBR) containing artificial wastewater. Different combinations of micro-predators with differing prey ranges and prey bacteria were assembled in mMBRs and studied over a four day period.

Predators included a protist (generalist predator), a bacteriophage (specialist "predator") and a predatory bacterium (*Bdellovibrio*-and-like organism, BALO) that only preys upon Gram-negative bacteria. *Klebsiella sp*., *Staphylococcus sp*., and *Pseudomonas putida* were included as typical wastewater bacteria.
We found that only the specialist predators (phages and BALOs) were able to drive their preferred prey bacteria to extinction when incubated with all three prey species. However, phages and/or BALOs in combination with the generalist predator were able to reduce all prey species to a similar extent. Here, all predator and prey species were able to coexist. We therefore conclude that the protist acted as a key player in our system and was able to balance the effects of the specialist predators. We also found that predator growth was higher when incubated with multiple prey species, independent of the predator's ability to utilize all those species.
Surprisingly, we observed a reduction in the preys´ generation time when exposed to the specialist predators; on the contrary, predation by the protist led to an increase in the generation time. We were able to link the change in *Klebsiella* generation time to the development of resistance to the phage, but only in settings with sufficient nutrient supply for the bacterium. Resource availability might be promoted by a generalist predator, most likely due to its removal of potential resource competitors, as we find a high rate of bacterial resistance in experiments that contain protists. This trade-off might therefore only develop in highly nutritious environments like wastewater, since it is likely coupled with higher energy requirements.
Our results imply that a generalist predator may not only directly reduce prey in a multi-predator system, but also indirectly by its impact on the development of bacterial resistance to a specialist predator. Considering this effect in process operations containing microbes, might therefore help to strengthen a process' robustness.

BEV02
The intestinal microbiome of root fly larvae – a source of isothiocyanate degrading enzymes
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Question: Plants of the Brassicaceae family produce toxic isothiocyanates to provide protection against herbivorous insects and mammals, as well as pathogenic bacteria. The larva of the cabbage root fly (*Delia radicum*) is one of few specialist insects that are able to feed on Brassicaceae. We hypothesized that *D. radicum* copes with high ITC concentrations with the help of microbes residing in the gut of the insect larvae.
Methods: The gut microbial community of lab-reared *D. radicum* larvae was profiled by 16S rRNA gene sequence analysis derived from a metagenome. ITC resistant strains were isolated from *D. radicum* intestines by successive clean streaking on 2-phenylethyl isothiocyanate (2PE-ITC) rich medium. Naturally occurring plasmids were isolated from these strains and subsequently sequenced, assembled and annotated. Plasmid-encoded genes were compared to a database of *sax* genes that are known to confer resistance against aliphatic isothiocyanate. A *saxA*-homologous gene sourced from one of those plasmids (pDrgb3) was transformed into competent *E. coli* cells and brought to expression. Transformed *E. coli* strains were tested for increased resistance against 2PE-ITC. Purified SaxA protein activity on 2PE-ITC was determined by GC-MS.
Results: Four strains capable of metabolizing 2PE-ITC were isolated from the gut. Plasmid pDrgb3, found in all four isolates, contained an operon with three genes with close homology to the *saxCAB* region associated with aliphatic ITC resistance. Heterologous expression of the *saxA* gene in *E. coli* transformants increased bacterial resistance to 2PE-ITC compared to empty vector controls. Purified SaxA protein was shown to catalyze the hydrolysis of several isothiocyanate species.
Conclusions: Four 2PE-ITC resistant bacterial strains in the *D. radicum* gut carry a natural plasmid containing genes that facilitate ITC breakdown. The SaxA protein is the first representative of a novel family of ITC hydrolase enzymes and is hypothesized to facilitate the first step in a previously undescribed ITC breakdown pathway. Our findings suggest that bacteria may aid in detoxifying the insect diet. The degree of the insect's dependence on gut microbes to overcome plant defenses awaits further study.

BEV03
Elucidation of the structural and functional diversity of the rumen microbiota
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The structure and functions of microbiota inhabiting the gastrointestinal tract are mainly shaped by the host's food intake. Apart the dietary impact, individuals maintain their own specific gut microbial composition. Describing the influence of various animal feedings on the inherent community arrangement and associated metabolic activities of the most active ruminal fractions (composed of bacteria and archaea) is of great interest for animal nutrition, biotechnology and climatology.
Samples were obtained from three fistulated Jersey cows rotationally fed either with corn silage, grass silage or hay and split into three sections (ruminal fluid, squeezed solid and solid matter). Microbial communities were examined by Illumina sequencing of the 16S rDNA (V1 and V2 regions) and by LC-MS/MS based metaproteomic analyses comprising specific sample preparation procedures (Deusch & Seifert, 2015). Further on the respective metabolomes were determined by 500 MHz-NMR spectroscopy.
Despite the predominating individuality of the microbiota structure, significant alterations in response to particular feedings were observed exploring the data obtained by metaproteomics and metabolomics. Species of the family *Succinivibrionaceae* showed higher abundance in corn-based samples whereas cellulolytic *Fibrobacteraceae* appeared in larger numbers in grass and hay diets. Fiber-degraders of the *Lachnospiraceae* family emerged in great quantities throughout the solid fractions. Comparing 16S rDNA based results with metaproteomic analyses, *Prevotellaceae* were found to be more abundant in the protein fractions. In contrast, proteins belonging to *Acidaminococcaceae* and *Ruminococcaceae* appeared to be less contributing. Enzymes involved in amino acid transport and metabolism increased in corn diets, proteins related to posttranslational modifications, protein turn-over and chaperons were less present in hay feeding.
Disregarding the diet-introduced changes in structure and function of the microbiota a host dependent microbiota composition was found to be prevailing. The microbial community of solid rumen matter was shown to be clearly distant from rumen fluids. The combination of *omics*-technologies represents a powerful tool to investigate the microbiota of complex ecosystems like the rumen. In order to retrieve deeper insight into the complicated network of gut microbial adaptation and to improve efficiency in livestock further investigations will be necessary.

Deusch & Seifert (2015) Proteomics 15(20):3590-3595.

BEV04
Contribution of uncultured *Planctomycetaceae* to the degradation of 4-chloro-2-methylphenoxyacetic acid in the drilosphere
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4-chloro-2-methylphenoxyacetic acid (MCPA) is a widely used phenoxyacetic acid herbicide in agriculture and a potential soil and ground water contaminant. Degradation occurs mainly under oxic conditions in soils. Earthworms dominate macrofauna in many soils, shape soil microbial communities by selective activation, and aerate soils by their burrowing activity. Thus, the impact of the anecic, litter feeding earthworm *Lumbricus terrestris* on the mineralization of MCPA and associated microbial community was assessed in soil columns that received agriculturally relevant concentrations of [^{13}C]MCPA via rRNA stable isotope probing combined with GC/C-IRMS and HPLC. Treatments with [^{12}C]MCPA served as controls. [^{13}C]MCPA disappeared and was mineralized within 45 and 60 days in the presence and absence of worms, respectively. Recoveries of MCPA-[^{13}C] in [^{13}C]O_2 approximated 45 % and 25 % in the presence and absence of worms, respectively. Illumina sequencing of 16S rRNA amplicons generated from [^{13}C]-labelled and unlabelled [^{12}C]-RNA suggested diverse active (family level) taxa in soil and drilosphere (i.e., earthworm impacted soil including burrows and cast). 16S rRNA affiliating with *Planctomycetaceae* was strongly [^{13}C]-labelled in cast and burrow walls. *Pseudomonadaceae, Comamonadaceae, Oxalobacteraceae, Rhodobacteraceae* and *Sphingomonadaceae* were of minor importance. *Comamonadaceae* and *Sphingomonadaceae* related 16S rRNA were strongly [^{13}C]-labelled in bulk soil. The collective data indicate that *L. terrestris* stimulates MCPA mineralization via activation of hitherto uncultured *Planctomycetaceae*, thus extending our knowledge on the physiological capabilities of an understudied, yet abundant family in soils.

BEV05
Genomic signatures of plant growth promoting *Bacillus*
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Variety of bacteria related to *B. subtilis* - *B. amyloliquefaciens* group are widely used in agriculture for plant growth promoting biocontrol of phytopathogens. Efficiency of these strains depends on the ability to colonize plants. Successful plant colonizers may be found among strains of different species. Identification of important genes and transcription regulation mechanisms ensuring the effective plant colonization will advance greatly our understanding of biology of plant growth promoting rhizobacteria (PGPR) and designing of new biopesticides. In this work, a holistic approach was used to compare whole genome sequence data generated for 12 model strains of *B. subtilis*, *B. amyloliquefaciens*, *B. atrophaeus* and *B. mojavensis* with bioassay data on activities of these strains in terms of plant growth promotion, competing against phytopathogens, improving draught tolerance in plants and shelf-life prolongation of crops in store room. Sixteen genes involved in oxidative stress response, sugar metabolism and transcriptional regulation were found to be shared by the most active strains despite their belonging to different species but absent in their non-active closest relatives. Involvement of horizontal gene transfer events was hypothesized. Designing genome specific barcodes allowed tracing the distribution of different lineages of these microorganisms in nature by mapping reads from publically available metagenomic datasets against the created barcodes. It was found that the active PGPR strains had different ecological preferences. While some of them were abundant in grassland, others were more frequent in forest rhisoshpere or in phyllosphere. These differences also should be taken into consideration when new biopesticides are designed. Another level of research was on gene transcription regulation. RNA-Seq comparison of gene expression profiles of an active PGPR strain *B. atrophaeus* UCMB-5137 stimulated by root exudate revealed the most important transcriptional factors involved in gene regulation during rhizosphere colonization. Analysis of the differential expression in intragenic spacer regions identified 49 putative non-coding RNA (ncRNA) and their mRNA targets. Despite being located in hyper-variable intragenic regions, the loci involved in ncRNA-mRNA interaction showed significant sequence conservation between organisms of *B. subtilis* group and also in more distant bacteria of *B. pumilus*, *B. licheniformis*, *B. halodurans* and *B. cereus*. It was hypothesized that the analysis of conservation in these loci also may help in selection the most active PGPR strains for future biotechnological application. Comparison of genomes of selected organisms on the levels of genomic sequences and genome functionalities allowed selecting of genetic signatures suitable for estimation of biological activities of PGPR strains and designing of new biopesticides.

BEV06
Distribution pattern of arbuscular mycorrhizal fungi in a tropical dry forest
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Arbuscular mycorrhizal fungi (AMF) are symbionts of the majority of vascular terrestrial plants and achieving a better understanding of their ecology could be invaluable due to their pervasive effects on plant fitness and NPP. Here we asked whether AMF communities on the top of rocky outcrops, termed inselbergs, could differ systematically from those of the surrounding plains. Our study area was located at a tropical dry forest in northeast semi-arid Brazil. We identified 12 rocky outcrops and we delimited surrounding sites of 5m x 20m. These consisted of a plot on top of the outcrop and three plots evenly dispersed over the surrounding plain with a minimum distance of 1 km to each other. From all plots, we obtained a single composite soil sample (ten soil cores 0-20 cm depth). AMF communities were identified based on spore morphological characteristics. We tested for systematic community shifts or diversity differences between the inselberg and plain AMF communities. For this reason, we used a range of multivariate approaches as well as paired *t*-tests. We found no evidence for AMF diversity or community shift differences in our plain-inselberg comparisons. A further forward-selection-backward-elimination redundancy analysis of 22 soil properties revealed that the only systematic differences other than a site effect present in our system resulted from soil sodium [Na] differences. The result was confirmed through a permANOVA and an indicator species analysis revealed that four AMF species were associated with soil of either low or high [Na]. The species *Gigaspora gigantea* was a good indicator of low [Na] and *Acaulospora scrobiculata*, *Acaulospora sp*. and *Rhizoglomus natalensis* of high [Na]. Our results suggest a potential role of [Na] as a factor affecting AMF community structure.

BEV07
Diversity and species recognition of the *Mucor circinelloides* complex
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The zygomycetous fungus *Mucor circinelloides* (*Mucorales, Mucoromycotina*) is a common saprobiont found in soil and all kinds of wet organic matter. As spoilage organism of food and beverage it is a permanent part of the human environment. It is used as fermenting agent of soybean based traditional Asian food. On the other hand, *M. circinelloides* is an opportunistic pathogen and the prevailing *Mucor* species involved in fungal infections (mucormycoses).
The last taxonomic revision of the *Mucor circinelloides* complex based on morphology and mating tests was performed nearly fifty years ago and resulted in the recognition of four formae: f. *circinelloides*, f. *griseocyanus*, f. *janssenii* and f. *lusitanicus*. Recent molecular phylogenetic analyses based on a single locus only or on a small number of strains revealed an unexpected high diversity and several taxonomic discrepancies. The formae formed supported groups in these analyses for which reason their taxonomic status is controversial currently.
In order to clarify the taxonomic status of the formae and to provide a reliable species concept we performed a polyphasic study of the *Mucor circinelloides* complex including multi-locus phylogenies, mating tests as well as morphological and physiological studies including about 80 strains. Using a phylome database of four mucoralean genomes new phylogenetic markers could be successfully applied for our molecular phylogenies. Mating experiments followed by a detailed analysis of the resulting zygospores were used to estimate biological species boundaries and to validate the results of the phylogenetic species recognition. Here we present the new taxonomic concept of the *Mucor circinelloides* complex that will contribute to a better understanding of epidemiology and ecology of these fungi.

BEV08
The troublesome life of microbes in leached slag
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The leaching of sulfur-containing minerals such as alum from soft coal was common until the end of the 19th century for early industrial applications. The leaching process yielded large amounts of leached soft coal slag as by-product. These slags were dumped into the environment without any precautions or remediation, which created an exceptional anthropogenic habitat.
We are interested in studying these slag deposits as unique environment for microbial life. Combining geochemical analyses with SSU rRNA community profiling and targeted metagenomics allowed us to assess the structure and genetic potential of residing microbial communities and to pinpoint the underlying environmental driving forces.
Slag deposits are acidic (pH ~ 3.5), highly enriched in aluminium, iron and sulfur and reduced in microbial biomass. The low pH is a consequence of sulfur oxidation and causes a high bioavailability of aluminium, which is in its free form highly toxic. Alpha- and beta-diversity analyses indicated a strong selection for highly adapted microbial populations and multiple correlation analyses identified aluminium to be the main driver for niche speciation. SSU rRNA community profiling revealed a dominance of uncharacterized groups including: DA052 (up to 16 % relative abundance), KF-JG30-18 (up to 3 %) [both *Acidobacteria*], TM214 (5-20 %, *Actinobacteria*), DA111 (7-8 %, *Alphaproteobacteria*) and JG37-AG-4 (15-20 %, *Chloroflexi*). The assembly of taxonomically binned metagenome contigs allowed us to reconstruct more than 50 putative genomes, of which 27 were of high quality for downstream analysis (> 85 % complete, < 10 % contaminated). These genomes were found to be

primarily affiliated with *Acidobacteria* (5), *Actinobacteria* (6), *Alphaproteobacteria* (5), *Chloroflexi* (6), and *Planctomycetes* (3), including genomes linked to the uncharacterized groups identified by SSU rRNA community profiling (DA052, KF-JG30-18, DA111). An detailed analysis of these genomes suggested that microbes residing in slag cope with high aluminium concentrations by combining strategies such as efflux systems, chelation, and aluminum-binding proteins. Although heavy metals and xenobiotics have not been detected in significant amounts in slag, microbes present harbor high numbers of genes linked to heavy metal detoxification and xenobiotics breakdown. The presence of these genes may provide us with a glimpse of the conditions that microbes were encountering while initially colonizing the slag.
Aluminium is a frequently overlooked abiotic factor. Given the unusual geochemical settings and observed broad metabolic capabilities, ongoing work involves metagenomic mining and tailored enrichment and isolation efforts to shed more light on the mechanisms facilitating the survival and persistence of microbes in this unusual habitat.

BMV01
The tetrathionate/thiosulfate reduction potential determined by catalytic protein film electrochemistry
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The interconversion of tetrathionate and thiosulfate is a two-electron process: $S_4O_6^{2-}$ + 2 e− ↔ 2 $S_2O_3^{2-}$. Both transformations can support bacterial growth since thiosulfate can be used as energy source by several microorganisms, while tetrathionate serves as respiratory electron acceptor [1, 2]. Interest in thiosulfate oxidation also arises from its widespread use in volumetric analysis of oxidizing agents and bleach neutralization during water treatment. Thus, exact knowledge of the tetrathionate/thiosulfate reduction potential is not only of huge interest for the field of microbiology but also contributes to a better understanding of industrial applications involving thiosulfate. However, considerable ambiguity exists concerning this potential with values between +24 and +300 mV versus SHE published over the last five decades. This variation stems largely from the irreversible nature of the thiosulfate/tetrathionate interconversion at an inert electrode precluding direct measurements. As a consequence, all previously reported values relied completely on calculations from relevant thermodynamic data.
Here, we solve the issue and report protein film electrochemistry as an experimental measure for the reduction potential of the tetrathionate/thiosulfate couple [3]. The reduction of tetrathionate to thiosulfate and the reverse reaction are catalyzed by enzymes of the thiosulfate dehydrogenase, TsdA, family adsorbed on graphite electrodes. Zero-current potentials measured with enzymes from two different bacterial sources (*Camyplobacter jejuni* and *Marichromatium purpuratum*), at three pH values, and multiple thiosulfate and tetrathionate concentrations together with the relevant Nernst equation resolved the tetrathionate/thiosulfate reduction potential as +198 ± 4 mV versus SHE. The value is considerably more positive than widely used in discussions of bacterial bioenergetics. As a consequence anaerobic respiration by tetrathionate reduction is likely to be more prevalent than presently thought in tetrathionate-containing environments such as marine sediments and the human gut.

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BMV02
A class C radical S-adenosylmethionine methyltransferase synthesizes 8-methylmenaquinone
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Question: The membranous quinone/quinol pool is an essential cellular component for the majority of life forms on Earth. The most important biologically relevant quinones are ubiquinone (UQ) and menaquinone (MK; vitamin K_2), which differ in their biogenesis pathways and midpoint redox potentials, thus making them adaptable redox mediators in electron transport chains (ETCs) [1].
Some microorganisms are known to produce a methylated form of MK, designated methylmenaquinone (MMK) or thermoplasmaquinone, which is anticipated to serve in low-potential ETCs involved in anaerobic respiration, such as polysulfide respiration of *Wolinella succinogenes* [2]. However, it remained unclear how MMK is synthesized in microbial cells.
Methods and Results: Here we show that a phylogenetically widespread class C radical SAM methyltransferase (RSMT) is employed to synthesize MMK in bacteria. Such enzymes, termed either MqnK or MenJ, are present in MMK-producing organisms that possess either the classical MK biosynthesis pathway (Men) or the futalosine pathway (Mqn) [3]. Quinones were extracted from the membrane of the model Epsilonproteobacterium *Wolinella succinogenes*, separated by HPLC and analyzed by UV absorption spectroscopy and mass spectrometry. An *mqnK* deletion mutant of *W. succinogenes* was unable to form 8-MMK-6 but its production was restored upon complementation using either the native *mqnK* gene or a homologous *menJ* gene from *Adlercreutzia equolifaciens* or *Shewanella oneidensis*. In addition, it is shown that each of the *menJ* genes enabled *Escherichia coli* cells to produce MMK-8 as well as a hitherto unknown methylated form of 2-demethylmenaquinone-8 (DMK).
Conclusion: The results expand the knowledge on quinone synthesis and demonstrate an unprecedented function for a class C RSMT enzyme in primary cell metabolism. The work also offers the prospect to design artificial quinones *in vivo* to operate low-potential anaerobic respiratory chains.

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BMV03
Occurrence and function of the Rnf complex in bacteria
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Question: Low-potential ferredoxin (E_0` ≈ -500 mV) is a common electron carrier in anaerobes [1]. It is used to drive endergonic reactions and it is also used as electron donor for a membrane-bound enzyme, the ferredoxin: NAD^+ oxidoreductase (Fno or synonymously), that couples exergonic electron transport from ferredoxin to NAD^+ to the translocation of Na^+ from the cytoplasm to the medium [2]. Here we want to (I) show unambiguously that the Na^+-translocating Fno activity is indeed encoded by the *rnf* genes that are present in many bacterial and a few archaeal species [3], (II) elucidate the enzymatic function in the metabolism of different bacteria and (III) characterize the enzymatic activity of the purified complex as well as single subunits.
Methods: Membranes from several bacteria were prepared and Fno activity was measured photometrically. A Δ*rnf* deletion mutant was constructed in the bacterium *Bacteroides fragilis*, the Rnf complex from *Thermotoga maritima* was purified and characterized biochemically, single subunits from *Acetobacterium woodii* were heterologously produced, purified and characterized biochemically.
Results: Membranes isolated from the *rnf* containing bacteria *A. woodii*, *T. maritima*, *B. fragilis*, *Clostridium tetanomorphum*, *Clostridium ljungdahlii*, *and Vibrio cholerae* catalyzed Fno activity. This activity was Na^+-dependent only in the former four organisms and activity in the Δ*rnf* deletion mutant from *B. fragilis* was abolished almost completely. No Fno activity was detected in the *rnf* containing bacteria *E. coli* and *Rhodobacter capsulatus*. Fno activity from *T. maritima* was enriched and an assignment as Rnf complex was enabled. The Rnf complex catalyzed Na^+-dependent Fno activity. Furthermore, the purified subunit RnfB from *A. woodii* was shown to accept electrons from ferredoxin and RnfC was shown to catalyze electron transfer from NADH to FMN, making them the presumable electron output and input site, respectively, of the Rnf complex.
Conclusion: Rnf is a widespread membrane-bound protein complex in bacteria. In some organisms, it couples electron transfer from reduced ferredoxin to NAD^+ to the Na^+ potential, in others it used H^+ as coupling ion. In other bacteria, the enzymatic activity of the Rnf complex remains to be elucidated.

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BMV04

Microbes with identity issues – the cell wall and energy-conserving prokaryotic organelle of anaerobic ammonium-oxidizing bacteria

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Anaerobic ammonium-oxidizing (anammox) bacteria belong to the phylum Planctomycetes and are recognized as major players in the global nitrogen cycle. It is estimated that anammox bacteria are responsible for up to 50 % of the nitrogen in the air that we breathe. In addition, anammox bacteria are extremely valuable for wastewater treatment where they are applied for the cost-effective and environment-friendly removal of nitrogen compounds. Besides their ecological and industrial importance, anammox bacteria defy some basic biological concepts. Anammox bacteria harbor a major intracellular compartment called the anammoxosome which is the location of the anammox reaction. In addition it was proposed that anammox bacteria and all other Planctomycetes lack peptidoglycan; a cell wall structure crucial for cell shape and integrity. Here we investigated both the function of the anammoxosome in energy conservation and the proposed absence of peptidoglycan. To this end, we used subcellular fractionation, proteome analysis, activity assays and complementary state-of-the art techniques such as cryo-transmission electron microscopy, peptidoglycan-specific probes and structured illumination microscopy and ultrasensitive UPLC-based muropeptide analysis. We show that peptidoglycan is present in the anammox bacterium *Kuenenia stuttgartiensis* after all. In addition, we isolated the anammoxosome from the cell and show it is able to perform the anammox reaction outside the confinements of the cell. Currently we are investigating membrane potential and ATP synthesis of the isolated prokaryotic organelle. We conclude that anammox bacteria have a peptidoglycan-containing cell wall [1] and a prokaryotic organelle involved in energy conservation [2].

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BMV05

Molecular model of the pIP501 type IV secretion system from *Enterococcus faecalis*

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Discovery void of novel antimicrobial substances and increasing antibiotic resistances in pathogenic bacteria present a serious threat for human health worldwide. Dissemination of antibiotic resistance genes often occurs through conjugative type IV secretion systems (T4SSs). An important conjugative model system in Gram-positive bacteria is the T4SS from broad-host-range plasmid pIP501, which has been often found in nosocomial pathogens, such as in clinical *Enterococcus faecalis* and *Enterococcus faecium* isolates. pIP501 shows self-transfer to virtually all Gram-positive bacteria, e.g., to enterococci, streptococci and staphylococci and additionally to *Escherichia coli*. This multiresistance plasmid encodes 15 putative transfer genes in a single operon. The corresponding proteins TraA-TraO are proposed to form a T4SS multiprotein complex. Putative key factors of the conjugative transfer complex are the relaxase TraA, two ATPases, TraE and TraI/TraJ, the first putative two-protein T4S coupling protein, the muramidase TraG, the putative channel factors, TraH, TraL and TraM and the surface factor TraO [1]. The functions of most of the transfer proteins and the mechanism of the conjugative T4SS are not known in detail. To elucidate the role of the pIP501 *tra* genes in T4S in Gram-positive pathogens we generated a number of single *tra* knock-out mutants in *E. faecalis* harbouring pIP501 using a markerless deletion method [2]. We have generated the deletion mutants: *E. faecalis* pIP501Δ*traE*, Δ*traF*, Δ*traG* [3], Δ*traH* [2], Δ*traM* and Δ*traN*. Biparental matings showed that TraE, TraF, TraG, TraH and TraM are essential for pIP501 conjugative transfer. Matings with *E. faecalis* pIP501Δ*traN* as donor demonstrated, that TraN has a special role as *oriT* DNA binding transfer repressor. Generation of the knock-outs *traJ, traL* and *traO* is in progress and will further help decipher the pIP501 conjugative transfer machinery. A molecular model on the pIP501 T4SS will be presented.

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BMV06

From substrate specificity to promiscuity – molecular analysis of a hybrid ABC transporter

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Question: The two closely related compatible solute ABC-transporters OpuB and OpuC (osmoprotectant uptake) are indispensable for acquiring a variety of compatible solutes under osmotic stress conditions in *Bacillus subtilis*. Whereas the substrate binding protein OpuCC recognizes a broad spectrum of compatible solutes, its 70 % sequence-identical paralogue OpuBC only binds choline [1][2]. Hence, the two closely related ABC-transporters possess strikingly different substrate spectra [3]. In this study we substituted the OpuBC substrate binding protein of the OpuB system with OpuCC in order to analyze whether the hybrid ABC-transporter is functional and what its substrate specificity might be.
Methods: We studied the hybrid transport system physiologically. Immunoblot analysis allowed detection of the OpuCC binding protein in the hybrid OpuB::OpuCC transport system. Kinetic parameters (K_m and v_{max}) for the uptake of various compatible solutes via the ABC-transporters were measured. By genetic enrichment, we screened for suppressor mutants that showed increased transport capacity via the hybrid OpuB::OpuCC system.
Results: Physiological growth experiments indicate that the substrate spectrum of the hybrid transport system is wider than that of the OpuB system. All compatible solutes transported by OpuC can be translocated through the hybrid system possessing the substrate binding protein of OpuC. OpuC exhibits high affinity towards glycine betaine (K_m= 4.8 µM) and substantial transport capacity (v_{max}= 100 nmol min^{-1} mg protein^{-1}), whereas the hybrid transporter exhibits a weaker transport capacity of glycine betaine (v_{max} = 20.2 nmol min^{-1} mg protein^{-1}) but the same high affinity (K_m= 5.6 µM). Several suppressor mutants exhibited enhanced transport capacity (v_{max}= 92-99 nmol min^{-1} mg protein^{-1}); each of these mutations were mapped in the coding region of *gbsR*, which encodes a repressor of the *opuB* operon. The mutant *gbsR* genes encode GbsR protein variants carrying only single aminoacid substitutions. These mutant GbsR proteins are not functional and are thereby enhancing *opuB* transcription.
Conclusion: Through molecular "micro-surgery" we were able for the first time to syntheticly develop a functional hybrid microbial ABC transporter. The starting system OpuB is an ABC transporter with high substrate specificity. By implanting the ligand binding protein of an ABC transporter with a broad substrate spectrum we converted OpuB into a promiscuous ABC transporter, arising interesting question about the evolution of the osmostress-adaptive OpuB and OpuC transporter.

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BMV07

The DNA translocator of *Thermus thermophilus* – *In situ* structure and structure/function correlation of a dynamic channel for DNA uptake and pilus extrusion

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Question: Uptake of free DNA, referred to as natural transformation, is a major mechanism of horizontal gene transfer. In many bacteria DNA transfer systems are linked to type IV pilus (T4P) machineries [1]. However, information with respect to the role of T4P in DNA uptake, *in situ* structures of T4P and DNA translocators as well as information concerning structure/function correlation of key components of both systems is scare.
Methods: We used electron cryo-tomography to unravel the first *in situ* structure of a T4P in the open and closed state. Dynamics is mediated by a secretin complex (PilQ) essential for both, T4P extrusion and DNA uptake. This complex was found to comprise of a cone and six staggered rings. To identify ring-forming domains in the secretin protein and unravel their function we generated a whole set of deletion derivatives by site directed mutagenesis and performed single particle analyses of purified secretin complexes and electron cryo-tomography to identify ring-forming domains. The structure/function correlation was verified by mutant studies.
Results: Our studies unraveled the first structure of a dynamic channel guiding T4P and mediating DNA uptake [2]. Comparison of the open and closed state revealed major conformational changes whereby the N-terminal domains of the secretin shift by ~30 Å leading to an opening of the two gates and pilus extrusion. Structural analyses of the secretin deletion derivatives led to the identification of a novel ββββαβ fold, required for gate and ring formation. Furthermore, four βαββα folds were identified as ring-building domains. Mutant studies revealed that each ring was important for pilus extrusion but dispensable for natural transformation.
Conclusions: Type IV pili machineries and DNA translocators are highly dynamic machineries. Distinct domains in the secretin PilQ are essential for ring formation and pilus extrusion. T4P are not required for DNA uptake.

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BMV08

The h-region of the TMAO reductase signal peptide – a major determinant for Tat-dependent protein translocation

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Question: The twin arginine translocation (Tat) pathway allows the translocation of folded and cofactor-containing proteins harboring a conserved twin-arginine motif in their signal peptides across the inner membrane of bacteria. While the importance of the Tat consensus motif for productive TatBC receptor binding has been demonstrated in various site-directed mutagenesis studies, the role of the hydrophobic h-region of Tat signal peptides in the binding process is still unexplored.
Methods: Here, we investigate the role of the h-region of the *Escherichia coli* TMAO reductase (TorA) signal peptide in the bacterial Tat translocation process using a genetic approach. Export-defective reporter protein variants function as starting point for mutagenesis studies aimed at identifying gain-of-function mutations which restore the export.
Results: Introduction of a negatively charged aspartate into the hydrophobic core resulted in a complete export block of the model Tat substrate TorA-MalE. Selection for restored export allowed the isolation of intragenic suppressors containing either replacements of the inserted aspartate by neutral/hydrophobic residues or deletions of discrete regions within the h-region. All mutant TorA-MalE variants were significantly accepted by the wildtype Tat translocase, however, the translocation efficiency increased with the length of an intact hydrophobic region suggesting that a minimal functional h-region in the signal peptide is required for productive binding to the TatBC receptor and thus for successful translocation. Moreover, we investigated the direct participation of the hydrophobic core in the binding process and identified intragenic mutations in the h-region that synergistically suppressed the export defect of the TorA[KQ]-MalE reporter, in which the crucial RR-motif was replaced by a lysine-glutamine pair. In all cases the overall hydrophobicity of the h-region was increased due to substitutions by more hydrophobic amino acid residues.
Conclusions: In this study we report mutations in the h-region which can compensate the loss of the crucial RR-motif and allow significant translocation of a normally transport-incompetent Tat precursor. Our results provide for the first time direct evidence for the importance of the h-region of Tat signal peptides as a major binding determinant of precursors to the TatBC receptor complex besides the Tat consensus motif.

BTV01

Model-based metabolic engineering of *Escherichia coli* for high yield itaconic acid production

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Question: In 2004 Werpy and Petersen proposed twelve top value added compounds derived from biomass [1]. One of these potential platform chemicals was itaconic acid, which is primarily used for polymer synthesis and has the potential to replace petrochemically derived methacrylic acid [2]. Itaconic acid is currently produced by *Aspergillus terreus*. Drawbacks of the fungal production are a low growth rate and the high impact of oxygen on the production performance. Therefore, the facultative anaerobic fast growing bacterium *Escherichia coli* seems to be a good organism for the heterologous itaconic acid production. However, titer and yields of itaconic acid production with *E. coli* are still low.
Methods: We expressed the cis-aconitic acid decarboxylase of *Aspergillus terreus* in *E. coli Mg1655* to allow itaconic acid production. Additionally we over-expressed the citrate synthase of *Corynebacterium glutamicum*, which is insensitive to NADH, to enable an improved flux through the tricarboxylic acid cycle. Then we applied a model-based metabolic engineering strategy (constrained minimal cut sets [3]) to further improve the itaconic acid production with *E. coli*. We used a stoichiometric network model [4] of the central metabolism of *E. coli* consisting of 113 reactions to calculate suitable intervention strategies. It was intended to reach an itaconic acid yield greater than 0.7 mol/mol while still allowing a minimum growth of 0.01 h^{-1}. One cut set was chosen as starting point and the gene knock-outs were iteratively introduced in *E. coli* by lambda red recombination or P1-transduction.
Results: After partially implementation of the chosen intervention set the *E. coli* strain produced 2.2 g/l itaconic acid with an excellent yield of 0.77 mol/mol and a minimum growth rate of 0.03 h^{-1}. A fed-batch cultivation with this strain led to the production of 32 g/l itaconic acid. This is the highest so far reported yield and product concentration for heterologous itaconic acid production
Conclusion: Our results demonstrate that itaconic acid can be produced with high yield and titer by a dedicated *E. coli* mutant strain and that constrained minimal cut sets are a suitable tool to identify intervention strategies for redirecting carbon flux to high product formation.

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BTV02

Construction of plasmid-free bacterial strains for the synthesis of human milk oligosaccharides

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Question: Beneficial effects of human milk oligosaccharides (HMOs) on infants' well-being raised attention for HMOs as potential nutritional additives for infant formula [1]. However, chemical or *in vitro* enzymatic syntheses are laborious or costly. Objective of this work was the construction of plasmid-free *Escherichia coli* strains capable of synthesizing HMOs using recombinant glycosyltransferases in combination with intracellularly generated nucleotide-activated sugars (e.g. GDP-L-fucose) to allow further research on these compounds.
Methods: Strain construction was based on the *E. coli* (JM109 or LJ110) using a site-specific λ-red recombineering technique for chromosomal integration of heterologous genes in combination with a screening on differential agar plates [2]. Strain evaluations and HMO syntheses were conducted in shake-flask cultivations and fed-batch fermentations,

allowing quantification and improvement of intracellular precursor molecule levels via HPLC.
Results: With up to 6 consecutive integrations in one strain, plasmid-free *E. coli* strains were constructed for the synthesis of different HMOs. During strain improvement, different copy-numbers of genes allowed leveling of gene expression in order to raise HMO productivity. Furthermore, chromosomal stability allowed syntheses in fed-batch fermentations without the need for antibiotics as selection markers, resulting in product titers of up to 20 g/L (2′-fucosyllactose) [3,4]. Utilizing chromosomal integration, we could also demonstrate the combination of specific glycosyltransferases (LgtA, WbgO) together with enhanced intracellular synthesis of UDP-nucleotide-activated sugars for the efficient preparative synthesis of oligosaccharide core-structures such as lacto-*N*-tetraose (LNT) [4,5]. Subsequent combination of these syntheses of core-structures with chromosomally integrated fucosyltransferases and enhanced intracellular supply of GDP-L-fucose resulted in fucosylated HMOs up to penta- and hexasaccharides such as LNF I and LNDFH II [6].
Conclusion: Chromosomal integration turned out to be a powerful tool for synthetic microbiology, allowing multiple rapid and site-specific insertions and construction of genetically stable strains. Using this method, we could construct strains for gram-scale syntheses of complex HMOs.

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BTV03
The 2-C-methyl-D-erythritol 4-phosphate pathway as a platform for isoprenoid formation – metabolic regulation and engineering of isoprenoid production in microbes
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Due to an increasing demand for sustainable energy and chemicals, new strategies have to be explored to produce these resources at low-cost and low environmental impact. Microbial produced terpenoids are a promising source for a broad spectrum of valuable molecules, including biofuels, fine chemicals and bulk chemicals. Terpenoids are the largest as well as most diverse class of chemical molecules synthesised by nature. Besides fulfilling vital functions in the producing organism (e.g. as protection from herbivores), terpenoids have important roles in human society as flavour & fragrances, pharmaceuticals, cosmetics, agrochemicals, industrial materials or chiral intermediates for chemical synthesis, many in scarce supply. The precursors of all terpenoids are synthesised through either one of two distinct metabolic pathways: The 2-C-methyl-D-erythritol 4-phosphate phosphate (MEP) pathway or the mevalonate (MVA) pathway. The MVA pathway is already used for commercial application. Even though the MEP pathway has a higher theoretical yield, attempts on the application of the MEP pathway in the production of terpenoids have met with limited success.
A combination of proteomics, metabolomics and fluxomics was applied to get insight into the regulation and control of the pathway in *Escherichia coli*. We successfully determined the quantity of the low abundant proteins of the MEP pathway in *E. coli*. Libraries of *E. coli* mutants with altered expression of the MEP genes were generated by randomization of the ribosome binding site (RBS) of each gene through recombineering. This method uses three proteins from the lambda phage to facilitate the homologous recombination of ssDNA oligonucleotides with the genomic target region and leads only to the wanted alteration in the genome in a wild type background. After screening the libraries by colony PCR for altered RBS, the altered expression was quantified by targeted proteomics. The metabolite concentration were quantified in all mutants by LC-MS/MS and the flux was measured by ^{13}C pulse-labelling experiments. The influence of the altered gene expression on the intermediate concentration and the flux through the pathway were investigated through metabolic control analysis.
Through the combination of these methods, we can generate an improved model to describe the MEP pathway. The data were used to generate a production strain for economical valuable terpenoid isoprene. The optimized production strain has highly improved titers above the non-optimized strains.
Our approach gives an understanding of the regulation of the MEP pathway that includes genetic and kinetic regulation under *in vivo* conditions, which enables the metabolic engineering of this pathway for the production of terpenoids.

BTV04
Enzymatic hydrolysis of macroalgae for the production of biobased chemicals
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Question: Increasing scarcity of fossil resources and climate change generate rising demand for renewable feedstocks. New concepts are needed to utilize plant biomass for the production of biofuels and biochemicals. As an alternative to lignocellulose or starch, macroalgae represent a promising feedstock due to their high content of carbohydrates and the lack of lignin. Moreover, special esterifications, sulfations and sugar residues bring carbohydrates from algae into the focus of the chemical industry.
Thus, the bioconversion of macroalgae polysaccharides into their small building blocks is a promising but challenging task. Therefore, the identification and characterization of novel efficient enzymes for the hydrolysis of special polysaccharides like alginate, laminarin and fucoidan is the bottleneck for the development of the future biorefinery.
Methods: By a sequence-based screening approach of a metagenomic dataset we identified new putative enzymes from extreme environments for the enzymatic hydrolysis of macroalgae polysaccharides. Candidates were produced heterologously in *E. coli* and were subsequently purified to homogeneity. Promising enzyme candidates were characterized biochemically and their activities were determined against polysaccharides and macroalgae biomass.
Results: Here we present new promising biocatalysts for the enzymatic hydrolysis of brown algae. We describe biochemical characteristics of new thermostable alginate lyases, which are able to decompose alginate, the major polysaccharide in the cell wall of brown algae. Moreover, we characterized thermoactive laminarinases for the hydrolysis of another key component of brown algae: the main storage glucan, laminarin. Altogether, these enzymes represent a suitable toolset for an effective bioconversion of brown algae biomass to high value products.
Conclusion: We reported various promising enzymes for the bioconversion of macroalgae to fine chemicals and high-value biobased products. These results were generated in the BMBF funded project LIPOMAR (FKZ 031A261), "lipids and surfactants from marine biomass", which was established by a consortium of academic and industrial partners.

BTV05
Acetoin production via unbalanced fermentation in *S. oneidensis*
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The aim of the described project is the realization of unbalanced anaerobic production processes in the proteobacterium *S. oneidensis*. Fermentation and respiration are the two metabolic processes used by bacteria to gain energy. The respiratory consumption of an organic carbon source is accompanied with its oxidation by using an external electron acceptor. In case of fermentation the oxidation state of the substrate is identical to the product´s oxidation state. Often, fermentative bacteria produce a mixture of products to achieve a balanced overall oxidation state of the products compared to the substrate. Typically, fermentative processes are of high biotechnological relevance since they offer high productivity and a low percentage of substrate consumption for anabolic processes. Nevertheless, the range of compounds that can be produced as sole endproduct of a fermentative process is rather limited, due to the above mentioned prerequisites regarding balanced oxidation states. This limitation could be overcome by the transfer of the surplus of electrons to an electrode surface, which of note is an anaerobic electron acceptor that cannot be depleted.
S. oneidensis is a model organism to study the biochemistry of extended respiratory chains to the cell surface. As a proof of principle we genetically engineered the organisms for the production of acetoin, a precursor on the biochemical route to 2,3-butanediol. First, a chassis

organism was produced that is due to the deletion of prophages more stable compared to the wild type. Moreover, this deletion strain produces more current when grown with an anode as electron acceptor. This higher current production is most likely due to increased biofilm production. The strain was further modified to contain codon optimized versions of acetolactate synthase and acetolactate decarboxylase. Hence, an acetoin biosynthesis pathway was introduced that branches of from the metabolite pyruvate. Without further modification, we achieved acetoin production with a carbon recovery of 30-40 %. Further modification of the strain will stop the production of side products and extend the range of substrates produced by unbalanced fermentation processes.

BTV06

Microbial synthesis of butadienes – a look into patent literature

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Question: Sustainability requires a shift in the production of intermediates for polymer synthesis from coal, cruide oil and gas to plant biomass. The large-scale production of butanediols (as intermediates for polyester production) and isobutene by fermenting processes has opened the field for the development of processes and enzymes producing butadienes. With an annual market volume of millions of tons butadiene worth billions of $, many companies are motivated to explore the production of butadiene from renewable resources.
Methods: Patent literature was reviewed for the production of volatile alkenes, especially butadiene and isoprene (methyl-butadiene).
Results: The patents explore a number of enzymes for the production of butadienes. The alkene formation seems to be most efficient with elimination reactions. Released are water molecules from alcohols or allylalcohols, carbon dioxide from a range of medium chain carboxylic acids, or water and carbon dioxide from beta-hydroxy carboxylic acids. Monoterpene biosynthesis is the elimination of an alcohol group after activation to a good leaving group in the form of pyrophosphate. An overview on these enzymes will be presented.
Conclusion: Synthesis of everyday polymers (tyres, nylon stockings, lego bricks) require butadienes as intermediates. In 2011, a shift in carbon source from cruide oil to natural gas in the chemical industry caused a production decrease and excess demand. Patents in the last years suggest that butadienes produced by fermenting microorganisms may fill the gap.

BTV07

Engineering industrial acetogenic biocatalysts – a comparative metabolic and genomic analysis

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Question: Syngas fermentation by acetogenic bacteria employing the Wood-Ljungdahl pathway is an anaerobic bioprocess for production of biofuels and biocommodities. To date, different strains of *Clostridium autoethanogenum, C. ljungdahlii*, *C. ragsdalei*, and *C. coskatii* are used for production of a variety of products by companies such as Coskata, INEOS Bio, or LanzaTech. However, these bacteria show very high similarities regarding their 16S rDNA sequences (99 to 100 %) and are therefore indistinguishable with this method. Thus, we performed a comparative metabolic and genomic analysis.
Methods: We compared autotrophic growth and product formation of the wild type (WT) strains and recombinant *C. ljungdahlii* strains in uncontrolled batch experiments. Furthermore, we sequenced the genomes of *C. ragsdalei* and *C. coskatii*. Afterwards, we performed a comparative analysis of the genome sequences of the four WT strains.
Results: Autotrophic growth behavior and product spectrum (acetate, ethanol, 2,3 butanediol) of *C. autoethanogenum*, *C. ljungdahlii*, and *C. ragsdalei* were almost identical. In contrast, *C. coskatii* produced significantly less ethanol and showed acetate production during the stationary growth phase. Additionally, we constructed recombinant isopropanol-producing *C. ljungdahlii* strains harboring plasmids carrying the genes *adc*, *ctfA*, *ctfB*, and *thlA* (encoding acetoacetate decarboxylase, acetoacetyl-CoA:acetate/butyrate:CoA-transferase subunits A and B, and thiolase) under the control of *thlA* promoter (P_{thlA}) from *C. acetobutylicum* or native *pta-ack* promoter ($P_{pta-ack}$) from *C. ljungdahlii*. Isopropanol production using both recombinant strains was confirmed under heterotrophic and for one strain also under autotrophic growth conditions.
Integration mutant *C. ljungdahlii adhE1*::intron was constructed using the ClosTron™ system and showed an increased acetate:ethanol ratio (7:1) compared to WT strain (2:1).
Comparative genome sequence analysis of the four WT strains revealed high similarities between *C. ljungdahlii* and *C. autoethanogenum* (99.3 %) and *C. coskatii* (98.3 %) using an average nucleotide identity (ANI) analysis. However, *C. ljungdahlii* and *C. ragsdalei* showed an ANI based similarity of 95.8 %. Regarding ethanol formation of the four WT strains we found that *C. coskatii* is the only one missing genes encoding tungsten-dependent aldehyde:ferredoxin oxidoreductases (Aor).
Conlusion: Our findings suggest that ethanol formation in acetogens is linked to acetate production via tungsten-dependent Aor that convert acetate into acetaldehyde that is further converted to ethanol by alcohol dehydrogenases. The results presented extend the toolbox for metabolic engineering of acetogenic bacteria and provide comprehensive insights into the genetic features of industrially most relevant biocatalysts fermenting syngas.

BTV08

α-Ketoglutarate production from pentose *in vitro* – one of the bedstones for hydroxyl amino acids production *in vivo*

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Introduction: Hydroxyamino acids (HAs) are important precursors for the production of pharmaceuticals like certain antibiotics and diabetes mellitus drugs and also represent valuable base chemicals for a variety of more sustainable potential industrial applications. However, so far HAs are mainly extracted from natural raw materials in complex and cost intensive processes which hamper the broader application of these compounds. A sustainable alternative is the enzymatic production of HAs via Dioxygenases converting amino acids with α-ketoglutarate (αKG) as electron donor and molecular oxygen yielding the corresponding hydroxyamino acid, succinate and CO_2
The mesophilic bacterium *Caulobacter crescentus* has been described to degrade pentoses [1], main constituents of lignocellulosic biomass, directly to αKG--the cosubstrate of the dioxygenase mediated amino acid hydroxylation.
Objectives: Aim of the Mercator Research Centre Ruhr funded project is to provide an enzyme cascade for αKG formation in the *in vitro* hydroxylation of amino acids via dioxygenases with the final goal to construct an *E. coli* based whole cell biocatalyst for the *in vivo* production of HAs based on lignocellulosic raw materials.
Methods & Materials: The genes encoding the enzymes for pentose conversion to αKG, i.e. sugar dehydrogenase, lactonase, sugar acid dehydratase, 2-keto-3-deoxy sugar acid dehydratase and αKG semialdehyde dehydrogenase, from *C. crescentus* were cloned and recombinantly expressed. The proteins were purified and characterized at optimal host strain conditions (37 °C, pH 7.5). The active proteins were reconstituted to the entire enzyme cascade and the αKG formation from D-xylose was analyzed. Afterwards, the cascade was applied together with the L-isoleucine dioxygenase from *Bacillus thuringiensis* for the hydroxylation of L-isoleucine and results were analyzed with HPLC.
Results: The enzyme cascade from *C. crescentus* was successfully reconstituted from the recombinant enzymes indicated by the αKG production from D-xylose. All of the single reactions in the cascade were defined to be irreversible and a complete conversion from D-xylose to αKG by the cascade was detected. Besides, the combination of the cascade with the L-isoleucine dioxygenase from *B. thuringiensis* resulted in 44 % conversion from L-isoleucine to Hydroxy-L-isoleucine after 3 hours of incubation.
Conclusion: αKG as cosubstrate for enzymatic hydroxylation of amino acids could successfully be produced from D-xylose using enzyme cascade derived from *C. crescentus*. Optimization of reaction conditions in combination with the dioxygenases is currently under way.

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BTV09

Metabolic engineering of *Corynebacterium glutamicum* for production of astaxanthin

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Corynebacterium glutamicum is a biotechnologically relevant bacterium used for the million-ton-scale production of amino acids. Fermentations with this bacterium are characterized by fast growth and high biomass concentrations. Its biotechnological potential to produce various other high-value products by metabolic engineering has been proven [1]. It was shown that *C. glutamicum*, as a natural producer of the rare C50 carotenoid decaprenoxanthin, is a suitable host for production of a range of carotenoids [2]. Astaxanthin is a red cyclic C40 carotenoid with a high anti-oxidant effect. Today, astaxanthin is mainly used as a colorant especially in aquacultures, and the demand for astaxanthin as a nutraceutical and pharmaceutical is increasing. In this work the potential of *C. glutamicum* to produce astaxanthin was analyzed.

In our work, the genomic background of a prophage-cured, genome-reduced *C. glutamicum* was engineered for enhanced production of carotenoids. Optimization of the supply of the precursor isopenthenyl pyrophosphate (IPP) by chromosomal promoter exchange of *dxs*, abrogating biosynthesis of the endogenous decaprenoxanthin by deleting of the endogenous carotenoid genes *crtEbY* and heterologous expression of the genes *crtE*, *crtB*, *crtI* and *crtY* from *Pantoea ananatis* yielded a *C. glutamicum* strain efficiently producing β-carotene [3].

In this work, astaxanthin production was established by metabolic engineering of β-carotene producing platform strains via two approaches. First a combinatorial gene assembly for *crtW* and *crtZ* was performed in order to optimize the ratio of enzyme quantities to improve the total catalytic enzymatic activity of heterologously expressed β-carotene ketolase (CrtW) and hydroxylase (CrtZ). For statistical coverage, a library with 8,000 transformants was generated and analyzed with respect to carotenoid production. Secondly, alternative *crtW* and *crtZ* genes from natural prokaryotic astaxanthin producers were heterologously expressed in a β-carotene accumulating platform strain to produce canthaxanthin or zeaxanthin, respectively. Co-expression of the most promising combination of *crtW* and *crtZ* resulted in the formation of astaxanthin in the mg/g CDW range in shaking flasks with volumetric productivities comparable to algal or yeast hosts currently used in astaxanthin production.

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BTV10

Systems metabolic engineering of *Corynebacterium glutamicum* for the production of bio-based nylon

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In light of a globally developing bio-economy for sustainable production of chemicals, materials and fuels, industrial biotechnology is facing tremendous challenges. For many decades, the natural pathway set of specific organisms, however, was limiting the complexity and outreach of economic harnessing [1]. Beyond the natural boundaries, the embedment of systems-level analysis, synthetic biology and advanced recombinant DNA techniques within comprehensive engineering concepts now, for the first time, promises streamlined cell factories for sustainable biorefinery applications [2].

This lecture will cover the development of engineered *Corynebacterium glutamicum* strain for the production of diaminopentane (DAP), a building block for polyamides, top-level industrial polymers with a market of several million tons [3]. Recruiting the *E. coli* enzyme lysine decarboxylase, the terminal lysine biosynthetic pathway was first extended to establish basic DAP production in lysine-producing *C. glutamicum* [4]. Based on comparative metabolome and transcriptome studies, production was improved by elimination of by-product formation [5] and transport engineering [6]. Extended systems-level engineering of carbon [7, 8] and redox metabolism [9, 10] combined with bioprocess engineering finally enabled DAP production with industrial-relevant performance, including high yield (50 %), titer (88 g L^{-1}) and productivity (2.2 g L^{-1} h^{-1}) [3]. Subsequent downstream processing and co-polymerization with sebacic acid provided a novel, completely bio-based polymer PA5.10 with excellent material properties for broad application sectors. This demonstration of a novel route for generation of bio-based nylon from renewable sources opens the way to production of sustainable bio-polymers with enhanced material properties.

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BTV11

Extracellular targeting of an active endoxylanase by a TolB negative mutant of *Gluconobacter oxydans*

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Gluconobacter oxydans strains have great industrial potential due to their natural ability of incomplete stereo- and regioselective oxidation of many alcohols and monosaccharides. One major limitation preventing the full production potential is the limited substrate spectrum of *G. oxydans*.[1] Hydrolysis of polysaccharides is not possible because extracellular hydrolases are not encoded in the genome of *Gluconobacter* species. Therefore, in a first step for the generation of exoenzyme producing *G. oxydans,* a leaky outer membrane mutant was created by deleting the TolB encoding gene *gox1687*.[2,3] Permeability of the outer membrane of the *ΔtolB* strain was quantified by measuring the alkaline phosphatase activity of a PhoA overproduction strain in the cytoplasmic, periplasmic, and culture supernatant fractions. Up to 46 % of the PhoA activity was localized in the culture supernatant in the TolB mutant, while the PhoA-expressing wildtype showed only minimal activity in the supernatant, confirming highly increased permeability of the outer membrane for periplasmic proteins in the TolB mutant. The *xynA* gene encoding an endo-1,4 β xylanase from *Bacillus subtilis* was fused to a pelB signal peptide and expressed in *G. oxydans ΔtolB* for periplasmic protein production. Nearly 75 % of the XynA activity was detected in the culture supernatant of the TolB mutant and only minor amounts of endoxylanase activity was observed in the XynA-expressing *G. oxydans* wildtype. These results showed that a *G. oxydans* strain with an increased substrate spectrum was generated that is able to use the renewable polysaccharide xylan as a substrate to produce the high-value chemical xylobiose.

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BTV12

A surprising diversity of solventogenic clostridia

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The production of solvents such as acetone, ethanol, and butanol (ABE) is one of the oldest industrial fermentation processes and has tremendous potential for chemical industry as these organic compounds are important intermediates for paints, plastics, coatings as well as for polymers. Solventogenic clostridia such as *C. acetobutylicum*, C. *saccharobutylicum*, *C. saccharoperbutylacetonicum* or *C. beijerinckii* are able to form ABE from a huge variety of sugar- and starch-based substrates. Various organic compounds such as glucose, fructose, saccharose, xylose, cellobiose, and even sorbitol, dulcitol, or inositol can be used. However, the single strains

and species differ with respect to the substrate spectrum they are able to use for ABE fermentation.
The genomes of 30 solventogenic clostridia from eight different species were sequenced to extend our knowledge on the biochemistry and physiology of these interesting organisms and for genome comparison with all publically available sequences.
Whole genome sequencing was either done by a combined approach using the 454 GS-FLX Titanium XL and the Genome Analyzer II or MiSeq or Illumina technique only. Hybrid *de novo* assemblies were performed with the Mira 3.4 and the Newbler 2.9 software and Illumina only assemblies by the SPAdes 3.5 software, respectively. In some cases remaining gaps were closed by PCR-based techniques and Sanger sequencing of the products.
The genomes of all solventogenic clostridia vary in size from 4.1 Mb for the *C. acetobutylicum* strains up to 7.2 Mb for some *C. beijerinckii* strains. Extrachromosomal elements could be identified in *C. acetobutylicum*, *C. roseum*, *C. aurantibutyricum*, *and C. saccharoperbutylacetonicum* as well as in some of the *C. beijerinckii* strains, but only in *C. acetobutylicum* is the *sol* operon, necessary for solvent production, located on the plasmid. A genome comparison of all sequences including the ten publically available genomes revealed a core genome of 638 and a pan genome of 26,944 orthologous groups, respectively. The different species complexes differ in the substrate spectrum they can use for fermentation, but also with respect to the acids and solvents they are able to produce.
Solventogenic clostridia are able to produce solvents from a variety of organic substrates, but genome comparison revealed species-specific capabilities.

BTV13
Proteotyping of biogas plant microbioms
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Question: Microbial conversion of biomass to methane and carbon dioxide in biogas plants (BGPs) represents a major renewable energy source. Besides chemical and technical parameters, methane yields as well as productivities are mainly influenced by the taxonomic and functional structure of the microbial communities. Understanding of these microbial communities is hampered, however, due to the lack of assays for identification of the composition and functional properties of the microbial communities. To overcome these limitations and to perform correlation analyses regarding community composition, process states, process parameters and process yields, a metaproteomics platform was established [1].
Methods: A large-scale screening of the microbial communities of 40 industrial BGPs was applied using LC-MS/MS based proteotyping. The applied workflow involved phenol extraction, tryptic digestion, peptide separation by liquid chromatography coupled to tandem mass spectrometry (Elite Hybrid Ion Trap Orbitrap), and data analysis using the *MetaProteomeAnalyzer [2]* software.
Results: Clustering and principal component analysis based on the identified metaproteins, taxonomies and biological processes (Uniprot keywords) revealed four main groups associated to mesophilic and thermophilic process temperature, upstream anaerobic sludge blanket reactors, and sewage sludge as substrate. Main microbial orders identified were *Bacillales, Enterobacteriales, Bacteriodales, Clostridiales, Rhizobiales, Thermoanaerobacteriales* as well as *Methanobacteriales, Methanosarcinales* and *Methanococcales*. The correlation study revealed several hints for trophic interactions, e.g. syntrophic hydrogen transfer between *Thermoanaerobacterales* and *Methanomicrobiales*.
Key functions (fermentation, secondary fermentation, methanogenesis) represented by metaproteins could be successfully assigned to taxonomies. The process parameters: (i) temperature, (ii) organic loading rate (OLR), (iii) total ammonia nitrogen (TAN) and (iv) sludge retention time (SRT) were identified as major factors shaping the microbial communities and metabolic functions in BGPs. Unfortunately, no significant correlations to methane yield and productivity were observed.
Conclusion: Application of large-scale microbiome proteotyping enabled the identification of correlations between the process parameters, process states and the microbial communities and thus, contributed to the understanding of the conversion of biomass into methane. This knowledge could be used to improve monitoring and control of BGPs.

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BTV14
Microbial electron uptake during biocorrosion and electrosynthesis
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Since its discovery, the ability of certain microorganisms to transfer electrons directly between the cell and the surrounding environment has gained much attention. Often, direct electron transfer (DET) is implied or reported based on kinetic arguments when tools for mechanistic studies are missing. This study aimed to elucidate the hydrogenase independent electron uptake mechanism during electro-methanogenesis of the methanogenic archaeon *Methanococcus maripaludis*. Surprisingly, filter sterilized culture medium of *M. maripaludis* was sufficient to take up cathodic electrons in a bio-electrochemical system and to form intermediates for methanogenesis like H_2, formate and CO at high rates. Genetic as well as biochemical experiments clearly showed that this catalytic activity is due to enzymes present in the culture medium. Thus, cell borne free enzymes (a) can mimic direct extracellular electron transfer in certain experimental designs, (b) constitute an overlooked put possibly important factor in biological electron transfer reactions and (c) might even present an opportunity for designing bio-electrochemical setups.

BTV15
Production of biobased fuels and plastics from CO_2 and light in defined mixed cultures
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Question: To bring the concept of a biobased economy to life, costs for the production of biotechnological commodities still have to drop considerably. A major hurdle for the mainly heterotrophic fermentation processes is the cost of the carbon source. There is great interest to replace traditional, crop-based feedstock like sugarcane, corn and wheat by carbohydrates derived from eukaryotic algae [1] and cyanobacteria [2]. These new sources of feedstock can be produced on non-arable land, with salty or brackish water and have potentially higher areal productivities than conventional crops.
Methods: Our approach is to take advantage of a genetically engineered *Synechococcus elongatus* PCC7942 *cscB* [2], releasing sucrose in the fermentation broth, which can be used by a second heterotrophic organism. Sugar production and cell growth in pure and mixed cultures was studied in shaking flasks and a 1.8-L photobioreactor by means of photometry, HPLC and flowcytometry.
Results: After one week, more than 3 g/L of sucrose in the culture medium were obtained. Sucrose containing culture supernatant was successfully used to support the growth of recombinant *Pseudomonas putida* KT2440 and *Ralstonia eutropha* H16, harbouring the *cscA* gene, a sucrase invertase, from *Escherichia coli W*. Additionally the oil producing yeast strain *Trichosporon oleaginosus* DSM 11815 was shown to grow with cyanobaterial growth medium containing sucrose. All three organisms offer great potential in biotechnology for the production of base and special chemicals, as well as biofuels [3-5].
Additionally to cultivating *S. elongatus* and the heterotrophic strain subsequently, a co-culture between both organisms could also be established in a photobioreactor. This defined mixed culture showed stable growth for both microorganism constituting artificial commensalism between *S. elongatus* as a donor for sucrose and the heterotrophic strain as the profiteer. This way sucrose was used in-situ as a substrate, reducing the risk of contamination in a one step cultivation.
Conclusion: On the whole, cyanobacterial carbohydrates were successfully used in this study as a carbon source in the fermentation of biotechnologically interesting strains. This platform technology thus flexibly offers a potentially cheap substrate for fermentation of a plethora of products and might be an option to make industrial biotechnology more cost-competitive in the long run.

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BTV16
Enoate reductase whole cell biocatalysis in *Synechocystis* sp. PCC 6803
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Question: Asymmetric reduction of C=C double bonds is a widely applied method in biocatalysis, which leads to the creation of up to two chiral centers. Enoate reductases [E.C.1.3.1.X] have regained interest as they are able to enantiospecifically reduce α,β-unsaturated aldehydes and ketones, acids, esters, nitro compounds and nitriles under mild reaction conditions. However the *in vitro* application of these enzymes is limited by the costly supply of the cofactor NAD(P)H. This issue has been addressed by using cofactor regeneration systems [1], artificial electron donors [2] and light-mediated photoredox biocatalysis [3]. Still, natural photosynthesis seems to be the most preferred method to regenerate NADPH. Therefore we introduced the native enoate reductase YqjM from *Bacillus subtilis* into the genomic DNA of the photoautotrophic cyanobacterium *Synechocystis* sp. PCC 6803 with the aim to circumvent the addition of sacrificial electron donors. We investigated the *in vivo* activity of the heterologously expressed YqjM in *Synechocystis*. A further task was the optimization of whole cell biotransformation regarding illumination, cell density and aeration.
Methods: YqjM was brought into the cyanobacterial genome by heterologous recombination, where it is controlled by a light-inducible promoter. We have previously shown that biocatalysts can efficiently be expressed in *Synechocystis* while maintaining their enantioselectivity [4]. The cultivation was carried out under different light intensities. The light-driven whole cell biocatalysis was performed at the desired OD_{750} with several substrates under various reaction conditions. Time samples were taken and analysed by GC-FID.
Results: The biocatalysis was optimized focusing on cell density, illumination and aeration. Interestingly, aeration (5 % CO_2) led to a significant decrease in cell viability upon addition of the substrates. Under optimized conditions, cyanobacterial cells expressing recombinant YqjM catalyzed the asymmetric reduction of prochiral enoates with excellent enantioselectivity (>99 %ee) and conversion (80 %) without the need for cofactor recycling.
Conclusion: Herein, we described the proof-of-principle of whole cell biocatalysis of the recombinant enoate reductase YqjM with regard to substrate consumption, product formation and cell viability.

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BEmV01
Mining the treasures of microbial diversity for industrial biotechnology with an optimized droplet-microfluidic screening platform
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The world's scarcely explored microbial biodiversity offers a practically inexhaustible source of naturally selected molecules and strains with high potential for application in industrial and pharmaceutical biotechnology. However, deep functional investigation of the vast microbial diversity in natural samples and metagenomic libraries (>10^9 cells/sample) is strongly limited in classic isolation methods. Droplet microfluidics has the potential to revolutionize classic biological discovery platforms by incrementing throughput up to several orders of magnitude, while diminishing time and costs. Microfluidic droplets can be tailored to function as picoliter counterparts of established larger scaled platforms (Petri dishes, shaking flasks and MTPs), thus transferring the knowledge from decades of microbiological research, yet surmounting the inherent reduced efficiency of classic approaches. Moreover, miniaturization provides important additional advantages: single-cells or simplified microbial communities can be used as the starting point for analysis; encapsulation in low volumes translates into higher cell and metabolite effective concentrations and the possibility to analyze rare and low-volume samples without major alterations of their natural conditions. Since thousands of droplets can be generated and processed within a second, millions of different cells and complex combinations of experimental variables can be tested and characterized per day.
To this end, we have established and optimized a droplet microfluidic platform suitable for detection and screening of novel microorganisms producing enzymes of industrial relevance. We have developed a strategy for culturing cells in ~ 100 pL-droplets, under homogeneous incubation conditions for more than 5 million droplets simultaneously. With this, clonal expansion of single cells can be used as a strategy to increase signal intensity while reducing stochastic noise, and also to screen for biomass and production yields. In particular, we are developing assays for the discovery of novel esterase and lipase producers, isolated from natural samples and metagenomic libraries. Model *E. coli* and *P. pastoris* strains that express the enzymes of interest have been used as positive controls. The catalytic activity is detected after precise addition of fluorescent substrates to each droplet in which the cells have been grown. Ongoing work addresses challenges associated with assay optimization and standardization such as selection of ideal fluorescent substrates and reaction conditions in order to maximize the signal window. Droplets containing highly active cells or enzymes can be sorted out and further analyzed at larger scales. Future work aims to expand the platform potential with heuristic experimental design and complementary read-out methods.

BEmV02
Development of a self-cleavable protein linker for the purification of fusion proteins
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Recombinant proteins are often produced as fusion proteins with an affinity tag for fast and easy purification. For functional analysis, the affinity tag has often to be removed by protease treatment after the purification [1, 2]. Here we present a novel method for the removal of the affinity tag from the target protein in a single step protocol, without the use of proteases.
The protein VIC_001052 of the coral pathogen *Vibrio coralliilyticus* ATCC-BAA450 possesses a metal ion-inducible autocleavage (MIIA) domain (formerly DUF1521). Previous experiments have shown that several divalent cations can induce the autocatalytic cleavage of the domain at the sequence motif GD'PH. The cleavage reaction is fast and stable over a large pH and temperature range [3]. The coding sequence of the MIIA domain was cloned in expression vectors to produce MalE and mCherry as MIIA-Strep fusion proteins in *E. coli*. The fusion proteins could be cleaved completely by the addition of calcium or manganese (II) ions within minutes. The autocatalytic cleavage was induced in immobilized proteins on different affinity columns. The obtained yield of purified protein after on-column cleavage was similar to conventional methods [4]. A fast single-step purification protocol was established. Our current efforts are directed towards reducing the number of amino acids in the linker (presently 44 amino acids) to a minimum without compromising the autocatalytic cleavage activity.

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BEmV03
Upgrading the toolbox for fermentation of (crude) syngas
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The bioliq® pilot plant at the KIT covers the complete process chain required for producing customized fuels from dry lignocellulosic biomass. The intermediate product, a tar-free, low-methane raw synthesis gas (syngas) is then used for further fuel synthesis. Since chemical synthesis has high requirements for gas purity and C/H ratio extensive conditioning of the crude syngas is necessary.
Acetogenic bacteria are able to grow on syngas as sole carbon and energy source under anaerobic conditions. They convert CO/CO_2 and H_2 to Acetyl-CoA and further to organic acids and alcohols using the reductive acetyl-CoA pathway. [Ragsdale and Pierce 2008] In contrast to catalysts used in chemical synthesis these bacteria can process a broad range of CO/CO_2 to H_2 ratios and tolerate impurities like sulphur or nitrogen compounds [Griffin and Schultz 20012, Vega *et al.* 1990].

One major challenge of this so called syngas fermentation is the poor solubility of CO and H_2 in the fermentation broth. To overcome this limitation one could increase the k_la-value for better mass transfer into the broth or increase the pressure in the bioreactor to obtain better solubility of the gases. On the other hand almost complete substrate usage can be enabled by recycling of the off gas.
To address the above named strategies a setup of multiple 2 L bioreactors with product analysis and online gas measurement was developed in our lab. With this setup it is possible to investigate k_la-values and substrate usage of different stirrer setups and aeration modes. It may also be possible to obtain more information about kinetic limitations of the metabolism of certain (genetically modified) acetogenic bacteria since the setup allows complete substrate usage and closing of the carbon balance of the process.

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BEmV04
A super competent *Bacillus subtilis* 168 strain enables the genome manipulation without using plasmid DNA
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Competence is a physiological state that enables *Bacillus subtilis* 168 to take up and internalize extracellular DNA. Using the transformation method of Spizizen and Anagnostopoulos (MG-medium), only a small subpopulation of *B. subtilis* 168 cells becomes competent. [1] ComK, the major transcriptional regulator of DNA-binding, -uptake and -recombination genes (*comG* operon), is responsible for the development of competence. The antiadaptor protein ComS protects ComK from proteolytic degradation by inhibiting the formation of a stable ternary ComK/MecA/ClpC complex. [2] The *comG* operon encodes a pseudopilus structure for binding and uptake of extracellular DNA, which is located at the cell poles. [3] Previously, a super competent *B. subtilis* 168 strain (REG19) together with a new transformation method in rich media was developed. Transformation efficiency of REG19 in LB-medium was higher compared to strain *B. subtilis* 168 in MG-medium. Here, by monitoring the competent cells carrying P_{comG}-*eGFP* in a confocal microscope, we could show that only 7 % of the *B. subtilis* 168 cells in MG-medium become competent, whereas 60 % of the REG19 cells in LB-medium (new method). Furthermore, most of the REG19 cells form a pseudopilus structure at both cell poles. Using REG19, different DNA fragments containing homologous sequences to the chromosome of *B. subtilis* were tested to show the optimal DNA fragment length for the transformation. In this way, we also observed that the localization of the point mutation within the DNA-fragment drastically influence the transformation efficiency. Strain REG19 also enables us to manipulate the genome without using plasmid DNA. All in all, due to its high transformation efficiency, REG19 is a promising strain which can be easily used in biotechnical industries to engineer a production strain.

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BEmV05
A grass associated microbial diversity and structure across the two successional stages of arctic inland dunes
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Plant associated microbes improve the host plant fitness, nutrient acquisition and help to overcome different biotic and abiotic stresses. Advanced sequencing technologies have provided some insight into plant associated microbiomes, but related studies in cold environment are lacking. In this study, we characterized the plant associated microbes of the circumpolar grass, *Deschampsia flexuosa* by ion torrent next generation sequencing of 16S rRNA (bacteria) and ITS (Fungi) genes. Plant associated microbial assemblages of different habitats such as rhizosphere and endosphere (leaf and root) in relation to bulk soil were studied in two successional stages of arctic Aeolian inland sand dunes. Microbial species richness and diversity were mainly affected by habitat but unaffected by successional stages. Microbial community profiling revealed that bacterial community structure was mainly determined by habitat, whereas fungal community structure was influenced by both habitat and successional stage. We identified a small, but statistically significant difference in the community composition between rhizosphere and bulk soil communities of both successional stages, in contrast endophytic communities were clearly different from their soil counterpart. Further, fungal endosphere community shared relatively more OTUs with rhizosphere soil than bacterial endosphere community. Co-occurrence network analysis revealed habitat and/or successional stage specific microbial groups in this ecosystem. The factors responsible for shaping up the plant associated bacterial community were different than fungal community, but the generality of our findings await confirmation from further studies.

BEmV06
Evolution of ecological diversity in *Acidobacteria* in German grassland soils
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Question: Due to their high abundance in soils, *Acidobacteria* may play a major role in soil ecology. The paucity of isolates of this phylum (~ 40 species) contrasts sharply with the large number (~ 13,000) of detected phylotypes. Therefore new approaches are needed to elucidate the ecological functions and evolution of *Acidobacteria*.
Methods: Large datasets of V3 16S rRNA Illumina reads generated for 150 German grassland soil samples, were used to identify ~ 4100 Operational Taxonomic Units (OTUs) of *Acidobacteria* at 97 % full length 16S sequence similarity threshold (along with a maximum likelihood phylogeny). Huisman-Olff-Fresco optimum-response models were calculated using ~ 60 environmental parameters, and mono- and multivariate statistics, network technology, coalescent models on macroevolution, and phylogenetic eigenvector regression were employed in the analysis.
Results: *Acidobacteria* currently occupy a huge range of ecological niches that could be determined based on the physiological activity for individual OTUs. From a macro-evolutionary perspective, the diversity of *Acidobacteria* in the population investigated is saturated, with signatures of extinction and a decay in speciation rate over time. The global worldwide population, however, is still expanding. We further determined the relative time points in evolution when specific environmental factors had their largest selective effect on the diversification of *Acidobacteria*. The life-time of phylogenetic lineages in their ecological niche is probably rather short (at the level of genera) and ends with a substantial ecological divergence of the descendants. As ecological niche space is not unlimited, multiple (re-) occupation of the same ecological niche by phylogenetically distinct descendants appears to occur frequently.
Conclusion: Given appropriate bioinformatic and statistical tools and the availability of environmental data, the high-throughput sequencing of 16S rRNA nevertheless enables a significantly improved insight into the evolution of ecological diversity of a dominant soil bacterial phylum that is otherwise barely accessible to science due to the paucity of cultured representatives.

BEmV07
Eco-systems biology of a rare biosphere member active in cryptic sulfur cycling of a model peatland
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Peatlands are regarded primarily as methanogenic environments significantly contributing to global methane emissions. Little attention is given to the fact that dissimilatory sulfate reduction is maintained by a cryptic sulfur cycle in these low-sulfate environments, with sulfate reduction rates being comparable to marine surface sediments. To deepen our understanding of sulfate reducers in peatlands, anoxic peat slurries

were supplemented with typical degradation intermediates of organic matter at *in situ* concentrations and either stimulated with low amounts of externally supplied sulfate or incubated under endogenous conditions. Changes in the microbial community were monitored by 16S rRNA gene and cDNA amplicon sequencing and correlated to substrate and sulfate turnover. OTUs most abundant in the native community (*Acidobacteria, Actinobacteria, Alphaproteobacteria, Planctomycetes*) showed no significant response to sulfate amendment. In contrast, small networks of natively low abundant bacteria strongly correlated with bulk sulfate turnover under lactate, propionate, and butyrate. Among the responsive OTUs affiliated to recognized sulfate reducers, members of the genera *Desulfomonile* and *Desulfovibrio* (*Deltaproteobacteria*) responded specifically to one of these three substrates, while a *Desulfopila* OTU (*Deltaproteobacteria*) and a *Desulfosporosinus* OTU (*Firmicutes*) were always responsive exhibiting a generalist lifestyle. Interestingly, the *Desulfosporosinus* OTU markedly increased its 16S rRNA and thus ribosome content but stayed at low abundance throughout the incubation period. This likely mirrors its ecological strategy also in the natural peat soil. Parallel sequencing of a metagenome enriched by DNA-stable isotope probing allowed almost complete reconstruction of the *Desulfosporosinus* population pan-genome. Extending this analysis by metatranscriptomics of the individual peat soil slurries linked the increase in ribosome content of the low-abundance *Desulfosprosinus* to transcription of genes involved in sulfate reduction and organic substrate degradation. Our results provide first insights into the metabolic activity and genetic make-up of a rare biosphere member actively involved in biogeochemical cycling and control of greenhouse gas production.

BEmV08

Microbiological assessment and prevalence of food borne pathogens from aprons of meat vendors in an abattoir, Awka Anambra Nigeria

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Food handlers with poor personal hygiene and inadequate knowledge working in Abattoirs could be potential sources of infections of many public health bacteria and parasites. This study evaluated the frequency and susceptibility profile of bacterial isolates from aprons of meat vendors an Abattoir in Awka, southeastern Nigeria. Randomly collected fresh swabs (100) from aprons of meat vendors were analyzed on different bacteriological media. Colonies were identified by basic identification techniques; and susceptibility profile was evaluated on Mueller-Hinton agar using agar diffusion. Of the 100 swab samples collected, 71 bacterial isolates were identified, *Staphylococcus aureus* were the predominant bacterial species (39.4 %), followed by *Escherichia coli* (26.8 %), *Shigella* (23.9 %) and *Salmonella* (9.8 %). *Staphylococcus aureus* showed 70 % and above susceptibility to gentamicin, ceftriaxone, levofloxacin, sparfloxacin and 70 % resistance to streptomycin and erythromycin. *Escherichia coli* showed over 70 % resistance to ampicillin, erythromycin, streptomycin, cefuroxime, sulphamethoxazole and moderately sensitive to penicillin and azithromycin. *Salmonella* was 100 % resistance to six antibiotics used and 50 % susceptible to azithromycin. *Shigella* was more than 80 % susceptible to levofloxacin and 60 % to ceftriaxone, it was resistant to sulphamethoxazole. This study confirmed the presence of *Staphylococcus aureus, Escherichia coli, Shigella and Salmonella* on the apron of meat vendors in abattoirs in Awka. These public health important bacteria from food handlers may pose significant risk on the consumers. Thus there should be periodic medical checkup and continuous monitoring of personal hygiene among the food handlers.

CMV01

Laws of attraction and repulsion – a novel family of bacterial chemosensors

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Question: Chemotaxis, mediated by membrane-embedded methyl-accepting chemotaxis protein (MCP) receptors, plays an essential role in the ecology of bacterial populations. We investigate the structural basis of how a novel, widespread family of bacterial chemoreceptors with a periplasmic tandem-Per-Ant-Sim domain (PTPSD) sense and discriminate between attractants and repellents, and transduce the signal across the membrane.

Methods: We have determined the first crystal structures of PTPSDs of several characterised MCP receptors. Analysis of these structures, in conjunction with mutagenesis, biophysical and molecular simulation studies, provided an insight into diverse mechanisms of ligand recognition by this protein fold.

Results: PTPSDs of amino acid chemoreceptors likely signal by a piston displacement mechanism. PTPSD fluctuates between the piston (C-terminal helix) 'up' and piston 'down' states. Binding of an attractant to the distal PAS domain locks it in the closed form, weakening its association with the proximal domain and resulting in the transition of the latter into an open form, concominant with a downward (towards the membrane) 4-Å piston displacement of the C-terminal helix.

Conclusions: Surprisingly, although the fold and mode of ligand binding of PTPSDs are distinctly different from the textbook example of the aspartic acid receptor Tar, our structural analysis suggests that PTPSDs of amino acid chemoreceptors may signal across the membrane by a similar piston displacement mechanism. Examples of PTPSDs with different ligand specificities and multiple mechanisms of signal recognition will be presented and discussed.

CMV02

How a bacterial cell detects the direction of light?

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The cyanobacterium *Synechocystis* sp. PCC 6803 exhibits flagellar-independent "twitching motility" using type IV pili. In order to use optimal light conditions for photosynthesis, they are able of directed movement towards light. Regulation of phototactic motility involves many different gene products, including various photoreceptors, second messengers and the RNA chaperone Hfq. Here, we show that individual *Synechocystis* sp. PCC 6803 cells do not respond to a spatiotemporal gradient in light intensity, but rather they directly and accurately sense the position of a light source. We use a range of optical techniques to show that directional light sensing is possible because the spherical cells of *Synechocystis* work like microscopic lenses. An image of the light source is focused with sub-wavelength resolution on the edge of the cell opposite to the light source. In contrast, the effects of shading due to light absorption or reflection are much smaller than the intensity difference due to the lensing effect. We then used highly-localized laser excitation to show that specific excitation of one side of the cell triggers movement away from the light, indicating that positive phototaxis results from movement away from an image of the light source focused on the opposite side of the cell. In our model for control of positive phototaxis in *Synechocystis*, the bright focused image of the light source is perceived by photoreceptors in the plasma membrane and/or locally excites pigments in the thylakoid membranes, resulting in a redox signal. This leads then to local inactivation of the motility apparatus. The motility apparatus therefore assembles at the side of the cell facing the light, resulting in movement towards the light source.

CMV03

Better together – a simultaneous tactic and kinetic response of the diatom *Seminavis robusta* in response to nutrient and pheromone gradients

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Microphytobenthic biofilm (MPB) communities are dynamic species assemblies that contribute to half of the primary production in estuaries.

Within the microphytobenthos, diatoms are key players. These microeukaryotes have a biomineralized silicate-based cell wall and a unique life cycle. During mitosis, the size of their rigid cell wall is reduced and it is only through sexual reproduction of two mating types that size restoration could be accomplished. Here we ask how diatoms respond to essential regulators of growth and reproduction success.
We show the function of signal molecules and nutrients in the structuring of the microphytobenthic environment. We investigated the movement patterns of cells of the benthic diatom *Seminavis robusta* exposed to gradients of the nutrient dissolved silicic acid (dSi) and the sex pheromone, L-diproline in separate experimental set-ups using a video monitoring and modeling approach.
Video track analysis revealed that the attraction of *S. robusta* towards dSi and pheromone sources is mediated by a simultaneous chemotactic and chemokinetic search mechanism. The cells showed a remarkable behavioral adaptation by changing their movement pattern towards signal gradients, thereby increasing their encounter rate with the perceived stimuli.
This behavioral adaptation allows diatoms to rapidly colonize sediments by selectively responding to dSi and pheromone gradients and exploit them. The active foraging and mate search of cells is therefore an important factor on ecosystem structuring. It can influence micro-scale patchiness in biofilm communities, as well as affecting sediment-water dSi fluxes and global biogeochemical cycles.

CMV04
How to analyse motility, chemoreceptors and chemotaxis in hyperthermophilic Archaea
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Question: Chemotaxis enables microorganisms to sense chemical stimuli in their environment and to direct their movement towards high concentrations of attractants or away from repellents. The structure, function and interaction of the proteins involved in the motility-chemotaxis network has been extensively studied in Bacteria, but data on the archaeal system are limited to a very few studies of mostly mesophilic species.
Hence, we aim to analyse the presence and localization of chemoreceptors in the very motile, hyperthermophilic Euryarchaeota *Methanocaldococcus villosus*, *Thermococcus kodakarensis*, and *Pyrococcus furiosus* and want to identify components or parameters that can be sensed by these organisms.
Methods: The genomes of *M. villosus*, *T. kodakarensis*, and *P. furiosus* were searched for genes homologous to bacterial chemoreceptors and chemotaxis genes. Selected (truncated) proteins were expressed recombinantly in *E. coli* and used for generation of polyclonal antibodies. For transmission electron microscopy, cells of the three species were either negatively stained or resin-embedded and ultrathin sectioned with/without subsequent immuno-labelling [1]. Swimming studies were performed using the temperature gradient-forming device (TGFD) that can be added to every upright phase contrast light microscope and allows analyses of anaerobic organisms at temperatures up to 110 °C [2]. Different media and conditions, e.g. changes in temperature, oxygen concentration or growth phase were tested for their influence on motility and the expression of flagella and chemoreceptors.
Results: *M. villosus* and *T. kodakarensis* possess five chemoreceptors, four classical transmembrane receptors and one cytoplasmatic receptor whereas no known chemotaxis genes are annotated in *P. furiosus*. The existence of chemoreceptors on protein level was proven in *M. villosus* and *T. kodakarensis* via Western Blots. In correlation with these data, a complex submembraneous structure resembling bacterial chemoreceptor arrays was identified in close proximity to flagella in ultrathin sections of *M. villosus* and *T. kodakarensis* but not in *P. furiosus*. Different growth conditions like temperature, osmolarity and the concentration of complex organic compounds were found to have an effect on the presence and number of chemoreceptors and flagella and thus the swimming behaviour.
Conclusion: This is the first study to investigate the link between motility, chemoreceptors and chemotaxis in hyperthermophilic Archaea. We suggest *T. kodakarensis* as a model organism to further analyse the complex network of sensing and movement in Archaea.

[1] R. Rachel *et al.* (2010) in "Methods in Cell Biology: Electron microscopy of model systems", 47-69
[2] M. Mora *et al.* (2014) Appl. Environ. Microbiol. 80, 4764-4770

CMV05
***In situ* structure of the archaellar assembly and motor complex**
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Since the archaeal lineage has split from the phylogenetic tree billions of years ago, archaea have conquered diverse habitats around the globe, ranging from the most extreme to more ambient environments, including the human body. Fundamental to their prolific diversification and ability to invade new habitats is the capability of archaeal cells to move. In the course of evolution, archaea have developed their own propulsion apparatus called the archaellum, which is distinct from bacterial and eukaryotic flagella in terms of molecular composition and function. To understand the molecular mechanism of the archaellum it is essential to study its structure, which in contrast to the bacterial flagellum remains largely unknown.
We employed electron cryo-tomography to investigate the *in situ* structure and organisation of the archaellum from the hyperthermophilic Euryarchaeum *Pyrococcus furiosus*. We found that most cells assemble a bundle of up to 50 archaella, which are held at one cell pole by a cytoplasmic protein sheet that resides ~40 nm below the plasma membrane. At the cell surface, the S-layer of *P. furiosus* forms holes of ~ 18 nm in diameter to accommodate each extruding filament. Using sub-tomogram averaging, we have determined a structure of the macromolecular motor and assembly complex at the base of each archaellum. We found that the complex consists of a ring-shaped assembly surrounding a central barrel-shaped unit that spans the cytoplasm between plasma membrane and cytoplasmic sheet. Using a combination of single particle electron microscopy, known X-ray structures, biochemical data and bioinformatics we can now identify the positions of individual protein components within the map in order to build a structural model of the entire machinery. This will provide invaluable and unprecedented insights into the function of this fascinating molecular device.

CMV06
The nucleotide-dependent interaction of FlaH and FlaI is essential for assembly and function of the archaellum motor
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Question: Motility is a crucial phenomenon among many prokaryotes. The archaeal motility structure, the archaellum, is a unique nano-machine, which shares structural homology with bacterial type IV pili while it functionally resembles bacterial flagella. The motor of the archaellum comprises of FlaX, FlaI, and FlaH. FlaX forms a 30nm ring structure that acts as a scaffold protein and was shown to interact with the bi- functional motor ATPase FlaI and FlaH. FlaH is a RecA/Rad51 family protein, which binds ATP with high affinity but is unable to hydrolyze it. We wanted to understand whether and how ATP binding of FlaH is important for its function and its interaction with other archaellum motor complex proteins.
Methods: The crystal structure of FlaH was solved at 2.3Å resolution. To illustrate the function of FlaH, point mutants were created in the ATP binding site and analyzed for *in vitro* ATP binding, interaction with motor ATPase FlaI and effects in swimming motility. Microscale thermophoresis was used to compare the binding affinity of wild type and mutant FlaH with FlaI. Pull down experiments using western blot analysis was

performed to isolate the binary FlaX and FlaH complex. Moreover chemical crosslinking was performed to show the oligomerization of FlaH and single particle analysis was performed in order to visualize the FlaX-FlaH complex.
Results: The crystal structure of FlaH reveals a RecA/Rad51 family fold with an ATP bound on a conserved and exposed surface, which presumably forms an oligomerization interface. FlaH does not hydrolyze ATP *in vitro*, but ATP binding to FlaH is essential for its interaction with FlaI and for archaellum assembly. FlaH interacts at the C-terminus of FlaX, which was earlier shown to be essential for FlaX ring formation and to mediate interaction with FlaI. Single particle analysis reveals that FlaH assemble as a second ring inside the FlaX ring *in vitro*. Collectively these data reveal central structural insight of FlaH function in archaellar basal body assembly.
Conclusion: FlaI is a dual function ATPase and we have hypothesized that the nucleotide dependent interaction with FlaH might be important for the switch between archaella assembly and rotation, hence FlaH can have a regulatory function in the archaellar basal body.

CMV07
The bacterial flagellum of *Salmonella* – length control and type-III protein export mechanisms of a macromolecular machine
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Question: Many bacteria - including *Salmonella* - use rotation of a helical organelle, the flagellum, for directed movement in various environments. The flagellum is composed of three main parts: a basal body (the engine), a flexible, curved adapter structure (the hook), and a long external filament (the propeller). This sophisticated nanomachine is functionally and structurally related to virulence-associated injectisome systems of pathogenic bacteria. Common features include a specific type-III secretion system at the base of the structures and a mechanism that controls the length of the flagellar hook or injectisome needle, respectively. The type-III export apparatus consists of a cytoplasmic ATPase-cargo delivery complex and six integral membrane proteins of unknown stoichiometry and functions. The molecular details of the type-III protein export process and the biological importance of hook length control remain obscure.
Results and Methods: We showed previously that a molecular ruler protein is intermittently secreted throughout assembly of the flagellar hook and by this mechanism controls the length of the hook structure in a stochastic process to a final length of 55 nm. Here, we generated deletions and insertions in the molecular ruler protein that resulted in shortened and elongated hook structures, respectively. Single-cell swimming motility analyses revealed that variations in hook length have a profound effect on motility performance.
In addition, we performed a genetic screen to dissect the minimally essential components of the core type-III export apparatus and characterized the role of the FliO component in assembly of the flagellar-specific T3SS. We found that most integral-membrane components were essential and all cytoplasmic components were dispensable for export of a reporter protein and that FliO participates in quality control of export gate assembly.
Conclusions: Our results demonstrate that flagellar hook length has evolved to match the requirements for optimal motility and that the PMF-dependent inner-membrane components constitute the core protein export machine of type-III secretion systems.

CMV08
Identification and characterization of minor pilin*s* and *PilY1* proteins involved in type IV pili-dependent motility in *Myxococcus xanthus*
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Type IV pili (T4P) are versatile, filamentous cell surface structures involved in a variety of processes including motility, biofilm formation, natural competence and virulence. T4P are highly dynamic and undergo cycles of extension, adhesion and retraction. How these events are accomplished by the T4P machine is not understood. We recently elucidated the architecture of the T4P machine in *M. xanthus* and mapped the 10 core proteins of the T4P machine to this architecture using electron-cryotomography (ECT) in combination with an informative set of mutants and fluorescent fusion proteins. Moreover, we have proposed a detailed model for the overall structure and function of this machine. Briefly, these 10 proteins assemble to form a macromolecular complex that spans from the cytoplasm and over the entire cell envelope to the outer membrane.
In addition to the 10 core proteins of the T4P machine, the so-called minor pilins and PilY1 are important for T4P function. The minor pilins are low abundance proteins and have been reported to be present in small amounts in the pilus fiber and have been suggested to prime pilus assembly and/or counteract retractions. PilY1 proteins are described as pilus-associated adhesins and are suggested to function in adhesion and/or anti-retraction. To understand the function of minor pilins and PilY1 inT4P biology, we identified the corresponding genes in *M. xanthus*. We identified three gene clusters each with four minor pilin genes and one *pilY1* gene. The corresponding proteins were named FimU-PilV-PilW-PilX_1-3, and PilY1_1-3 following the nomenclature of the homologous proteins of *Pseudomonas aeruginosa*.
To analyze the function of the minor pilins of *M. xanthus* we systematically deleted the three *fimU-pilV-pilW* clusters in the genome and analyzed for T4P-dependent motility and T4P formation in the mutants. Whereas deletion of single *fimU-pilV-pilW* clusters did not affect T4P-dependent motility lack of two or all three clusters abolished T4P-dependent motility and T4P formation. Moreover, all 10 core components of the T4P machine accumulate at wild-type levels in total cell extracts of the nine-fold mutant. Using ECT on intact cells of the nine-fold mutant, we only detected non-piliated T4PM machines and these empty machines lacked a short periplasmic stem structure that is present in non-piliated T4P machines in wild-type and connects to the inner membrane. We suggest that this short stem functions as a priming complex for T4P assembly and that the minor pilins are part of this complex.
In the case of PilY1 proteins, *single deletions* did not affect T4P-dependent motility whereas lack of two or all three PilY1 proteins abolished T4P-dependent motility. We are currently analyzing if this defect in T4P-dependent motility is also caused by a defect in T4P assembly. We will report our progress in understanding the function of minor pilins and PilY1 proteins of *M. xanthus*.

EMV01
The sRNA NsiR4 is involved in nitrogen assimilation control in cyanobacteria by targeting glutamine synthetase inactivating factor IF7
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Glutamine synthetase (GS), a key enzyme in biological nitrogen assimilation, is regulated in multiple ways in response to varying nitrogen sources and levels. Here we show a small regulatory RNA, NsiR4 (nitrogen stress induced RNA 4), which plays an important role in the regulation of GS in cyanobacteria [1]. NsiR4 expression in the unicellular *Synechocystis* sp. PCC 6803 and in the filamentous, nitrogen-fixing *Anabaena* sp. PCC 7120 is stimulated through nitrogen-limitation via NtcA, the global transcriptional regulator of genes involved in nitrogen metabolism. NsiR4 is widely conserved throughout the cyanobacterial phylum, suggesting a conserved function. *In silico* target prediction, transcriptome profiling upon pulse overexpression and site-directed mutagenesis experiments using a heterologous reporter system showed that NsiR4 interacts with the 5'UTR of *gifA* mRNA, which encodes glutamine synthetase inactivating factor IF7. In *Synechocystis*, we observed an inverse relationship between the levels of NsiR4 and the accumulation of IF7 *in vivo*. This NsiR4-dependent modulation of *gifA* (IF7) mRNA accumulation influenced the glutamine pool and thus NH_4^+ assimilation via glutamine synthetase. As a second target, we identified *ssr1528*, a hitherto uncharacterized nitrogen-regulated gene. Competition experiments between wild type and an NsiR4 knock-out mutant showed that the lack of NsiR4 led to decreased acclimation capabilities of *Synechocystis* towards oscillating nitrogen levels. These results suggest a role for NsiR4 in the regulation of nitrogen metabolism in cyanobacteria, especially for the adaptation to rapid changes in available nitrogen sources and concentrations. NsiR4 is the first identified bacterial sRNA regulating the primary assimilation of a macronutrient.

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EMV02
Unprecedented hydrogen production of free-living Epsilonproteobacteria (*Sulfurospirillum* spp.)
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Introduction: While the microbial production of hydrogen is well known for a wide range of bacteria and several algae, Epsilonproteobacteria like *Wolinella succinogenes* were assumed to exclusively consume H_2 for catabolic purposes so far. A membrane-bound [NiFe] hydrogenase was shown to be responsible for H_2-oxidation in those bacteria. Here, H_2 production of free-living Epsilonproteobacteria belonging to the S*ulfurospirillum* genus, known for their variable catabolism including pyruvate fermentation and organohalide respiration, is reported for the first time. Therefore, the ecological role of *Sulfurospirillum* spp. as a potential H_2-producing partner in syntrophic communities is discussed.
Objectives: This study focusses on the H_2 production capability of various *Sulfurospirillum* spp. during growth on fermentable substrates.
Methods: H_2 production of *Sulfurospirillum* spp. was measured gas-chromatographically during growth on pyruvate as sole carbon and energy source and hydrogenase transcript levels were compared via quantitative reverse transcriptase (qRT) PCR. Fermentation product formation was measured via HPLC.
Results: Comparative genome analysis revealed the presence of up to five hydrogenases (four [NiFe], one [FeFe]-type) encoded by *Sulfurospirillum* spp. While [FeFe] hydrogenases are in general known to be H_2-evolving hydrogenases, two of the [NiFe] hydrogenases are most likely H_2-producing hydrogenases as well, bearing high similarities to characterized H_2-evolving [NiFe] hydrogenases. Due to its environmentally relevant ability to dechlorinate organohalides, *S. multivorans* was chosen as model organism in this study. Transcript levels of the hydrogenases were compared to already available proteome data[1] and besides the periplasmic H_2-oxidizing MBH, a multi-subunit cytoplasmic membrane-bound [NiFe] hydrogenase similar to the H_2-evolving hydrogenase 4 of *E. coli* was expressed. Fermentation experiments revealed H_2 production during pyruvate fermentation for all tested *Sulfurospirillum* spp, while one non-dechlorinating species, harbouring the additional [FeFe] hydrogenase, showed the highest amount, assuming to be responsible for the higher production.
Conclusion: Unprecedented H_2 production capability of free-living Epsilonproteobacteria is shown here for the first time. Considering the importance of hydrogen in anaerobic ecosystems, *Sulfurospirillum* spp. and other Epsilonproteobacteria might be unconsidered hydrogen producers and thus contribute to the microbial anaerobic food web.

[1] Goris *et al.*, *Scientific Reports* (2015), doi: 10.1038/srep13794.

EMV03
Where to dig for exoelectrogens or is there any ecological niche of electroactive microorganisms?
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Question: The core of microbial electrochemical technologies (METs) is the ability of the electroactive microorganisms, also known as exoelectrogens, to interact with electrodes *via* extracellular electron transfer (EET), allowing an immediate wiring of electric current flow and microbial metabolism. MET applications range from wastewater treatment to production of chemicals with *Geobacter sulfurreducens* and *Shewanella oneidensis* being the model organisms. Many other microorganisms were reported for being electroactive, but are often only sparsely characterized. As for an industrial application of METs broader understanding of the ecological requirements of these electroactive microorganisms is required, we assessed, if there is any "typical" ecological niche of electroative microorganisms, or more bluntly speaking: Where to dig for finding new ones?
Methods: The environmental preferences and natural habitat characteristics of 88 electroactive species (bacteria and archaea) described in literature were combined with their physiological as well as EET characteristics. Based on 20 individual descriptors an extensive meta-analysis was performed.
Results: All in all, 43 species displayed anodic, 25 cathodic (13 of them including autotrophic biomass formation) and 17 species both ways of EET with electrodes. The meta-analysis revealed correlations between some of the investigated parameters, e.g. different electron transfer characteristics (direct vs. mediated) and the preference for soluble or flexible use of soluble and solid electron acceptors, whereas no obvious linkage to any specific habitat was found.
Conclusion: These results strongly indicate that significantly more electroactive species exist in nature as well as in already established strain collections. But current standard cultivation techniques certainly impede the identification of their EET capacities. In the light of specific traits required for industrial application like metabolic functions performed under defined temperature or salinity optima, microbial resource mining (of strain collections as well as natural habitats) based on ecological knowledge bears an enormous potential for future developments of METs.

EMV04
Environmental distribution and enrichment of anaerobic methanotrophs from Italian paddy fields
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Rice agriculture is a major source for the greenhouse gas methane. It is produced by anaerobic methanogenic archaea in the bulk soil. However, not all produced methane is also emitted to the atmosphere. Methane oxidizing microorganisms act as a bio-filter reducing the emissions substantially. For a long time, research has focussed on aerobic methane oxidisers, however, it has become apparent that anaerobic methanotrophy might also play a significant role in flooded rice fields. Most paddy fields are heavily fertilized with nitrate which can be used as alternative electron acceptor. To get insights into the importance of anaerobic oxidation of methane (AOM) in Italian paddy fields, we studied the distribution using 16S rRNA gene amplicon sequencing and qPCR. *Methanoperedens*-like nitrate reducing archaea and *Methylomirabilis*-like nitrite reducing bacteria were both present in the bulk soil as well as in the rice rhizosphere. Nevertheless, the abundance of *Methanoperedens*-like archaea was an order of magnitude higher than *Methylomirabilis*. Soil slurries converted ^{15}N-nitrate to dinitrogen gas and ^{13}C-methane to $^{13}CO_2$ thereby confirming the potential for nitrate-dependent AOM (at 17 nmol g-1dw d-1). Furthermore, the AOM community was enriched using a bioreactor fed with methane and nitrate. After 1.5 years of incubation, the culture was dominated by a *Methanoperedens*-like archaea (approximately 20 % of total microbial community). After coverage and GC % based binning, the analysis of the metagenome of the enrichment culture revealed furthermore a high abundance of a *Methylomirabilis*-like bacterial species and a *Chloroflexi* species. FISH analysis and qPCR confirmed the successful enrichment of the anaerobic methanotrophs.

EMV05
***Methylomagnum ishizawai* gen. nov., sp. nov., a mesophilic type I methanotroph isolated from rice rhizosphere**
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Methods: Rhizosphere sample was collected from a rice paddy field in Anjo, Aichi, Japan. Details of the sampling and enrichment procedure were described previously (Dianou *et al.*, 2012). The ribulose monophosphate serine and ribulose 1,5-bisphosphate carboxylase/oxygenase pathways were determined.as described by Maeda *et al.* (2002). Cellular fatty acids was determined using the Sherlock Microbial Identification (MIDI) system. DNA was isolated and purified as described by Marmur (1961) and DNA G+C content was determined by HPLC according to the method of Katayama-Fujimura *et al.* (1984). Amplification of the *pmoA* gene and *mmoX* gene was carried out by PCR with primers universal and mmoX206f/mmox886r, respectively. Amplification of the 16S rRNA gene was carried out with primers 27f/1492r. Phylogenetic trees were constructed with the neighbour-joining method.
Results: Description of *Methylomagnum* gen. nov.: Cells are aerobic, Gram-negative, motile rods with a single polar flagellum. Cells possess the typical intracytoplasmic membrane system for gammaproteobacterial methanotrophs (type I) forming bundles of membrane vesicles. Resting stages are observed. Cells utilize only methane or methanol as sole carbon and energy sources. Methane is oxidized via pMMO and C1 compounds are assimilated via the ribulose monophosphate and/or ribulose bisphosphate pathways. Cells are neutrophilic, mesophilic and non-thermotolerant. The major cellular fatty acids are $C_{16:1}\omega7$, $C_{16:0}$ and $C_{14:0}$ and the major respiratory quinone was 18-methylene-ubiquinone-8. The strain belonged to the family *Methylococcaceae* (*Gammaproteobacteria)*. The type species is *Methylomagnum ishizawai*.
Description of *Methylomagnum ishizawai* sp. nov.: Exhibits the characteristics as described for the genus. In addition, cells are 2.0-4.0 µm

long and 1.5-2.0 µm wide. Colonies appear white and resting stages are observed. Cells grow optimally at 31-33 °C (range 20-37 °C) and at an optimal pH of 6.8-7.4 (range pH 5.5-9). Cells utilize methane and methanol as sole carbon and energy sources, but are sensitive to methanol concentrations higher than 0.05 %. Grows with nitrate, ammonium, glutamic acid and tryptophan as sole nitrogen sources, but not with N_2 and tolerates NaCl up to 0.2 %. Cultures are positive for cytochrome *c* oxidase, but negative for catalase activity.

Conclusion: Japanese rice paddy field is a biological resource for description of a novel species. The type strain, RS11D-Pr^T was isolated from rhizosphere of rice. The DNA G+C content of the type strain is 64.1 mol %.

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EMV06

Nutrient and increasing temperature effects on the microbial community structure and function in streambed sediments

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Central European streams are affected by multiple stressors as global warming and pollution which act on the structure and activity of microbial communities in streambed sediments. To investigate the effects of increasing temperature and nutrient levels on bacteria, sediment samples from two streams with different pollution levels (low: Breitenbach (Btb) and high: Vollnkirchener Bach (Vkb)) were incubated in the laboratory at three different temperatures (10, 15 or 20 °C) using a perfused core technique. The activity of five extracellular enzymes and the abundance of seven different bacterial groups using CARD-FISH were determined in the beginning and after 2 weeks of incubation. Differences in extracellular enzyme activity and bacterial abundance were more pronounced between the streams than caused by different temperatures. Total bacterial abundance increased significantly with increasing temperature (23 % (15 °C), 28 % (20 °C) for Btb and 11 % (15 °C) 45 % (20 °C) (Vkb) compared with 10 °C) in both streams. The bacterial community structure changed with the temperature. In the Breitenbach *Actinobacteria* dominated the bacterial community at higher temperatures. In the Vollnkirchener Bach the proportion of *Gammaproteobacteria* were highest at 20 °C and *Archaea* increased significantly at higher temperatures. The extracellular enzyme activities were strongly temperature dependent but showed also that nutrient and microbial composition were more important than temperature. This study illustrates that increasing temperatures (5 °C) and higher nutrient levels induce effects on microbial communities and their function in streams.

EMV07

The dark side of the Mushroom Spring microbial mat – life in the shadow of chlorophototrophs

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Microbial communities inhabiting the hot spring phototrophic microbial mats in the effluent channels of Octopus Spring and Mushroom Spring within the Lower Geyser Basin at Yellowstone National Park have been studied for nearly 50 years (*1*, *2*). In these studies, the emphasis has generally been focused on the chlorophototrophic bacterial populations, i.e., chlorophyll-based phototrophs of the phyla *Cyanobacteria* and *Chloroflexi* and the newly discovered phototrophs *Chloracidobacterium thermophilum* (*Acidobacteria*) and "*Candidatus* Thermochlorobacter aerophilum" (*Chlorobi*). In contrast, the diversity and metabolic functions of the heterotrophic community in the anoxic/microoxic region of the mat are not well understood. In this study we analyzed the orange-colored undermat of the phototrophic microbial mat at Mushroom Spring using metagenomicand rRNA-amplicon (Itag) analyses.
Members of the oxygen-tolerant, filamentous anoxygenic phototroph *Roseiflexus* sp. were identified as the dominant populations in the microbial undermat community. The second most abundant organisms were *Thermotoga* sp., whose predicted predatory lifestyle might contribute to the availability of biological substrates to the heterotrophic community, and which have been hypothesized to constitute a major source of H_2 by anaerobic fermentation that could enable photomixotrophic metabolism by *Chloroflexus* and *Roseiflexus* spp. (*3*, *4*). Further key-players are two different members of the phylum *Armatimonadetes* (former Candidate phylum OP10), *Thermocrinis* sp., two potentially phototrophic and one heterotrophic member of the *Chloroflexi*, one member of the *Atribacteria* (former Candidate phylum OP9), a sulfate-reducing member of the genus *Thermodesulfovibrio*, a *Planctomycetes* member, a member of the EM3 group tentatively affiliated with the *Thermotogae*, as well as a putative member of the former Candidate phylum OP8, *Arminicenantes*. *Archaea* were not detected in abundant numbers in the Itag analysis, and no metagenome bin representing an Archaeon was recovered from the metagenome.
Highly similar *Synechococcus* sp.variants in these mats were shown to represent ecological species populations with specific ecological adaptations (*5-7*). A similar microdiversity and existence of putative ecotypes is suggested by this study for members of the undermat community, and in particular for *Roseiflexus* sp., the mostdominant member of the *Chloroflexi* and overall species in the undermat.

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EMV08

Bacteria dominate the short-term assimilation of plant-derived N in soil

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Fungi dominate the degradation and assimilation of plant-derived polymeric organic C [1]. Both antagonistic and synergistic relations of fungi to bacteria have been reported [2, 3] but it was generally assumed that bacteria feed on low molecular weight compounds released by the fungal breakdown of complex polymers. In comparison to the well-understood cycling of inorganic N [4], only little is known about the assimilation of organic N in soil. For the first time, we characterize the bacterial and fungal populations involved in the cycling of 15N-labeled plants using amplicon sequencing (16S and 18S rDNA), metaproteomics and protein-SIP. Copiotrophic behaviour was revealed for *Rhizobiales* belonging to *Proteobacteria*, *Actinomycetales* belonging to *Actinobacteria* and *Chroococcales* belonging to *Cyanobacteria* as these phylotypes immediately incorporated N from the added plant tissue. Otherwise, the fungal *Saccharomycetales* and the bacterial *Enterobacteriales*, *Pseudomonadales*, *Sphingomonadales* and *Xanthomonadales* displayed oligotrophic 15N-assimilation as incorporation of plant-derived N was reported during the later stages of short-term decomposition. Our study unveiled that, in contrast to the dominance of fungi in the degradation of complex compounds and bacteria feeding on fungi-released low molecular weight compounds, mostly bacteria were involved in the short-term assimilation of plant-derived N in soil.

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EMV09
A novel central carbon assimilation pathway in the marine Alphaproteobacterium *Erythrobacter* sp. NAP-1
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Question: Many carbon sources in the environment, such as fatty acids, alcohols, esters, waxes, alkanes, polyhydroxyalkanoates, and C1-compounds are initially converted into the central metabolite acetyl-CoA. Acetyl-CoA is then transformed into biomass through anaplerotic assimilation. For more than fifty years, the glyoxylate cycle of *Escherichia coli* has been the only known acetyl-CoA assimilation pathway. Recently, however, we discovered the ethylmalonyl-CoA pathway (1) and the methylaspartate cycle (2) that operate in environmentally and ecologically highly relevant microorganisms, such as Haloarachea, soil-born Streptomycetes, and plant-associated, as well as aquatic alphaproteobacteria. Here, we report on a novel acetyl-CoA assimilation strategy that operates in the marine organism *Erythrobacter* sp. NAP-1 and that essentially differs from all other acetyl-CoA assimilation strategies described so far. This novel central metabolic pathway seems to be specifically adopted to the lifestyle of *Erythrobacter* sp. NAP-1 in a carbon- and energy-limited environment.
Methods: Bioinformatics was used to identify the pathway reaction sequence. Enzyme assays were developed to test and quantify the proposed reactions *in vitro* and *in vivo.* Key enzymes of the proposed pathway were expressed and biochemically investigated. *Erytrobacter* sp. Nap 1 was cultivated under different growth conditions to confirm the functioning of the proposed novel acetate assimilation pathway by cell extract assays and proteomics.
Results: A new propionyl-CoA synthase (3) that possesses an unusual carboxylation activity was demonstrated as key enzyme of the proposed pathway. Other enzymes involved in the proposed novel acetyl-CoA assimilation pathway were demonstrated by enzyme measurements in cell extracts and validated by proteomics.
Conclusions: *Erythrobacter* sp. NAP-1 uses a completely novel pathway for acetyl-CoA assimilation that features an unusual CO_2-fixation reaction, which allows for the assimilation of acetyl-CoA and efficiently saves ATP. This study extends our knowledge beyond the *E. coli* "textbook" example of acetyl-CoA assimilation by describing (yet) another acetyl-CoA assimilation strategy that seems to enable the specific adaption of the central carbon metabolism of a microorganism to its environmental niche.

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(2) Khomyakova *et al.* Nature 2011
(3) Alber & Fuchs Journal of Biological Chemistry 2002

EMV10
Biological significance of glucosinolate break-down products on soil microbiome
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Glucosinolates (GSLs) are a group of secondary metabolites in plants of the Brassicales order. The GSLs and the hydrolytic enzymes, the myrosinases, constitute a natural plant defense system against diverse pathogens due to their allelopathic properties. Potential negative long-term effects of the highly bioactive break-down products on beneficial soil microorganisms have not been studied sufficiently. However, this would be worthwhile with regards to a sustainable agricultural use of Brassicaceae-dominated producing areas for the subsequent cultivation of different crops, especially legumes. In order to address this issue a combination of approaches was used to systematically characterize the changes in the soil microbiome. Bioassays were based on analyzing growth suppression of selected pathogenic and beneficial soil fungi by GSLs and their hydrolysis products. The results obtained revealed that GSL and its derivatives do not specifically target pathogenic fungi, but also have detrimental effects on beneficial fungi.
Lipid and RNA analyses were carried out to systematically characterize GSL derived changes of the soil community structure. To this end, soil of a selected test site (organic farming) was analyzed after exposure to GSLs utilizing quadrupole time of flight mass spectrometry and gas chromatography to provide lipid fingerprints. Signature lipid biomarker (SLB) analysis used in determining changes in community structure of soil microorganisms revealed significant changes after exposure to GSLs. These changes in SLBs are accompanied by changes in different enzyme activities in soil extracts. Here, increase of relative activities for 20, 4 and 10 folds for esterases, phospholipases and proteases, respectively, compared to the control samples were determined. Information obtained by 16S metagenomic analyses of soil samples exposed to a 4-week-GSLs application revealed a tremendous reduction of fungal and bacterial diversity. This shift was accompanied by a massive reduction in biological diversity concerning bacteria and especially fungi. After 4 weeks of incubation, a certain yeast of the genus *Trichosporon* accounted for over 98 % of the total fungal biomass found in the test soil. Similar results were obtained concerning the bacterial community. Specific bacteria, which were found to be associated to the root microbiota of Brassicaceae, became more abundant, while in general the biological diversity decreased profoundly during incubation.
Conclusively, a time-dependent shift in microbial community structure, and significantly decreased levels of fungal biomass due to application of GSL breakdown products were detected. The project thus revealed unprecedented negative effects of GSLs on the soil microbial biodiversity, which has an impact on growth and germination of other crop plants e.g. Fabaceae.

EMV11
Metabolic labor united – complete nitrification by a single microorganism
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Nitrification, the step-wise oxidation of ammonia to nitrate, is a process catalyzed by two physiologically distinct clades of microorganisms. First, ammonia-oxidizing bacteria and archaea convert ammonia to nitrite, which subsequently is oxidized to nitrate by nitrite-oxidizing bacteria. This division of labor was already described by Winogradsky in 1890 and is a generally accepted characteristic of the biogeochemical nitrogen cycle. Even though the existence of a single organism capable of catalyzing complete nitrification was not reported to date, this reaction is energetically feasible and was postulated to occur under conditions selecting for microorganisms with lower growth rates, but higher growth yields than canonical ammonia and nitrite-oxidizing bacteria (Costa *et al.*, 2006). Thus, these organisms could have a competitive advantage in biofilms and other microbial aggregates growing at low substrate concentrations. In this study, we enriched for microorganisms responsible for nitrogen transformations in an ammonium-oxidizing biofilm, which was sampled from the anaerobic compartment of a biofilter connected to a recirculating aquaculture system. This enrichment culture contained two *Nitrospira* species that had all genes required for ammonia and nitrite oxidation in their genomes. Batch incubation experiments indicated that these *Nitrospira* indeed formed nitrate from the aerobic oxidation of ammonia, and FISH-MAR confirmed their ability to use the energy derived from ammonia and nitrite oxidation for carbon fixation. Their ammonia monooxygenase (AMO) enzymes were phylogenetically distinct from canonical AMOs, thus rendering recent horizontal gene transfer from known ammonia-oxidizing microorganisms unlikely. Instead, their AMO subunit A displayed highest similarities to the "unusual" particulate methane monooxygenase from *Crenothrix polyspora*, thus shedding new light onto the function of this largely uncharacterized sequence group. Our results show by the recognition of a novel *amoA* type that a whole group of ammonia-oxidizing microorganisms was previously overlooked and thus will lead to a better understanding on the environmental abundance and distribution of this functional group. Furthermore, the discovery of the long-sought-after comammox process will change our perception of the nitrogen cycle.

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EMV12
Microbial communities in extremely oligotrophic sediments of the South Pacific Gyre – Do they eat phages?
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Microbial communities in deep subsurface sediments are limited by energy and electron donor availability. In the extreme oligotrophic sediments of the South Pacific Gyre (SPG) the viral biomass represents a

considerable amount of bioavailable organic matter in relation to the microbial biomass especially in deeper sediments. Thus, the question emerges which strategies indigenous microorganisms have developed for the acquisition of nutrients. Hydrolysis of polymeric substances by exoenzymes is a prerequisite of substrate incorporation. To assess a feasible of microbial virus degradation, potential exoenzyme activity measurements of aminopeptidase and alkaline phosphatase were measured with pure cultures and bulk sediments using substrate analogues. Additionally, SPG isolates were cultivated with concentrated Rhizobiophages as sole food source. Viral and bacterial numbers were followed by direct counts, q-PCR and flow cytometry. Potential exoenzyme activities were detected for all tested isolates and bulk sediment samples from various depths and SPG sites. Potential aminopeptidase activities generally decreased with depth, which points to diminished microbial activities in deeper layers. In contrast, potential alkaline phosphatase activities increased with sediment depth and were inversely correlated to the phosphate concentrations present in the sediments. Above the oceanic crust, the diffusion of phosphate-bearing fluids into the sediments lead to diminished activities indicating an active response rather than conservation by particle attachment. The putative utilization of virus particles as a food source was supported by the results of the feeding experiment. Enumeration by direct counts and q-PCR analysis showed a clear trend of phage degradation over time. In conclusion, our experiments revealed the presence of active microbial communities within the deep subsurface of SPG sediments. Diminished exoenzyme activity might explain the increase in virus-to-cell ratio in deeper sediment layers. In conclusion, our experiments infer that indigenous microorganisms might have developed a survival strategy to use viruses as a food source.

EMV13

Regulatory network of *Dinoroseobacter shibae* DFL 12^T for the adaptation to low oxygen tension

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Question: *Dinoroseobacter shibae* DFL12^T is capable of performing nitrate respiration and subsequent denitrification under anaerobic conditions. *D. shibae* possesses genes encoding several homologs of the dissimilatory nitrate respiration (Dnr) regulator (DnrD, DnrE and DnrF) and one gene encoding afumaratnitrateregulator (Fnr) homolog (FnrL). What is their role within the fine-tuned regulatory network guiding the adaptation to low oxygen tension?
Methods: We generated knock-out mutants of the *dnrD*, *dnrE*, *dnrF* and *fnrL* regulator genes and performed shift experiments from aerobic to anaerobic growth conditions in the presence of 25 mM KNO_3. Samples for RNA preparation and subsequent DNA array hybridization and RNA Sequencing were taken before the shift and after 30 and 60 min of anaerobiosis. Furthermore, DnrF was recombinantly produced and purified as a His-tagged fusion protein under anaerobic conditions. To study hemin binding UV/ Vis and electron paramagnetic resonance (EPR) spectroscopy was performed. DNA binding of the anaerobically purified DnrF was analyzed using electro mobility shift assays (EMSA).
Results: Using transcriptome analysis the regulons of DnrD, DnrE, DnrF and FnrL were determined. The genes encoding proteins involved in denitrification processes were found upregulated under anaerobic conditions. The periplasmatic dissimilatory nitrate reductase (Nap) was found expressed even under aerobic conditions. A repression by DnrD, DnrE and DnrF was found under aerobic and anaerobic conditions. The anaerobic expression of the *nir* and *nor* operon was induced by FnrL and fine-tuned by DnrD. The *nos* operon was anaerobically activated by DnrD and FnrL combined with a fine modulation of DnrF. Moreover genes involved in electron transport like the cytochrome bc_1 and NADH dehydrogenase are regulated by FnrL. The promoter region of the *nap* operon was used for EMSA to study binding of DnrF. A K_d of 3.45 nM by addition of 4 nM DNA was determined. The conserved binding motif (TTGAc-N_4-gTCAA) was identified and the specificity of DnrF binding was shown in competition experiments. This binding site was found additionally within promoter regions of other DnrF target genes we identified by transcriptome analysis. Furthermore, we used anaerobically produced and purified DnrF regulator protein for biochemical characterization. In UV/Vis spectroscopy the addition of hemin to DnrF resulted in a typical absorption spectrum, which indicates specific hemin coordination. Exposure of the DnrF/hemin complex to NO blue shifted the UV/Vis spectrum indicating a possible NO sensing mechanism for DnrF.
Conclusion: The Crp/Fnr family members FnrL, DnrD, DnrE and DnrF form a fine-tuned regulatory network and mediate the anaerobic expression of denitrification genes. Specific binding of DnrF to DNA was shown. The coordination of hemin to DnrF indicates a possible NO signal perception mechanism.

EMV14

Diversity and function of bacterial communities in sublittoral marine surface sediments

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The benthic bacterial community in sublittoral sediments is persistently supplied with water column-derived organic matter and oxygen. A concept for the breakdown of algal biomass in the water column has been proposed and specialized *Bacteroidetes* and *Gammaproteobacteria* have been identified as main mineralizers [1] but only little is known about benthic-pelagic coupling and microbes involved in degradation processes in sublittoral sediments.
Here, we studied temporal and lateral changes (7 sites) of the microbial community structure in North Sea surface sediments by Illumina tag sequencing of the 16S rRNA gene and CARD-FISH. Further, functions of dominant populations were addressed by metagenomics.
The bacterial communities in permeable surface sediments differ significantly from overlaying bottom waters (ANOSIM R=0.98, p>0.005) and exhibit a greater evenness. In contrast to the water column, which is dominated by the SAR11 clade, actinobacterial *Candidatus* 'Actinomarina spp.' and flavobacterial NS5, NS7 and NS9 groups, dominant benthic bacterial groups were gammaproteobacterial *Xanthomonadales* and *Chromatiales*, deltaproteobacterial *Sandaracinaceae* and the acidobacterial group Sva0725. No significant differences between subsampled depth layers of the first two centimeters were observed. The bacterial diversity varied greatly between sites with highest values observed for two sampling sites furthest offshore. The portion of shared $OTU_{0.05}$ ranged from 11 % to 29 % across all samples. Despite their great variability, the bacterial communities differed significantly between permeable and non-permeable sites (ANOSIM R=0.4973, p<0.001) and the orders *Campylobacterales* and *Desulfobacterales* were identified as characteristic for non-permeable sediments. Although less pronounced than in the water column, seasonal changes in the bacterial community were observed. The $OTU_{0.05}$ richness and evenness was highest in winter and lowest in summer while absolute cell numbers were three times higher in summer compared to following winter (5×10^8 and 1.3×10^8 cells/mL). Only 17 % of $OTU_{0.05}$ were shared between all sampling points. CARD-FISH showed high abundances of *Holophagae* (15 % of total cells), *Planctomycetes* (19 %), *Blastopirellula* (4 %), *Phycisphaeraceae* (5 %) and *Sandaracinaceae* (7 %) for investigated sediments of the German Bight. Additionally, first insights from metagenomes will be presented and discussed in the context of degradation of complex organic substrates, in particular of polysaccharides.
This study shows that oxic sublittoral surface sediments harbor a distinctively different community than the water column despite its close coupling. Such communities underlie temporal and lateral influences but are dominantly shaped by the sediments physical nature.

[1] Teeling H., *et al.*, Science 336 (2012) 608-611

EMV15

A targeted mutation system is active in the filamentous N_2-fixing cyanobacteria *Trichodesmium erythraeum*

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Blooms of the dinitrogen-fixing marine cyanobacterium *Trichodesmium* considerably contribute to new nitrogen inputs into tropical and subtropical oceans. Intriguingly, only 60 % of the *Trichodesmium erythraeum* IMS101 genome sequence codes for protein, compared with ~ 85 % in other sequenced cyanobacterial genomes.
Using primary transcriptome sequencing, we demonstrated recently that *T. erythraeum* has the highest percentage of transcription start sites yielding non-coding RNAs of any bacterium examined to date (Pfreundt *et al.*, 2014). Up to 86 % of the intergenic sequence space is transcribed into RNA in culture and its sequence is highly conserved in metagenomic

datasets of *Trichodesmium* (Walworth *et al.*, 2015). Adding another detail to this exceptional genome architecture, we provided evidence for the sequential splicing of a group II twintron (nested introns) interrupting a conserved cyanobacterial protein-coding gene (Pfreundt and Hess, 2015). This is the only twintron known to interrupt a protein coding gene in bacteria, raising the question about possible special adaptations of the *Trichodesmium* genome to mobile genetic elements.
This idea is also in line with our finding of a highly transcribed retroelement (a Diversity Generating Retroelement, DGR) that serves as a template for the targeted mutation of at least 12 different genes by mutagenic homing (Pfreundt *et al.*, 2014). This is of special interest in view of the generally high genome stability of Trichodesmium species all over the world's oceans. Using individually picked *Trichodesmium* colonies from the Gulf of Mexico and amplicon sequencing, we have generated first evidence that some of the putative target genes are indeed highly diverse even within a few individual *Trichodesmium* colonies, indicating high mutation rates at these genomic sites. While there is no functional annotation for these genes, a role in viral defense, sensing of diverse molecules, or cell-to-cell interactions can be imagined.

Pfreundt, U., and Hess, W.R. (2015). Sequential splicing of a group II twintron in the marine cyanobacterium *Trichodesmium*. Sci. Rep. *5*, 16829.
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EMV16
Low wind speed induces strong bacterial community changes in the sea surface microlayer of a wind-wave system
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Covering two-thirds of the Earth's surface, the sea surface microlayer (SML) forms the boundary layer between the atmosphere and the underlying oceanic water mass (ULW). The SML evokes our interest due to its unique physicochemical and biological properties that differ from deeper waters. Its atmospheric proximity makes the SML a controlling zone for climate-related gas exchange processes and comprises a vast microbial ecosystem. The bacterioneuston, i.e. the bacterial community within the SML, inhabits a harsh environment dominated by wave action, turbulences by wind, upwelling and advection as well as increased UV radiation. Calm wind has been identified as a driver for the formation of distinct bacterial populations within the SML, but knowledge about effects on the species level and a mechanistic explanation of how the wind speed affects the neustonic habitat is still scarce. Experiments at the Heidelberg Small-Scale Air-Sea Interaction Facility (Aeolotron) filled with sea water from the Atlantic Ocean allowed us to study the bacterioneuston under controlled wind speed ranging from very calm to stormy conditions as well as three different pCO_2 levels (600-630, 740-900 and 900-1200 µatm). We used 16S rDNA fingerprinting analysis, i.e. Denaturing Gradient Gel Electrophoresis, for bacterial community analysis and flow cytometry to determine the bacterial abundance in the SML and the ULW. The results show that bacterial numbers in the SML were up to twice as high as in the ULW below a wind speed threshold of 5.5 m s^{-1}. Highest enrichment was observed at high pCO_2 levels. Additionally, the wind speed also affected the bacterioneuston diversity causing increasingly different bacterial communities between SML and ULW, both of which were generally dominated by members of the *Alpha-* and *Gammaproteobacteria*. At low but not at high wind speed we identified abundant SML-specific bacteria e.g. belonging to the *Alteromonadaceae*. In contrast, a member of the class *Flavobacteriia* (*Bacteriodetes*) was absent from the SML at low wind speed and could be found in the ULW at high wind speed. Ongoing work aims to investigate whether our identified wind speed threshold of 5.5 m s^{-1} holds also true for natural bacterioneuston communities, which were sampled in the central Baltic Sea during summer 2015.

FBV01
Intracellular and potential extracellular roles of the *Ustilago maydis* Acyl-CoA-binding protein Acb1
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Conventionally secreted effectors play a crucial role during the biotrophic interaction between the smut fungus *Ustilago maydis* and its host plant *Zea mays*. In the last decade it has been established that proteins without a signal peptide can also be targeted to the outside of the cell in an ER/Golgi independent manner. Many of these unconventionally secreted proteins have been shown to be 'moonlighting' proteins with an extracellular function distinct from their intracellular function. One candidate for an unconventionally secreted protein in *Ustilago maydis* is the UmAcb1 protein, previously detected in the apoplastic fluid of infected maize leaves. UmAcb1 is a homolog to the Acyl-CoA binding protein of *Dictyostelium discoideum* (AcbA), for which intracellular functions as Acyl-CoA shuttle protein and Acyl-CoA pool former are described. In addition, AcbA is unconventionally secreted as full-length protein and extracellularly processed into a small peptide (SDF-2) which triggers terminal spore differentiation upon interaction with a membrane receptor.
In our work we could show that *U. maydis* Acb1 can be secreted and processed into an SDF-2 like peptide. This peptide was able to trigger terminal spore differentiation in *D. discoideum*. When *acb1* is deleted in a solopathogenic strain virulence is unaltered, however, when *acb1* is deleted in compatible haploid strains, these mutants are unable to mate and cause disease. At present, we are trying to establish whether the absence of disease symptoms is linked to the intra- or extracellular function of Acb1.

FBV02
Soil amoeba impose predatory selection pressure on environmentally acquired pathogenic fungi
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Opportunistic systemic fungal infections (mycoses) are frequent threats of severely immunocompromised patients. They are manifested as an acute pneumonia, fungemia or in worst cases as a life-threatening progressive dissemination.
Some fungal pathogens, like *Candida albicans*, are present as a commensal of the mucous membranes; however, the number of cases, where *Candida sp.* acts as opportunistic nosocomial pathogen is rapidly increasing. Other yeasts and also filamentous fungi, like *Aspergillus fumigatus,* are acquired from environmental sources. In their natural environment, fungi are daily exposed to the selection pressure from the side of their predators, such as soil amoebae. Amoebae are professional phagocytes that are abundant in the nature, grazing on the many bacteria, yeasts and filamentous fungi. Their role as agents to control terrestrial microflora is significant.
We hypothesize, that parts of the multifactorial virulence potential of some fungi have emerged during the co-evolution with soil inhabitants, as a result of long-term interaction with predatory amoebae. With all their enormous biodiversity, *Dictyostelium discoideum* has become a leading non-mammalian model for host-pathogen interactions and presents a powerful tool for investigating the evolutionary origin of virulence. During *in vitro* confrontations with the spores of *A. fumigatus*, phagocytic interactions between both organisms showed similarities to *A. fumigatus* encounters with macrophages. While white, naked conidia of *pksP* mutant were rapidly ingested by *Dictyostelium*, uptake of those covered with the green pigment DHN-melanin was drastically reduced. Beside the phagocytic interactions, both amoeba and fungus secrete cross-inhibitory compounds, which suppressed the fungal growth or induced amoeba aggregation with subsequent cell lysis, respectively. Using LC-MS analysis we identified gliotoxin, a non-ribosomal peptide with immunosuppressive properties, as the major amoebacidal metabolite of *A. fumigatus*. On the side of the predatory amoeba, we are currently working on the identification of an antifungal metabolite.
We have recently extended our infection model to a natural isolate of the abundant soil amoeba *Protostelium mycophaga,* which has not been studied in detail but lives exclusively fungivorous. Our results indicate a broad food range within the fungal kingdom and we have further observed the ability of several yeast species to undergo reversible phenotypic switching to escape the phagocytosis by invasive growth. This

phenomenon was specific to amoebae predation and therefore might represent an adaptive trait of yeasts acquired in the environment.

FBV03

Molecular background of virulence in human pathogenic Mucoralean fungi

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Background: Members of Mucoromycotina (formerly zygomycetes) have great biotechnological and industrial importance, e.g. in the production of organic acids (lactic acid), unsaturated fatty acids and carotenoids, and oriental fermented food (soy sauce or tempeh). On the other hand, several species are important as opportunistic human pathogens, which can cause frequently fatal systemic infections in immunocompromised patients. In the recent years, the number of patients with zygomycoses has significantly increased.
Question: Little is known about virulence factors and genes playing role in survival of human pathogenic zygomycetes within the host.
Methods: In this study, our goal is the functional characterization of selected genes of *Mucor circinelloides* and *Lichtheimia corymbifera*, which may have effect on virulence potential of these fungi. Genes of *L. corymbifera* up and down regulated during interaction with macrophages were studied. HMG-CoA reductase mutants of *M. circinelloides* isolated previously were also involved in the study.
Results: Several genes (involved in iron transport, encoding hydrophobic surface and heat stress proteins, etc.) were cloned and disrupted in *L. corymbifera*. The interaction of *Lichtheimia* and *M. circinelloides* mutants with macrophages is still in progress.
Expected results: Our results will greatly contribute to knowledge of the virulence of *Mucor* and *Lichtheimia*. Exploration of the role of the potential virulence factors in the infections would help to find new therapeutic targets against pathogenic fungi. The applied comparative approach could reveal whether there are any differences and/or similarities in the pathogenicity and defensive ability of different zygomycetous species.

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FBV04

***Parasitella parasitica*, an experimental laboratory system for studying horizontal gene transfer**

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The *Mucor*-related zygomycete *Parasitella parasitica* is a parasite of various mucoralean fungi that has developed characteristics pointing towards an endosymbiotic relationship. During infection, host and parasite undergo fusion, thus leading to the transfer of organelles including nuclei. Looking at the morphology of infection structures, the transfer of nuclei and, consequently, of genetic information from the parasite to the host is highly favoured. However, the opposite direction, gene transfer from hosts into the parasite is not completely impossible.
Parasexual gene transfer from the parasite into one of its hosts, *Absidia glauca*, has been proven by complementation of host auxotrophic mutants following infection with prototrophic parasites. Transfer rates range between 10^{-2} and 10^{-4}. For several of these so-called para-recombinants, DNA analyses have recently been performed (1).
In order to find the putatively rare parasexual events in the parasite, induced by DNA coming from the host, we isolated a mutant of *P. parasitica*, auxotrophic for adenine. This mutant has a reddish phenotype, as expected for fungal mutants defective in either *ade1* or *ade2*. A single para-recombinant among 10^8 spores has been isolated. The transfer rate is thus 10^6 to 10^4 times lower than for the normal direction.
At the evolutionary scale, the reverse direction of gene transfer has recently been supported by the appearence of a rRNA repeat unit, strongly resembling the rDNA of the *A. glauca* (-) mating type in the genome of a (+) mating type strain of *P. parasitica*. This mating type attribution matches with the infection behaviour: (+) strains of *P. parasitica* exclusively infect (-) strains of *A. glauca*. Host/parasite combinations belonging to identical mating types never form infection structures and, consequently, never undergo fusion.
We also analysed the behaviour of mitochondria during infection. Until now, we can not provide evidence for transfer of complete organelles or defined parts of the chondriome in laboratory experiments, but by analysing the mtDNA sequences of *P. parasitica* (2) and *A. glauca* (unpublished), we see ample indications for sharing mobile mtDNA elements between the organisms.
The physiology of communication between host and parasite strongly resembles the sexual recognition principles, based precursors and derivatives of trisporic acid (3) and has recently been reviewed.

(1) Burmester *et al.* (2013) Microbiology 159, 1639-1648
(2) Ellenberger *et al.* (2014) Genome Announcements 2
(3) Wöstemeyer *et al.* (2016) The Mycota I 3rd ed, Chapter 10

FBV05

Redox regulation of hypoxic response in *Aspergillus fumigatus*

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The mould *Aspergillus fumigatus* can cause life-threatening invasive infections in immunocompromised patients. In the site of infection the fungus faces many environmental stresses such as a dramatic drop in oxygen concentration. The ability to tolerate severely hypoxic environments represents an important virulence trait of *A. fumigatus*. Currently, many reports suggest that adaptation to hypoxia may be regulated by elevated production of ROS. In this project we aim to gain more insights into the mechanisms of how *A. fumigatus* maintains redox homeostasis during exposure to low oxygen levels. In particular, we want to reveal proteins that are specifically modified and thus can be responsible for hypoxic adaptation. Our results confirmed increased amounts of intracellular reactive oxygen intermediates in *A. fumigatus* exposed to low oxygen levels. Moreover, by applying gel-free redox proteomic approach we could identify proteins, which get reversibly oxidized by ROS after shifting oxygen content in the culture from 20 % to 0.2 %. For instance, proteins with a putative role in cellular copper ion homeostasis, assembly of respiratory chain complex IV and oxidative stress regulation were modified in one hour of hypoxic cultivation. Further investigation of biological functions of reversibly oxidized proteins will help us to elucidate essential pathways involved in sensing hypoxia by *A. fumigatus*.

FBV06

Post-transcriptional regulation impacts on iron metabolism regulation in *Candida glabrata*

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Question: Iron is a trace element essential for many cellular processes but also harbors a toxic potential when present in excess. Due to this dual role and to prevent microbial growth of invading pathogens the host restricts iron availability during infection. Thus, successful pathogens such as the fungus *Candida glabrata* must have developed effective strategies to obtain iron to prevail in the host, which have to be tightly controlled. Although well characterized in the related non-pathogenic yeast *Saccharomyces cerevisiae* and the human pathogen *Candida albicans*, regulation of iron metabolism has not been studied in detail in *C. glabrata*. Interestingly, *S. cerevisiae* and *C. albicans* differ greatly in their iron regulation strategies. The aim of this project is to elaborate the regulation of iron homeostasis in *C. glabrata* and to identify potential unique strategies present in *C. glabrata*.
Methods: To identify potential iron regulators in *C. glabrata,* we searched for orthologs of genes relevant for iron metabolism in *S. cerevisiae* or *C. albicans.* Deletion mutants were constructed and growth assays under stress and iron limitation were carried out. Further, expression analyses

using qRT-PCR and microarrays were performed to elucidate the role of the selected genes in the regulation of iron metabolism and to identify their target genes.
Results: Our data suggest that Aft1 is the main iron regulator in *C. glabrata* inducing iron uptake (*FTR1*, *FET3*, *SIT1*) and iron recycling genes (*HMX1*) under iron limiting conditions. An *aft1*Δ mutant depicts a severe iron dependent growth defect and susceptibility against oxidative stress, high pH, high metal and cell membrane stress. Importantly, our data point to a post-transcriptional degradation of iron consumption pathway related gene products under iron limitation, such as mRNAs from *ACO1*, *CTA1* and *HEM15*, modulated by the potential RNA-binding protein Tis11. These results match with observations made in *S. cerevisiae*, but not in *C. albicans* [1,2]. In contrast, Sef1, the main iron uptake initiator in *C. albicans*, harbors no such function in *S. cerevisiae.* However, we observed a distinct iron- related role of *SEF1* in *C. glabrata,* related to glutamate biosynthesis, in particular the regulation of *ACO1*, *IDH1* and *IDH2*.
Conclusion: Taken together, these results indicate an iron-regulation system for *C. glabrata* closely resembling *S. cerevisiae* with Aft1 being the main regulator under iron starvation. This is to our knowledge the first description for a pathogenic species utilizing this particular setting of regulation. However, the involvement of *SEF1* indicates an evolutionary intermediate step to the *SEF1* dependent regulation system of the pathogen *C. albicans*. Thereby, *C. glabrata* has evolved a unique system for the regulation of iron metabolism.

[1] Puig *et al.* 2005. Cell, Jan. 14, Vol. 120, 99-110.
[2] Wells *et al.* 2015. Mol. Microbiol., 95(6), 1036-1053.

FBV07
A glimpse into the role of the fungal rhodopsins CarO and OpsA in *Fusarium fujikuroi*
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Question: In filamentous fungi green light is sensed by fungal rhodopsins, a family of membrane-standing photoreceptors, which exhibit a highly conserved, heptahelical structure. The central chromophore all-*trans*-retinal is covalently bound to the protein via a protonated Schiff-base. Depending on the class of fungal rhodopsins the conformational change of retinal from all-*trans* to *13-cis* upon light activation may either mediate proton-pumping or sensory function. While the research in bacterial rhodopsins has a long history, in contrast, the information about fungal rhodopsins is sparse and their biological function is almost unknown. In the present work we aim to gain more insights into the role of rhodopsins and their importance for fungi.
Methods: For our investigation we chose the rice pathogen *Fusarium fujikuroi*. This filamentous ascomycete contains two rhodopsin-encoding genes, *carO* (Prado *et al.*, 2004) and *opsA* (Estrada & Avalos, 2009). The *carO* gene is linked to and co-regulated with genes coding for enzymes for retinal synthesis, whose expression is strongly induced by light. Also the *opsA* gene is upregulated by light. We fused CarO and OpsA to fluorescent proteins and expressed the fusion constructs in *F. fujikuroi* mycelia (endogenous promoter) and mammalian cells (CMV-promoter) and analyzed them by confocal laser scanning microscopy (cLSM) and patch-clamp techniques, respectively (García-Martínez *et al.*, 2015). In addition, we analyzed the germination of rhodopsin-deficient mutant *F. fujikuroi* strains and performed rice plant infection experiment.
Results: We observed that in light-exposed mycelia CarO and OpsA are mainly expressed in conidia but to some extend also in growing hyphae. The rhodopsins are located in the cytoplasm membrane but also in the membranes of inner organelles. CarO is a very efficient proton pump and it becomes even more active when chloride in the extracellular solution is substituted by gluconate or acetate. In contrast, OpsA does not provide any net charge transfer under those conditions which is in accordance with observations in the related fungal rhodopsin nop-1 from *Neurospora crassa*. Software-assisted, video-microscopic analysis of conidia germination revealed that the conidia of the CarO-deficient strain germinate more slowly in light than the one from the CarO-expressing strain. In contrast, we did not observe such distinct phenotype in the OpsA strains.
Conclusion: In plant tissue green light is highly accessible and, in accordance, fungal rhodopsins are found in many phyto-associated fungi. Rhodopsins are involved in the regulation of the conidia germination and, by that, may also play a role in the regulation of the plant-fungus interaction. Besides that, as efficient proton-pump, CarO could also contribute to the maintenance of the trans-membrane proton-gradient.

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García-Martínez, J., Brunk, M., Avalos, J. & Terpitz, U. 2015. *Sci. Rep.* 5: 7798.
Prado, M.M., Prado-Cabrero, A., Fernández-Martín, R. & Avalos, J. 2004. *Curr. Genet.* 46(1): 47-58.

FBV08
Regulatory networks of the gibberellin cluster in *Fusarium fujikuroi*
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The filamentous fungus *Fusarium fujikuroi* is known to produce a variety of structurally diverse secondary metabolites such as the plant hormones gibberellins (GAs), pigments and mycotoxins. The GAs are a virulence factor of its producer and causer of the so-called 'Bakanae' (foolish seedling) disease but otherwise they are also commonly used in agri-, horti- and viticulture as plant growth regulators. Thus, the understanding of the regulation of the GAs is of great biotechnological interest.
During the past decade of years, we identified a complex regulation network for GA biosynthetic genes. They are repressed by high amounts of nitrogen and were the first secondary metabolites for which a strict dependency on the GATA-type transcription factor AreA was shown. Recently, we revealed that a second GATA-type transcription factor, AreB, is also essential for expression of the GA genes. Besides these two transcription factors the fungal-specific Velvet complex is also involved in the regulation of the GA cluster. Deletion of FfVel1 and the putative methyltransferase FfLae1 led to downregulation of the respective biosynthetic genes while overexpression of FfLae1 resulted in significant upregulation. Furthermore chromatin modification, e.g. deletion of the histone deacetylase FfHda1, strongly affects GA gene expression and production. In addition, the active histone mark H3K9Ac (acetylation of lysine 9 at histone 3) at the GA cluster is enriched under nitrogen-limiting conditions and this is consistent with gene expression of this cluster. This study will give an overview of the complex regulation of the GA cluster.

FBV09
Coordinated process in polarized growth of the filamentous fungus *Aspergillus nidulans*
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The highly polarized growth of filamentous fungi requires a continuous supply of proteins and lipids to the hyphal tip. This transport is managed by vesicle trafficking via the actin and microtubule cytoskeletons and their associated motor proteins. Particularly, actin cables originating from the hyphal tip are essential for hyphal growth. Although specific marker proteins to visualize actin cables have been developed in filamentous fungi, the exact organization and dynamics of actin cables has remained elusive. Here we visualized actin cables using tropomyosin (TpmA) and Lifeact fused to fluorescent proteins in *Aspergillus nidulans* and studied the dynamics and regulation. Comparison of actin markers revealed that high concentrations of Lifeact reduced the actin dynamics. Simultaneous visualization of actin cables and microtubules suggests temporally and spatially coordinated polymerization and depolymerization between the two cytoskeletons. In addition, Ca^{2+} gradient oscillation was visualized at hyphal tips using the Ca^{2+} sensor, cameleon. The frequency of this oscillation correlated with that of actin cable disassembly and microtubules reaching hyphal tips. Our results provide new insights into the molecular mechanism of ordered polarized growth regulated by actin cables and microtubules.

FBV10
Role of the autophagy-related gene *Smatg12* in fruiting-body development of the filamentous ascomycete *Sordaria macrospora*
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In filamentous fungi, autophagy functions as a catabolic mechanism to overcome starvation conditions and to control diverse developmental processes under normal nutritional conditions. Autophagy involves the formation of double-membrane autophagosomes engulfing cellular

components for the degradation in the vacuole. Two ubiquitin-like conjugation systems are essential for the expansion of the autophagosomal membrane. We recently showed that in the homothallic ascomycete *Sordaria macrospora*, autophagy related genes encoding components of the conjugation systems are required for fruiting body development or are essential for viability. Here, we cloned and characterized the *S. macrospora (Sm)atg12* gene, encoding a ubiquitin-fold protein. To examine its role in detail, we replaced it with a hygromycin resistance cassette and generated a homokaryotic ΔSmatg12 strain, which displayed a decreased vegetative growth rate and was unable to form fruiting bodies. GFP-labeled SmATG12 was detected as punctured structures and co-localized with DsRED-SmATG8 at some places in the cytoplasm. We also showed that lipidation of SmATG8 and proper autophagosome formation depends on SmATG12.

FBV11

In-depth characterization of the *Aspergillus fumigatus* mating-type system

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Sexual reproduction of the human fungal pathogen *Aspergillus fumigatus* was assumed to be absent or cryptic until fertile crosses among geographically restricted environmental isolates were described in 2008 by O'Gorman *et al.* The existence of cryptic sexuality in this species had been proposed before, based on genomic and genetic analyses revealing the presence of mating-type idiomorphs (*MAT1-1* and *MAT1-2*) and of several putative genes orthologous to recognized determinants of pheromone signalling, mating, karyogamy, meiosis, or fruiting body formation in the fertile species *Aspergillus nidulans*. Furthermore, the products of *A. fumigatus MAT1-1* and *MAT1-2* genes were shown to be functional in *A. nidulans*. We provide evidence for mating, fruiting body development, and ascosporogenesis accompanied by genetic recombination between unrelated clinical isolates of *A. fumigatus*, which reveals the generality and reproducibility of this long-time undisclosed phase in the lifecycle of this heterothallic fungus. Furthermore, we demonstrate that successful mating requires the presence of both mating-type idiomorphs *MAT1-1* and *MAT1-2*, as does expression of genes encoding factors presumably involved in this process. Comprehensive transcriptional profiling studies reveal the depth of the *MAT1*-driven transcriptomes. Functional categorization of genes that are significantly up- or down-regulated in these transcriptomes led us to further investigation of candidate genes and gene clusters that are under control of the bipolar mating-type system, especially those involved in secondary metabolism, which are ideal for validation on the product level. Secondary metabolite profiling of recombinant strains that are deregulated or mis-regulated in sexual development confirms the association. Furthermore, functional analysis of a novel presumed mating-type gene *MAT1-2-4* associated with the *MAT1-2* idiomorph indicates its necessity for fruiting body formation, assigning the corresponding gene product a functional role in the mating process. With the help of yeast two-hybrid screening we were able to identify an interactor with the product of *MAT1-2-4*, and further investigation of this genuine interaction is ongoing.

FBV12

Impact of light on differentiation and virulence of the plant pathogen *Botrytis cinerea*

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Botrytis cinerea has a worldwide distribution and is the causal agent of gray mold diseases in more than 200 plants species including high-value crops such as grape vine and strawberry. The fungus may reproduce asexually by forming macroconidia for dispersal (summer cycle) and sclerotia for survival (winter cycle); the latter also participate in sexual reproduction by bearing the apothecia. Light induces the differentiation of conidia and apothecia, while sclerotia are exclusively formed in the absence of light. Field populations are highly diverse with regard to different phenotypic traits and can be divided into two groups: strains that undergo photomorphogenesis ("*light-responsive*") and "*blind*" strains that produce either sterile mycelia ("*fluffy*") or the same reproductive structures under different light conditions ("*always conidia", "always sclerotia")*. However, the inability to form certain reproductive structures such as conidia as the major source of inoculum or sclerotia as prerequisite for sexual recombination is expected to decrease the overall fitness of the pathogen, and thus, it is questionable why "*blind*" strains are found in the field. In fact, the "*blind*" phenotype may accompanied by reduced virulence, as shown for the "*always conidia*" phenotype of strains T4 and 1750 that is caused by single nucleotide polymorphisms (SNPs) in the VELVET gene *bcvel1*. The identification of *bcltf1* (Light-responsive Transcription Factor 1) and *bckdm1* (Histone Demethylase 1) as new virulence-associated genes by random mutagenesis, further supports our hypothesis of an interrelationship between light signaling and virulence.

FBV13

Why some like it on the rocks – recurring stresses select for organisms with manifold protective pigments

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Microcolonial fungi (MCF) are the most stress-resistant eukaryotes known to date. These ascomycetes are able to colonize bare rock surfaces and have evolved mechanisms to cope with multiple stresses like high solar irradiation, temperature extremes, low water activity and spare nutrient availability. They are ubiquitous in terrestrial ecosystems like weathered rocks and material surfaces. Black fungi are an interesting object to study mechanisms of stress resistance and to perform applied research to prevent material colonization and biodeterioration.
Pigments, like melanin and carotenoids, have been proven to contribute to the unique robustness of MFCs. We study how these pigments ameliorate oxidative stress responses - one of the most significant environmental challenges encountered by MCF - using the model rock fungus *Knufia petricola* (Chaetothyriales) strain A95. This non-pathogenic fungus possesses all characteristic features of MCF, including meristematic growth, melanised cell-walls and extensive secondary metabolite production.
The melanin knockout mutants ΔSDH and ΔPKS were compared concerning physiology, stress resistance to H_2O_2 and UV radiation, TEM of the cell wall and analysis of the extracellular polysaccharides (EPS), to elucidate mechanisms of cell wall maturation. Deletion of the polyketide synthase type I in *K. petricola* (ΔPKS) leads to a complete loss of melanin, showing the carotenoids which are normally hidden beneath the melanin. Colonies of scytalone dehydratase mutants (ΔSDH) appear darker than ΔPKS strains because of the incomplete disruption of the melanin synthesis pathway.
In comparison to the wild type strain, treatment with the oxidative agent H_2O_2 (up to 30 mM) did not show any dose-dependent growth inhibition in any melanin mutant strain. Analysis of the extracellular polysaccharide content showed an increased development of EPS in the melanin mutants which is also proven by TEM.

FBV14

Identification of novel factors involved in dimorphism and pathogenicity of *Zymoseptoria tritici*

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Introduction/objective: *Zymoseptoria tritici* (teleomorph: *Mycosphaerella graminicola,* syn. *Septoria tritici*) is the causal agent of septoria leaf blotch disease of wheat. Septoria wheat diseases have increased in incidence over the last few decades, which may be seen as a result of an increased emergence of strains evolving fungicide resistance. Especially *Z. tritici* has been referred to as the most destructive foliar pathogen of wheat in Europe. *Z. tritici* is considered to be a dimorphic plant pathogen displaying environmentally regulated morphogenetic transition between yeast-like and hyphal growth. Considering the infection mode of *Z. tritici*, the switching to hyphal growth is essential for pathogenicity allowing the fungus the host invasion through natural openings like stomata. In order to understand the molecular mechanism behind dimorphic transition and thus to identify novel pathogenicity associated genes, a forward genetics approach was applied.
Methods and results: *Agrobacterium tumefaciens*-mediated transformation (ATMT) method was used in order to generate a mutant

library by insertional mutagenesis including more than 8000 random mutants, which were screened for the ability/deficiency to perform a dimorphic switch upon nutrient-poor environment. In our screening 10 dimorphic switch deficient random mutants could be identified, 9 of which exhibited a yeast-like mode of growth and one mutant predominantly growing filamentously, producing a high amount of mycelium under different incubation conditions. Within this study four insertion mutants could be characterized in detail. Three of them were non-pathogenic and one mutant exhibited a drastically impaired virulence within infection assays on whole host plants. Moreover further phenotypical investigations on macroscopic and microscopic level revealed different pleiotropic effects. Using the genome walking approach the T-DNA integration sites could be recovered and the disrupted genomic loci of corresponding mutants were identified and validated within reverse genetics experiments. Finally a transcriptome analysis by RNA-Seq was performed in order to examine the transcriptional reprogramming during dimorphic switch.
Conclusion: With this study we present four novel and functionally characterized genes, which are involved in the dimorphic switch of the fungal plant pathogen *Z. tritici*. Exploring transcriptome data with respect to differences in gene expression under switch-inducing condition unveils new insights to precisely analyze the dimorphism related processes in *Z. tritici.*

FBV15
Environmental decisions in early steps of fruiting of *Coprinopsis cinerea*
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Coprinopsis cinerea is a model fungus to study developmental processes in the Agaricomycetes. We use agar and horse dung cultures of the fungus to study the cellular processes of mushroom development with microscope techniques.

Fruiting body morphogenesis of *C. cinerea* begins in dikaryotic cultures with simple hyphal growth and localized intense hyphal branching within freshly grown mycelium leading to hyphal aggregation. A knot-like structure, the primary hyphal knot is formed as a first step in the fruiting body developmental process. Under dark condition, the leading hyphae branch and sub-branch to give a bush-like structure that in further growth may incorporate neighboring lateral hyphae. The size of a primary knot measures approx. 20 µm. When kept in dark for longer time, primary hyphal knots transform into dark brown melanized resting structures called sclerotia. These are oval or globular, symmetrical, multi-cellular resting bodies of about 0.1 to 0.2 mm in Ø. Sclerotia have been noticed in cultures of mono- and dikaryons within aerial and submerged mycelia. They can be categorized on the basis of color. The young sclerotia look white. With maturity they become light brown and eventually dark brown. A mature sclerotium comprises of an outer hyphal layer named rind and an inner medulla. The rind is built by compact cells with usually brown stained cell walls. The medullary part is filled by large non-stained round to oval cells with thick cell walls that are tightly wrapped by swollen hyphae. The thick-walled cells resemble chlamydospores which normally appear in the submerged mycelium of aging cultures. Under sufficient illumination, primary hyphal knots in dikaryotic cultures cluster together within the hyphal lattice to form a three-dimensional secondary hyphal knot (0.2 - 0.3 mm) which is the second step in the process of fruiting body development and the first step that is specific to the fruiting body. Subsequently under light restriction, it elongates abnormally and grows into a long slender elongated stipe-like structure with a poorly developed cap. However, with correct amount of light and nutrient conditions in a 12 h light / 12 h dark rhythm, a secondary hyphal knot grows to a young primordium that eventually can develop into a mature mushroom. Illumination further induces asexual sporulation (oidia formation) in the dikaryon on young hyphae in contrast to monokaryons that constitutively produce masses of oidia in their freshly grown mycelium.

FBV16
Towards making *Agrocybe aegerita* a modern model basidiomycete for mushroom formation
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Introduction: Model basidiomycete-based fundamental research in the field of edible mushroom production aims at raising mushroom yield and quality by optimising strains and breeding strategies [1]. Currently, mushroom formation-related aspects can only be studied in depth with a few well-established model mushrooms, all having a number of major individual upsides but also certain considerable downsides [2].
Question: Hence, the commercially cultivated edible mushroom *Agrocybe aegerita* is suggested to become a modern model basidiomycete mostly combining the individual advantages from the present models.
Methods: A short 'text-book' basidiomycete life cycle involving dikaryotic and monokaryotic fruiting as well as asexual sporulation on agar media plus a good accessibility for molecular genetics were considered as must-haves of suitable standard strains for the future model mushroom.
Results: According to these criteria, a set of standard strains was selected for establishing *A. aegerita* as a model basidiomycete suitable to investigate mushroom formation by molecular genetics. This set is comprised of a parental dikaryon, *A. aegerita* AAE-3, and a set of standard monokaryons, generated from single basidiospores harvested from AAE-3 fruiting bodies. Each of these monokaryons exhibits one of the known degrees of monokaryotic fruiting and each of them is mating compatible with at least one sibling monokaryon. With the whole-genome of the parental dikaryon AAE-3 sequenced and *in silico* annotated, we were able to design a first tool to enable molecular genetics with *A. aegerita*. In the course of this, we have also been able to successfully establish an *A. aegerita* transformation protocol, using protoplasts originating from asexual monokaryotic spores. As a result, we were able to artificially express a dominant selection marker in one of the standard monokaryons derived from *A. aegerita* AAE-3.
Conclusions: Making use of the selected set of standard strains, the genome sequence of the strain *A. aegerita* AAE-3, our *A. aegerita* transformation protocol and our first *A. aegerita* molecular genetics tools, the prerequisites are met for the establishment of *A. aegerita* as a modern model mushroom.

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IBV01
CRASP11 recruits soluble human complement regulators and mediates complement evasion of *Candida albicans*
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Question: *Candida albicans* is the most important fungal pathogen threatening human health. The fungus blocks and evades the destructive actions of the human innate immune system and in particular attack by the complement system. To this end, *C. albicans* expresses several CRASPs (complement regulator-acquiring surface proteins) which recruit human complement regulators to the fungal surface. As complement and innate immune evasion of *C. albicans* is yet not fully understood, my project aims to identify and isolate new complement evasion proteins of *C. albicans*. Here I describe CRASP11 as a new fungal protein that binds the complement regulator Factor H.
Methods: Recombinant protein expression (*Escherichia coli*), ELISA, Western Blot, Flow cytometry, Immunofluorescence, Cell culture (primary cells)

Results: CRASP11 was cloned, recombinantly expressed in *E. coli* and purified. Binding of recombinant CRASP11 to the human plasma proteins and complement regulators, Factor H, Factor H-like protein 1 (FHL-1), Factor H related protein 1 (CFHR1), C4 binding protein (C4BP) and plasminogen was shown by ELISA. Each of these human complement regulators bound to candida CRASP11 in a dose dependent manner.
CRASP11-bound plasminogen was activated by uPA and newly generated plasmin cleaved a synthetic substrate, as well as natural substrates: C3b, fibrinogen and vitronectin.
CRASP11-bound Factor H retained cofactor activity for complement Factor I mediated cleavage of C3b.
CRASP11 was localized on the surface of *C. albicans* using a new specific antiserum. Specificity of this antiserum was confirmed as it did not detect the fungal protein on the surface of a candida CRASP11 knock-out strain.
This CRASP11 knock-out strain recruited the soluble human complement regulators Factor H and plasminogen with lower intensity. However, binding of Factor H and plasminogen was restored in the corresponding *C. albicans* CRASP11-revertant strain.
The CRASP11 knock-out strain, when challenged with complement active human serum had more complement C3b deposited on its surface, as compared to the wild-type and the CRASP11-revertant strain. The enhanced opsonization of the knock-out strain correlated with the increased phagocytosis by human neutrophil granulocytes.
Conclusion: Taken together, CRASP11 is a novel candida immune evasion protein that binds four soluble human complement regulators. CRASP11 contributes to complement evasion of *C. albicans* as the CRASP11 knock-out strain was more susceptible for complement damage. CRASP11 recruits human complement regulators to the surface of *C. albicans* and these regulators block activation of the human complement system. Thus, CRASP11 limits C3b-deposition on the cell surface (opsonization) and also phagocytosis of the fungal cells.
Thereby, CRASP11 is a new fungal immune evasion protein that protects *C. albicans* from host innate immune attack at several levels.

IBV02
Candida albicans modulates the immune response of human blood monocytes
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The human pathogenic yeast *Candida albicans* (*C. albicans*) is a dimorphic fungus, which can cause superficial as well as systemic infections. *C. albicans* inhibits host innate immune reactions by recruiting human complement inhibitors, such as factor H or FHL-1 to the surface. Thereby the host regulators reduce complement opsonisation of the fungus by dissociating the C3 convertase and inhibiting the subsequent recruitment of immune cells. Whether factor H on the *Candida* surface also influences the response of immune cells is still unknown. Upon incubation in normal complement active human serum *C. albicans* is immediately opsonized with human complement protein C3b and induces a strong inflammatory response in monocytes as measured by the induction of IL-1b and IL-6, but not IL-10. However, plasma purified factor H bound to the surface of *C. albicans* prior to incubation with the monocytes substantially inhibited the pro-inflammatory response of monocytes. This inhibitory function of factor H on monocytes was confirmed when factor H bound to apoptotic human cells had the same effect and did not induce inflammation. Moreover factor H recruited via Pra1 to microbeads also inhibited IL-1b and IL-6 and clearly induced the anti-inflammatory cytokine IL-10. This regulatory activity of factor H on the surfaces was dependent on active human serum, indicating that not factor H alone is mediating the inhibitory effect. Indeed, when C3b was transformed by factor H together with factor I to iC3b, the same inhibitory effect on monocytes was observed. Altogether the results demonstrate that factor H is recruited via Pra1 to the *Candida* surface to generate iC3b, which by itself acts anti-inflammatory. This new function of factor H mediated iC3b explains why *C. albicans* and many other pathogens recruit factor H to their surfaces. Notably the new identified iC3b function is relevant in many human diseases like atypical hemolytic uremic syndrome or age related macular degeneration, which are characterized by reduced factor H functions on self surfaces due to genetic mutations.

IBV03
The killing of macrophages by _Corynebacterium ulcerans_
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Corynebacterium ulcerans is a pathogenic species of the genus *Corynebacterium* and closely related to the human pathogen *Corynebacterium diphtheriae* and the animal pathogen *Corynebacterium pseudotuberculosis. C. ulcerans* is mainly associated with mastitis in cattle, non-human primates and other animals and is also known as commensal in various domestic and wild animals. However, during the last decade, human infections associated with *C. ulcerans* appear to be increasing in various countries and can most often be ascribed to zoonotic transmission. Toxigenic *C. ulcerans* harbor lysogenic β-corynephages coding for diphtheria toxin and can cause respiratory diphtheria-like illness. However, also non-toxigenic strains can cause disease symptoms in humans, indicating that there are additional factors contributing to virulence of this bacterium.
Despite rising numbers of infections and potentially fatal outcomes, data on the molecular basis of pathogenicity are scarce. In this study, *C. ulcerans* 809, isolated from a fatal case of human infection and BR-AD22, isolated from an asymptomatic dog, were characterized in respect to their interaction with human macrophages as important part of the innate immune system in order to get deeper insight into the pathogenicity of this emerging pathogen. THP-1 cells were infected with *C. ulcerans* and the uptake and intracellular survival of the bacteria was analyzed by counting colony forming units. Furthermore, Lysotracker staining and fluorescence microscopy was carried out to investigate co-localization of bacteria with acidic compartments. The reaction of the host cell to the bacterial infection was investigated measuring cytokine levels by ELISA and NF-κB activation with the reporter cell line THP1-Blue™ NF-κB. Through 7-AAD staining and FACS analysis as well as LDH release measurement, detrimental effects of *C. ulcerans* on host cells were analyzed.
C. ulcerans strains were able to multiply within macrophages at least 8 hours post-infection and survive at least 20 hours. Uptake of the bacteria leads to delay of phagolysosome maturation which might be an effective mechanism for immune evasion and spreading of *C. ulcerans* within the body supporting the establishment and progress of infections. Upon bacterial infection, the host cells produce high amounts of IL-6 and G-CSF and NF-κB is activated. Furthermore, *C. ulcerans* induces a lytic cell death in THP-1 macrophages as deduced from cytotoxicity measurements and FACS analyses.
In summary, the data presented here indicate a high infectious potential of this emerging pathogen.

IBV04
Identification of biomarkers for invasive aspergillosis in the urine
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Question: *Aspergillus fumigatus* is one of the major causes of fungal lung infections for patients with immune suppressive treatment after organ or stem cell transplantations and with leukocyte deficiencies. Invasive aspergillosis (IA) is associated with high mortality despite antimycotic treatment. While early diagnosis is regarded as critical for increasing the chances of survival, the unspecific symptoms of fungal-mediated pneumonia, the presence of fungal DNA and anti-fungal antibodies due to environmental exposition and insufficient sensitivity of systems detecting fungal markers in blood or bronchial lavage fluid make early diagnosis difficult. We propose that fungal antigens secreted in patient urine could serve as a promising target for diagnostic screening, allowing non-invasive diagnosis of IA.
Methods: As human cases of proven IA are rare and IA is commonly diagnosed at late stages only, we chose to use murine models for the identification of fungal biomarkers in urine. We established mouse models mimicking either neutropenic patients (e.g. undergoing stem cell transplantation) or patients under corticosteroid treatment. Immunocompetent mice, which do not develop IA upon exposure, serve as

controls, allowing the identification of infection-specific fungal antigens. To identify fungal biomarkers in urine we used two approaches: (1) liquid mass spectrometry (LC-MS) and (2) ELISA/western blotting.
Results: We have developed methods to sample urine from exposed and infected animals throughout the course of infection. In combination with *in vivo* imaging of bioluminescent fungi, this set up allows collection of samples from defined stages of infection. Sample preparation has been successfully optimized to allow detection of fungal proteins in murine urine by both LC-MS and by ELISA / western blotting.
Conclusion: We have successfully established a workflow for the analysis of mouse urine. Experiments using urine from infected animals are currently under way to determine which fungal antigens are secreted in urine and whether they might provide suitable targets for novel diagnostic tools.

IBV05
The extracellular adherence protein (Eap) of *Staphylococcus aureus* affects proliferation and migration of eukaryotic cells by altering the adhesive and morphological properties of the host cell
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The clinically important human pathogen *Staphyloccocus aureus* is equipped with a large arsenal of virulence factors promoting/facilitating attachment to various host cell structures, and to modulate the immune response of the host. One of these factors is the extracellular adherence protein Eap, a member of the "secretable expanded repertoire adhesive molecules" (SERAM) protein family that possess adhesive and immune modulatory properties. We have previously shown that Eap impairs wound healing by interfering with host defense and repair mechanisms, and that this secreted protein abates cell proliferation and migration of keratinocytes and endothelial cells.
Here we report that Eap affects the proliferation and migration capacities of eukaryotic cells by altering the cell morphology and adhesive properties. By challenging non-confluent HaCaT cell cultures with Eap, a profound reduction in cell proliferation and migration was observed, accompanied with alterations in cell morphology. Eap-treated HaCaT cells changed their appearance from an oblong to trapezoid form with normally three to five distinct filopodia to a fried-egg like morphology with numerous lamellipodia like excrescences but lacking clear filopodial structures. These changes in morphology were accompanied by decreases in cell volume and cell stiffness. The Eap-challenge, on the other hand, significantly increased the adhesive properties of the cell towards its substratum. Similar effects were observed with endothelial and cancer cells, respectively, indicating that these Eap-induced effects are likely to occur with a broad range of eukaryotic cell types. Our data suggest that Eap might interfere with host cell migration and proliferation by impairing filopodia protrusion and by enhancing the cell-substratum adhesion.

IBV06
Quantitative proteomics reveals the dynamics of protein phosphorylation in human bronchial epithelial cells during internalization, phagosomal escape and intracellular replication of *Staphylococcus aureus*
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Internalization of *Staphylococcus aureus* by non-professional phagocytic cells is a major suspected cause of persistent and difficult-to-treat infections including pneumonia. In this study, we established an infection model with 16HBE14o- human bronchial epithelial cells and demonstrate internalization, escape from phagosomal clearance and intracellular replication of *S. aureus* HG001 within the first four hours post infection. We use quantitative phosphoproteomics to identify characteristic signaling networks in the host at different stages of the infection. Whereas we found only minor changes in the abundance of proteins, the infection was accompanied by highly dynamic alterations in phosphorylation events primarily in proteins that are associated with pathways of cytoskeleton dynamics, cell-cell and cell-matrix contacts, vesicle trafficking, autophagy as well as GTPase signaling. Analyses of host protein kinases by kinase-substrate mapping, immunoblotting of activity-regulatory sites and prediction algorithms highlight known and novel host kinases with putative critical roles in *S. aureus* infection-accompanied signaling including FAK, PKA, PKC and CDK. Targeted pharmacological inhibition of these kinases resulted in a significant reduction of intracellular *S. aureus* cells. The current study constitutes a valuable resource for a better understanding of the infection-relevant molecular pathomechanisms of airway cells and for the development of novel host-centric anti-infective strategies in *S. aureus* infections.

IBV07
The cystic fibrosis lower airways microbial metagenome
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Chronic airway infections determine most morbidity in people with cystic fibrosis (CF). Here we present unbiased quantitative data about the frequency and abundance of DNA viruses, archaea, bacteria, molds and fungi in CF lower airways. Induced sputa were collected on several occasions from children, adolescents and adults with CF. Deep sputum metagenome sequencing identified on average about ten DNA viruses or fungi and several hundred bacterial taxa. The metagenome of a CF patient was typically found to be made up of an individual signature of multiple lowly abundant species superimposed by few disease-associated pathogens such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* as major components. Anaerobes are characteristic inhabitants of the upper and lower airways of healthy non-CF humans. In our cohort of CF patients the proportion of anaerobes in the microbial metagenome decreased with age. The viral community consisted primarily of phages, a few human pathogens, primarily herpes virus and adenovirus, and rare cases of viruses infecting non-mammalian eukaryotic hosts. Dominant species in the mycobiome were Aspergillus species and Saccharomycetes including Candida sp. consistent with current knowledge of CF mycology. Whole metagenome analysis resolved the microbial signature of the individual patient. The spectrum ranged from a normal flora via an intermediate stage when the normal community is perturbed by *Haemophilus influenzae* or *S. aureus* to a final stage of a low-diversity community dominated by *P. aeruginosa*. This shift from a normal highly diverse metagenome indistinguishable from that of a healthy subject to the CF-typical end-stage of an almost pure culture of *P. aeruginosa* was correlated in our patient cohort with disease severity, but not with age. Thanks to the high accuracy of sequencing-by-ligation in the color space of 99.94 % we could resolve the clonal diversity of *S. aureus* and *P. aeruginosa* in CF airways metagenomes. The *S. aureus* and *P. aeruginosa* populations were composed of one major and numerous minor clone types. The rare clones constitute a low copy genetic resource which could rapidly expand as a response to habitat alterations such as antimicrobial chemotherapy or invasion of novel microbes. Results were obtained with a novel in-house software pipeline of filtration and normalization techniques necessary for accurate quantification of microbial organisms in metagenome samples.

IBV08
Clinical *Streptococcus pneumoniae* isolates from patients with pneumococcal hemolytic uremic syndrome efficiently control host innate immune attack
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Question: *Streptococcus pneumoniae* is an important human respiratory pathogen that is also associated with thrombotic microangiopathy and hemolytic uremic syndrome (HUS). In this study we characterized two clinical *S. pneumoniae* isolates derived from young patients with pneumococcal HUS. In order to elucidate how these bacteria overcome the human complement attack and contribute to pathogenesis we characterized their complement and innate immune evasion in detail.

Methods: ELISA, Flow cytometry, SDS-PAGE/Western Blot, Cytotoxicity assay
Results: The two clinical pneumococcal strains bound several complement regulators from human serum, including Factor H, FHL1, CFHR1 and plasminogen. Both clinical isolates recruited the human complement regulator Factor H efficiently to their surface and bound Factor H more prominent as the virulent reference strain D39. Factor H bound to the pneumococcal surface retained its complement inhibitory activity and contributed to enhanced complement resistance of the two clinical strains. When challenged with complement active human serum, less C3b was deposited on the surface of the clinical isolates as compared to the reference strain D39. In addition also degradation and inactivation of C3b to iC3b was more efficient. Thus, the clinical isolates efficiently control complement by exploiting the human plasma regulator Factor H at their surface.
Expression levels of the pneumococcal Factor H binding proteins PspC and Tuf were analyzed. PspC surface levels of the clinical strains were two times higher than in D39 explaining the efficient Factor H recruitment. A new PspC variant for clinical isolate B and a rare PspC variant for isolate A were revealed by sequence analysis.
In addition, plasminogen which was bound to the surface of the clinical isolates was still converted to the active protease plasmin and cleaved the synthetic substrate S-2251 and the natural substrates Fibrinogen and C3b. Thus, plasminogen binding supports pneumococcal immune evasion and in addition plasmin reduces the viability and surface attachment of human endothelial cells.
Conclusion: By recruiting high amounts of Factor H to their surface the two clinical *S. pneumoniae* isolates effectively control complement activation as they enhance C3b inactivation and reduce opsonization. Thereby, the pathogenic bacteria are more resistant to host complement attack. Furthermore, the clinical isolates exploit the human protease plasmin to inhibit complement activity and also to damage human endothelial cells. Concluding from these results, we hypothesize that plasmin mediated damage of human endothelial cells promotes cell retraction and exposure of the underlying extracellular matrix, thereby generating a prothrombotic environment that favors coagulation, leading to thrombus formation, thrombocytopenia, hemolytic anemia and ultimately to renal injury, the key features of HUS.

IBV09
The biochemical RNA landscape of a cell revealed by Grad-seq
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High-throughput sequencing can rapidly profile the expression of theoretically all RNA molecules in any given organism but the primary sequence of these transcripts is a poor predictor of cellular function. This has been particularly evident for the regulatory small RNAs of bacteria which dramatically vary in length and sequence within and between organisms.
Here, we establish a new method (gradient profiling by sequencing; Grad-seq) to partition the full ensemble of cellular RNAs based on their biochemical behavior. Our approach enabled us to draw an RNA landscape of the model pathogen Salmonella Typhimurium, identifying clusters functionally related noncoding RNAs irrespective of their primary sequence. The map revealed a previously unnoticed class of transcripts that commonly interact with the osmoregulatory protein ProQ in Salmonella enterica. We show that ProQ is a conserved abundant global RNA-binding protein with a wide range of targets, including a new class of ProQ-associated small RNAs that are highly structured, and mRNAs from many cellular pathways. By its ability to describe a functional RNA landscape based on expressed cellular transcripts irrespective of their primary sequence, our generic gradient profiling approach promises to aid the discovery of major functional RNA classes and RNA-binding proteins in many organisms.

IBV10
The RNA chaperone Hfq mediates post-transcriptional regulation of adhesins in the enteropathogen *Yersinia enterocolitica*
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Question: Bacterial infections are initiated by molecular interactions between host cells and bacterial surface molecules, named adhesins. The proper deployment of adhesins, i.e. their expression or repression under specific cues, is crucial for colonization and survival within the host. In *Enterobacteriaceae*, the RNA chaperone Hfq mediates the interaction of many small non-coding RNAs (sRNA) with their target mRNAs, thereby modulating transcript stability and translation. This post-transcriptional control is believed to help bacteria adapt quickly to changing environmental conditions. We previously demonstrated that Hfq impacts growth, metabolism as well as stress resistance of the gastrointestinal pathogen *Y. enterocolitica* [1]. Moreover our proteomic analysis revealed that Hfq inhibits the production several outer membrane proteins, including OmpX which is 56 % similar to the adhesin Ail [1]. In this study we investigated whether Hfq and the conserved sRNA CyaR mediate post-transcriptional repression of OmpX. Moreover, we assessed the role of Hfq in the expression of surface pathogenicity factors such as the non-fimbrial adhesins Ail, YadA and InvA and the Myf fimbriae.
Methods: Immunoblotting and translational fusions with GFP were used to monitor protein production and gene expression. Bacterial fluorescence was measured by flow cytometry. Translocation of a type III secretion effector into mouse splenocytes was measured using a reporter fusion with beta-lactamase.
Results: Using immunoblotting and bacterial strains carrying translational fusions with GFP we demonstrated that Hfq inhibited the production of Ail, the Ail-like protein OmpX and the MyfA pilin post-transcriptionally, whereas it promoted production of the two major autotransporter adhesins YadA and InvA. Upon overexpression of the sRNA CyaR, expression of *ompX* was inhibited in a wild-type but not in an *hfq*-negative strain, suggesting that Hfq potentiates the CyaR-dependent post-transcriptional repression of *ompX* expression. While protein secretion *in vitro* was not affected, *hfq* mutants exhibited decreased protein translocation by the type III secretion system into host cells, consistent with decreased production of YadA and InvA. The influence of Hfq on YadA resulted from a complex interplay of transcriptional, post-transcriptional and likely post-translational effects. Finally, Hfq regulated *invA* by modulating the expression of the transcriptional regulators *rovA*, *phoP* and *ompR*.
Conclusion: Taken together, our results suggest that Hfq is a global coordinator of bacterial surface virulence determinants in *Y. enterocolitica*. Therefore, this conserved protein constitutes an attractive target for developing new antimicrobial strategies.

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IBV11
tRNA modifications – a novel virulence factor in pathogenic *Candida* species
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The human fungal pathogens *Candida albicans* and *Candida dubliniensis* are closely related sister species, but strongly differ in their clinical prevalence and their virulence. Focusing on genetic differences, we aimed to identify factors that contribute to their specific virulence profiles. These factors were then analyzed in *in vitro* and *in vivo* settings to answer the question: Which genes are important for species-specific pathogenicity-related traits?

To identify novel virulence factors of *C. albicans,* a library of *C. albicans* DNA fragments integrated into the *C. dubliniensis* genome [1] was screened under various conditions. Under nitrogen deprivation, integration of *C. albicans HMA1* significantly altered the growth phenotype of *C. dubliniensis*.

For detailed phenotypic characterizations, *HMA1* orthologs were deleted in *C. albicans* and *C. dubliniensis*. These included *in vitro* tests under stress and morphology-related conditions and *ex vivo* assays to determine adherence to and invasion of oral epithelial cells. Virulence was determined by infection of embryonated chicken eggs. To elucidate the molecular function of Hma1, tRNA was measured using quantitative liquid chromatography mass spectrometry analysis.

Hma1 (**H**yper**m**odified **A**denine) was identified as a novel determinant of fungal growth morphology: Genomic integration of *C. albicans HMA1* into *C. dubliniensis* inhibited *C. dubliniensis*-specific pseudohyphae and chlamydospore formation on SLAD agar and phenocopied the yeast-only morphology of *C. albicans* on this medium. This nitrogen-starvation induced phenotype was linked to nutrient-controlled pathways, as a *C. albicans hma1*Δ mutant displayed higher resistance against the TOR pathway antagonists, rapamycin and caffeine. The *C. albicans hma1*Δ mutant also exhibited diminished hyphal length, a phenotype that was not observed for *C. dubliniensis* Cd*hma1*Δ mutants. Moreover, *C. albicans hma1*Δ formed shorter hyphae on oral epithelial cells with concordantly decreased invasion. These virulence-associated attributes of *C. albicans hma1*Δ were confirmed by significantly lower mortality after infection of embryonated chicken eggs. Hma1 encodes a tRNA threonylcarbamoyladenosine dehydratase, and a dramatic reduction in ct^6A_{37} modification of tRNA was detected in *hma1*Δ mutants.

The tRNA modifying enzyme Hma1 is a novel contributor to morphogenesis and virulence of the human fungal pathogen *C. albicans*. We did not find a similar role for CdHma1 in *C. dubliniensis*, indicating that the gene could play a role in *C. albicans*-specific pathobiology. To our knowledge, this is the first time that a tRNA modifying enzyme has been shown to support virulence in a fungus. These results help to expand our understanding of pathogenicity mechanisms and their regulation beyond transcriptional networks.

1 Staib and Morschhäuser, *Differential expression of the NRG1[…]* Mol Microbiol, 2005.

IBV12

The extracellular adherence protein (Eap) of *Staphylococcus aureus* exhibits DNase activity

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The clinically important human pathogen *Staphyloccocus aureus* is equipped with a number of virulence factors allowing it to modulate or circumvent the immune responses of the host. One of these factors is the extracellular adherence protein Eap, a member of the "secretable expanded repertoire adhesive molecules" (SERAM) protein family that possesses adhesive and immune modulatory properties. We and others have previously shown that Eap interferes with the host innate immune system by reducing NFκB activation in leukocytes, decreasing neutrophil extravasation, and blocking neutrophil serine protease activity.

Here we report that Eap also provides exonuclease activity: Incubation of double-stranded DNA with Eap in the Ca^{2+} containing cell culture medium RPMI 1640 led to a rapid degradation of linearized plasmid DNA and PCR-amplified DNA fragments, respectively. Atomic force microscopy confirmed that Eap binds to and degrades linearized DNA in a time-dependent manner, while circular DNA did not interact with Eap and remained undegraded. Eap binding preferentially occurred to the termini of the double-stranded polynucleotide chains of DNA and was not affected by the type of overhang. In a dose-dependent manner, Eap also inhibited/prevented formation of bacteria-killing "neutrophil extracellular traps" (NETs), which represent the entire chromatin content of neutrophils that becomes ejected by incubation of cells with various agonists. These data indicate that Eap, via its DNase-associated activity, appears to express another immune-evading function by degrading NETs and thereby destroying an effective anti-microbial mechanism of the host.

IBV13

The phospholipases of *A. baumannii* – role in interbacterial competition and pathogenicity

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Question: *Acinetobacter baumannii* is an emerging threat in hospital environments due to its increasing resistance to antibiotics. Even though knowledge with respect virulence mechanisms and survival of *A. baumannii* in the human host is scarce. Phospholipases are known to contribute to interbacterial competition and have been identified as virulence factors in different pathogenic bacteria. Phospholipases are implicated in the specific destruction of endosomal or phagosomal membranes, which releases the bacterium into the nutrient rich host cell cytoplasm. This together with the identification of potential phospholipase C and D genes in the genome *of A. baumannii* led to the suggestion that phospholipases might contribute to the survival and metabolic adaptation of *A. baumannii* to the human host.

Methods: To analyze the role of phospholipases in metabolic adaptation and virulence of *A. baumannii* ATCC19606^T we developed a marker-less mutagenesis system. A set of different phospholipase C and D mutants was generated and analyzed in *E. coli* killing assays, in a *Galleria mellonella* infection model as well as in invasion of A549 lung epithelial cells.

Results: Here we present the development of a highly efficient markerless mutagenesis system for *A. baumannii* using a suicide plasmid encoding for 1500 bp of the up- and down-stream region of the target gene as well as for a kanamycin resistance for positive selection and the levansucrase form *Bacillus subtilis* for negative selection. With this technique we generated a whole set of phospholipase single, double and triple mutants. Bacterial competition assays and *Galleria mellonella* infection studies revealed that the *A. baumannii* phospholipases are not crucial for interbacterial competition but play an important role in infection of *G. mellonella.* Furthermore we tested the effect of phospholipases on invasion of human lung epithelial cells, showing that all of the tested phospholipases are necessary for full invasion efficiency.

Conclusions: The phospholipases of *A. baumannii* ATCC19606^T are not essential for interbacterial competition, but function as specific tools to target eukaryotic cells.

IBV14

Flotillin controls the assembly of protein complexes related to staphylococcal virulence

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Bacteria organize membrane related-signaling and transport processes in discrete membrane microdomains, similar to Eukaryotic lipid rafts. Those membrane rafts are constituted by specific type of lipids and carry a specific subset of membrane proteins. Flotillin is a chaperone protein that exclusively localize in lipid rafts and acts by facilitating protein-protein interaction within the rafts. Whilst most of research has been carried out in the model organism *Bacillus subtilis* [1-7], the presence of flotillin is widely detected in bacteria and Archaea. In this work, we have extended our research to explore the existence of bacterial lipid rafts to other bacterial species, like *Staphylococcus aureus. S. aureus* is human pathogen with a huge impact on the global health system by causing hard-to-treat acute and chronic infections. To mediate virulence *S. aureus* delivers a plethora of virulence factors and toxins to its host via different protein secretion machineries. Here we show that flotillin plays an important role in facilitating the oligomerization and stabilization of these protein secretion machineries and that the absence of flotillin causes a dysfunctionality in the secretion of proteins that are related to these protein secretion machineries that eventually develop an infection. Altogether, we will provide evidence that the organization of functional membrane microdomains in S. aureus is important for the correct functionality and stability of membrane-bound protein megacomplexes that are necessary to develop an infection process.

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IBV15

Structure of the bacterial cell division determinant GpsB and its interaction with penicillin binding proteins

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DivIVA proteins are curvature-sensitive membrane binding proteins essential for cell division, growth or virulence of Firmicutes and Actinobacteria. Firmicutes contain a second DivIVA protein, named GpsB. GpsB is implicated in the coordination of cell wall biosynthesis in *Bacillus subtilis* and essential for septal ring closure in *Streptococcus pneumoniae* (1, 2). While DivIVA of *L. monocytogenes* is indispensable for cell division and virulence (3, 4), listerial GpsB was so far uncharacterized. *L. monocytogenes* GpsB was found to be an oligomeric membrane binding protein and its deletion conferred pronounced growth defects to *L. monocytogenes*. Δ*gpsB* mutants were prone to cell lysis, impaired in cell wall biosynthesis and formed extremely long filaments, when combined with a Δ*divIVA* deletion. Bacterial two hybrid and biochemical experiments revealed that GpsB binds penicillin binding protein PBP A1. The structures of the N- and C-terminal domain of GpsB were solved independently and support a hexameric arrangement of the full-length protein. Genetic experiments were used to map the interaction site with PBP A1 to a conserved surface stretch of the N-terminal GpsB domain and showed that PBP A1 binding and hexamer formation are important for GpsB function. Deletion of *pbpA1* partially suppressed the *gpsB* phenotype, but PBP A1 became non-functional in the absence of GpsB, which indicates that GpsB may restrict PBP A1 and simultaneously is required for its activity. Interestingly, attenuation of *L. monocytogenes* virulence upon *gpsB* deletion in an animal infection experiment is as strong as upon the deletion of known virulence factors (5). This further strengthens the idea that DivIVA/GpsB proteins might be useful targets for novel chemotherapeutics.

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IBV16

Identification of a pneumococcal enzyme essential for anchoring of lipoteichoic acid to the bacterial cell surface

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Typical structures of Gram-positive cell walls are wall teichoic acids (WTA) and lipoteichoic acids (LTA). These surface associated glycopolymers are highly diverse, often species- and sometimes even strain-specific. The role of WTA in cell morphology and division, regulation of autolysis and adhesion to host cells is e.g. well characterized for *Staphylococcus aureus* and *Bacillus subtilis*, while the role of LTA remains elusive. Teichoic acids of *Streptococcus pneumoniae* (pneumococci) are unique in several aspects. First, pneumococcal WTA (pnWTA) and LTA (pnLTA) are identical in their complex repeating unit structures[1,2], suggesting a common biosynthesis pathway. Second, pneumococcal teichoic acids are highly decorated with phosphorylcholine (*P*-Cho), which bind non-covalently choline-binding proteins (CBPs) to the surface and are essential for bacterial growth[3]. Based on the *in silico* analysis of the *S. pneumoniae* R6 genome, putative enzymes involved in the biosynthetic pathway of teichoic acids have been proposed[4]. Inactivation of *spd_1672*, a gene in strain D39 encoding an enzyme with homology to O-antigen ligases from different Gram-negative bacteria, demonstrated the involvement of this enzyme in pnLTA anchoring to the surface of pneumococci. Chemical analysis of the cell wall of mutant D39Δ*cps*Δ*spd1672* indicated a total loss of pnLTA compared to the isogenic wild-type. The phenotypic characterization of the mutant revealed an impact on growth in complex and chemically defined medium, a decrease in the *P*-Cho content of the cell wall and an increased autolytic activity. Alterations in cell morphology of pneumococcal wild-type and mutants were illustrated by fluorescence, scanning electron and transmission electron microscopy. Furthermore, quantification of selected teichoic acid associated CBPs and capsule was investigated by flow cytometry. Finally, the effect of loss of function of SPD_1672 on phagocytosis was analyzed using human monocytes (THP-1-cells). In summary, we present here the identification of an enzyme involved in cell surface anchoring of pnLTA. Furthermore, we performed a phenotypic and functional characterization of pneumococci deficient in pnLTA and indicate for the first time that the lack of pnLTA impairs growth but not per se viability of these human pathogens.

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IbSV01

Dual proteome analysis towards understanding neutrophil interaction with *Aspergillus fumigatus*

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The opportunistic filamentous fungus *Aspergillus fumigatus* is a major cause of high morbidity and mortality rates in immunocompromised hosts. Neutrophils orchestrate the front-line defense against fungal "intruders" and deploy various strategies for immune clearance such as: degranulation, phagocytosis, formation of reactive oxygen species (ROS) - and -nitrogen intermediates (RNI) as well as **n**eutrophil **e**xtracellular **t**rap (**NET**) formation. We found that neither ROS, nor RNI are direct mediators of fungal killing due to the strong upregulation of the fungal ROS/RNI efficient detoxifying systems (catalase, superoxide dismutase, flavohaemoproteins) regulated by the transcription factors Af*yap1*, Af*Skn7*. These factors were dispensable for fungal virulence in infection mouse models.

We demonstrated that NETs occur both *in vitro* and *in vivo* but exhibit only fungistatic effects. Dual transcriptome efforts resulted in high coverage of the fungal transcriptome and low coverage of the human transcriptome. Thus, the key signalling networks remain to be elucidated. We therefore conducted a simultaneous dual proteome study of the activation of human neutrophils by *A. fumigatus* CEA10 hyphae without prior separation of the two organisms at a time point of NET formation.

We developed a protocol for parallel extraction and enrichment of both NETs covering hyphae and secreted proteins during NET formation. Besides a lysis-based approach for the cellular fraction consisting of hyphae entrapped in NETs, secreted proteins were enriched by C4 solid-phase extraction of the supernatant fraction. Using a multiplexed isobaric labelling approach and TiO_2 phosphopeptide enrichment combined with nLC-MS/MS analysis, we were able to identify and quantify 273 differentially regulated proteins of 856 proteins in total on the fungal side and 298 differentially regulated proteins of 1950 proteins in total on the host side compared to NET controls induced with PMA and hyphae grown alone.

We showed that neutrophils trigger higher expression of fungal signal transduction proteins from the GPCR-cAMP and CalA axis as well as Ste-20 tyrosine kinase. Neutrophil activities repressed proteins of the 60S ribosome and proteins responsible for siderophore-and-ergosterol biosynthesis. Fungal Af*yap1* dependent and independent heat-shock and ROS response was generally repressed.

Antifugnal NETs were highly enriched in core and alternative histones with changed stoichiometry and contained in higher abundance the calprotectin complex, lactotransferrin and PTX3 versus control NETs. We observed that there is a fungal specific neutrophil activation by detecting unique 275 interactome-specific proteins and 50 PMA-specific proteins. The results of the phosphopeptide enrichment were implemented in a model of the signal transduction cascade of NET formation.

IbSV02

Real-time imaging of the bacillithiol redox potential in the human pathogen *Staphylococcus aureus* using a novel genetically encoded redox biosensor

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Question: Bacillithiol (Cys-GlcN-Malate; BSH) is the major thiol-redox buffer of *Bacillus subtilis* and *Staphyloccoccus aureus*. Under oxidative stress, BSH forms mixed disulfides with proteins, termed as S-bacillithiolation which functions as thiol-protection and redox-control mechanism [1]. Two glutaredoxin-like enzymes YphP (BrxA) and YqiW (BrxB) were characterized as bacilliredoxins in the reduction of BSH-mixed protein disulfides [2]. In eukaryotes, glutaredoxin-fused roGFP2 biosensors have been applied for dynamic live-imaging of the glutathione redox potential. Here we have constructed the first genetically encoded bacilliredoxin-fused redox biosensor for dynamic live-imaging of the BSH redox potential in *S. aureus*.
Methods: The bacilliredoxin (Brx) of *S. aureus* was fused to roGFP2 and purified as His-tagged fusion protein from *E. coli* cells to analyse the response to BSSB, ROS and antibiotics *in vitro*. In addition, *S. aureus* COL and USA300 cells expressing Brx-roGFP2 fusions were used for live-imaging of the BSH redox potential *in vivo* using microplate reader measurements and confocal laser scanning microscopy as well as infection assays using THP-1 macrophage cell lines.
Results: The purified Brx-roGFP2 biosensor showed a specific and rapid response to low levels bacillithiol disulphide (BSSB) *in vitro* which required the active-site Cys of Brx. Dynamic live-imaging revealed fast and dynamic responses of the Brx-roGFP2 biosensor in *S. aureus* cells under hypochlorite and H_2O_2 stress and constitutive oxidation of the probe in the *bshA* mutants of the MRSA COL and USA300 isolates. Furthermore, we found that the Brx-roGFP2 expression level and the dynamic range are higher in *S. aureus* COL compared to the USA300 strain. Using confocal laser scanning microscopy, the changes in the BSH redox potential in *S. aureus* were visualized. Infection experiments of THP-1 macrophages with *S. aureus COL* revealed the specific oxidation of the biosensor inside the macrophages as measured by flow-cytometry. Notably, Brx-roGFP2 expressed in *S. aureus* USA300 and COL was 80-90 % oxidized during the phagocytosis assays.
Conclusion: Our studies demonstrate that this novel Brx-roGFP2 biosensor catalyzes specific equilibration between the BSH and roGFP2 redox couples and can be used for live imaging of the BSH redox potential in *S. aureus*.

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IbSV03

Prognostic model of urinary tract infections

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Urinary tract infections (UTIs) are considered to be the most common bacterial infection and are responsible for 155,000 cases per year in Germany alone, making it one of the biggest health concerns of today.[1] *Escherichia coli* still remains most common infecting organism in patients with uncomplicated UTI[2], although other pathogens include genera: Aerococci, *Proteus*, Staphylococci, *Pseudomonas*, Enterococci and *Klebsiella*.[3] Positive culture combined with patients' symptoms has been a gold standard for diagnosis.[4] Contamination of the collected urine and misinterpretation of asymptomatic bacteriuria remain pitfalls in clinical diagnosis[5] which often results in patients' unnecessary antibiotic exposure and overtreatment indicating that novel methods and approaches are much needed.[6] To overcome some of the clinical bottlenecks, prognostic models play a major role in recent years and are frequently being used to estimate an outcome of patients with a disease or health condition. Here, we compared metabolic urine profile of 92 subjects of whom 44 were diagnosed with UTI and 48 subjects with no signs of an infection. Single Strand Conformation Polymorphism (SSCP) analysis has been applied in the urine analysis to get insights into microbial communities present. Non-targeted LC-MS based analysis of patients' urine provided high quality mass spectral data which was used for prognostic modelling in the training set. Despite different UTI causing pathogens, all UTI patients were characterized by a significantly higher concentrations of putative metabolites heneicosanoyl-glycero-3-phospho-(1'-glycerol), benzothiazole and 2,2,6,6-tetramethyl-4-piperidone. To the best of our knowledge this is the first study in which statistical model was successful in infection prognosis on a randomized and unlabelled sample set with the help of UPLC-MS-based urine metabolomics.

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IbSV04

Sorting of Vancomycin BODIPY FL labeled *Staphylococcus aureus* from infection experiments – fast and easy enrichment of *S. aureus* isolates for analysis by mass spectrometry

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In host-pathogen infection experiments comprehensive proteomic analyses require the enrichment of the internalized bacteria to avoid interference with prevailing host proteins. Green fluorescent protein (GFP) labeling of *S. aureus* allows such separation of the microorganism from host cells after infection experiments. However, such labeling of bacterial isolates depends on the ability for genetic manipulation of the respective strains. Specific labeling of *S. aureus* with low molecular weight compounds such as vancomycin would avoid the need for such genetic manipulation and allow effective analysis of clinical isolates.
S. aureus HG001 was stained either during *in vitro* cultivation or in cell lysates of previously infected epithelial cells with the commercially available glycopeptide antibiotic vancomycin conjugated to BODIPY FL (VMB) at sub-inhibitory concentrations of vancomycin. Labeled cells were subsequently either counted in a Guava easyCyte flow cytometer or sorted with a FACSAria IIu cell sorter and collected on a membrane filter device. Afterwards, bacteria were digested on-filter with trypsin and subjected to mass spectrometry to elucidate the influence of VMB on the proteome level. In addition, we infected human bronchial epithelial S9 cells with *S. aureus* HG001 or clinical *S. aureus* isolates to prove the usability of VMB labeling. Furthermore, we established a staining protocol employing VMB for tracing intracellular bacteria by fluorescence microscopy.
In initial experiments the VMB concentration which resulted in the highest fluorescence intensity was determined. At sub-inhibitory concentration of 0.2 µg/ml VMB we found only a few unspecific proteome changes in labeled bacteria compared to non-labeled bacteria. Moreover, cell sorting provoked stronger changes compared to the staining. Having tested the suitability of VMB labeling for proteomics, we first used this dye for counting labeled *S. aureus* HG001 by flow cytometry - a method faster and more reliable than counting colony forming units. We counted a pure bacterial culture as well as bacteria recovered from lysed human cell lines and obtained comparable results to a *S. aureus* HG001 strain which continuously expresses plasmid-encoded GFP. Also bacterial enrichment by cell sorting was possible due to a clear separation of labeled bacteria and host cell debris, and the enriched bacteria were utilized for the analysis of the adaptation of *S. aureus* to the intracellular milieu.
We provide a fast and reproducible staining strategy which allows the counting, microscopic analysis, and separation of *S. aureus* in infection settings, which is, moreover, favorable compared to plasmid-encoded GFP production when working with clinical isolates since genetic manipulations are avoided.

IbSV05

Predicting compositions of microbial communities from stoichiometric models with applications for the biogas process

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Question: Microbial communities play a major role in ecology, medicine and various industrial processes. In order to investigate relevant interactions and to identify factors that influence the community we used stoichiometric metabolic models of a three-species biogas producing community.
Methods: We first constructed and validated stoichiometric models of the core metabolism of *Desulfovibrio vulgaris*, *Methanococcus maripaludis* and *Methanosarcina barkeri*, representing acetogenesis and methanogenesis. Those models were then assembled to community models. The community was simulated by applying the previously described concept of balanced growth [1] assuming an equal growth rate for all organisms. For predicting community compositions we propose a novel hierarchical optimization approach: first, similar to other studies, a maximization of the specific community growth rate is performed. This, however, often leads to a wide range of optimal community compositions. Therefore, in a secondary optimization, we also demand that all organisms must grow with maximum biomass yield (optimal substrate usage) reducing the range of predicted optimal community compositions.
Results: Simulating two-species as well as three-species communities using the representative organisms we gained several important insights. First, using our new optimization approach, we obtained predictions on optimal community compositions for different substrates which agree well with measured data. Second, we found that the ATP maintenance coefficient influences significantly the predicted community composition, especially for small specific growth rates. Third, we observed that maximum specific methane production rates are reached i) under high specific community growth rates, and ii) if at least one of the organisms converts its substrate(s) with suboptimal biomass yield. On the other hand, the maximum methane yield is obtained at low community growth rates and, again, when one of the organisms converts its substrates with suboptimal biomass yield. Apparently, maximum values for these key process parameters of biogas plants can only be reached if some species in the process waste substrate and energy. However, this would be accompanied by lower biomass yields negatively affecting the volumetric productivity. Finally, simulations of the three-species community clarify exchangeability and essentiality of the methanogens in case of alternative substrate usage and competition scenarios.
Conclusions: In summary, our study presents new methods for stoichiometric modeling of microbial communities in general and provides valuable insights in interdependencies of bacterial species involved in the biogas process.

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IbSV06

Anatomy of the bacitracin resistance network in *Bacillus subtilis*

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The protection against antimicrobial peptides (AMPs) is key for bacterial survival - both in their natural habitats and during immune evasion. While this defense often involves the parallel production of multiple, well-characterized resistance determinants, less is known about how these resistance modules interact and how they jointly protect the cell. To close this gap, we studied the interdependence between different layers of the resistance network of *Bacillus subtilis* against the lipid II cycle-inhibiting AMP bacitracin, involving the production of the ABC transporter BceAB, the UPP phosphatase BcrC and the phage-shock proteins LiaIH. Our systems-level analysis of the functional and regulatory interactions reveals a clear hierarchy, allowing us to discriminate between primary (BceAB) and secondary (BcrC and LiaIH) layers of bacitracin resistance. Strikingly, in a mutant devoid of the primary layer, the secondary layer is strongly induced and partially compensates for this mutation, thereby revealing the first direct role of LiaIH in the bacitracin stress response of *B. subtilis*. Conversely, deletions in the secondary layer give us novel clues about feedback regulation in the Lia system, and underline a pivotal role of BcrC in maintaining cell wall homeostasis in the presence and absence of AMPs. Lastly, we show that the compensatory regulation between resistance layers can also explain how gene expression noise propagates amongst them. We suggest that the redundant organization of the bacitracin resistance network of *B. subtilis* is a general principle to be found in many antibiotic resistance networks throughout the bacterial world.

IbSV07

Bacterial phase diagrams – using engineering concepts to predict cell-to-cell heterogeneity of microbial gene expression

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Microbial phenotypic heterogeneity is known to be naturally present in isogenic populations and can be attributed to the stochastic nature of biochemical reactions or changing environmental conditions. Cell-to-cell heterogeneity might be beneficial for adaptation of bacterial cultures to changing environmental conditions but is under reasonable suspicion to be responsible for failure, missing robustness, reduced control and lower yield in bacterial production processes. Thus novel analytical methods and strategies need to be developed to understand, predict and control bacterial heterogeneity [1].
In this contribution, we introduce to the first time the concept of "Bacterial phase diagrams" (BPDs). Similar to chemical phase diagrams, BPDs can be used to predict the state/behavior of microbial gene expression (e.g. homogeneous or heterogeneous gene expression) at certain environmental conditions. In order to determine single-cell responses, novel picoliter bioreactor cultivation platforms were used [2,3].
We demonstrate the concept for T7-RNA-Polymerase based EYFP production in *E. coli* BL21(DE3) [4], one of the most prominent expression strains in biotechnology. It generates a non-gradual and partially inhomogeneous induction behavior over a bacterial population. Depending on the inductor (here IPTG) and repressor concentration (here glucose), the expression behavior can be manipulated. Especially for low amounts of inducer molecules, an inhomogeneous all-or-nothing expression response can be observed. Here we present the manipulation of expression response, by means of varying inductive and repressive components. Latest results, potential application and ongoing research directions will be shown.
Our results show that the concept of "bacterial phase diagrams" has the potential to predict bacterial behaviour in gene-expression. This paves the way for an improved understanding of cell-to-cell heterogeneity and could lead to novel strategies of controlling microbial bioprocesses.

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IbSV08

Transcriptional, proteomic and metabolic networks of the Fur regulated iron metabolism of *Clostridium difficile*

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Clostridium difficile is known as a human pathogen causing diarrhea among patients in hospitals and health care units in Germany. *C. difficile* is a strict anaerobic, Gram-positive bacterium which is able to form spores in an unfavorable environment. In contrast to its clinical importance, almost nothing is known about the gene regulatory networks employed by the bacterium during host colonization and infection. *C. difficile* is confronted with iron-limitation during the infection process, therefore complex iron-uptake and iron-regulation systems are essential. Our group is particularly interested in elucidation of *C. difficile* iron homeostasis. For that purpose we us a combined genetic and molecular systems biology approach. The presence of multiple iron-uptake systems in *C. difficile* illustrates the importance of iron acquisition for clostridial growth and the ability of the bacterium to adapt to both iron-overloaded and iron-

restricted environments. In order to measure the iron homeostasis in *C. difficile* a *fur* mutant using a group II intron based technology was constructed. Subsequently, compared growth experiments of the wild type and the *fur* mutant displayed a clear growth deficiency of the *fur* mutant. To define *C. difficile* iron and Fur regulon, a transcriptomic, proteomic and metabolomic approaches were applied. Results displayed that iron transporters are highly upregulated in the *fur* mutant strain compared to wild type. Interestingly, the butanoate metabolism was strictly downregulated, indicating a Fur and iron dependent mechanism. Further studies will be employed to determine the role of Fur in iron homeostasis in *C. difficile*. This systems biology approach will contribute to a better understanding of the role of iron in the infection cycle of *C. difficile.*

MCV01

Architectural transitions in *Vibrio cholerae* biofilms at single-cell resolution

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Many bacterial species colonize surfaces and form dense three-dimensional structures, known as biofilms, which are highly tolerant to antibiotics and constitute one of the major forms of bacterial biomass on Earth. Bacterial biofilms display remarkable changes during their development from initial attachment to maturity, yet the cellular architecture that gives rise to collective biofilm morphology during growth is largely unknown. Here, we use high-resolution optical microscopy to image all individual cells in *Vibrio cholerae* biofilms at different stages of development, including colonies that range in size from 2 to 4500 cells. From these data, we extracted the precise cellular arrangements, cell shapes, sizes, and global morphological features during biofilm growth. We discovered several critical transitions of the internal and external biofilm architectures that separate the major phases of *V. cholerae* biofilm growth. Optical imaging of biofilms with single-cell resolution provides a new window into biofilm development that will prove invaluable to understanding the mechanics underlying biofilm formation.

MCV02

Spatial segregation in *Bacillus subtilis* biofilm allows the emergence of growth yield strategists

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Every living population aims to achieve a maximum fitness in terms of reproduction, survival and optimal use of resources in the environment. Bacterial populations are no exception. It is theoretically predicted that clonal lineages within a population show heterogeneous tradeoff between growth rate and growth yield strategy. When resources are abundant, maximizing growth rate favors the population level fitness. However, in nutrient limiting and spatially structured environment, slow growing but higher yield strategists manifest in the population.

To study experimentally the emergence and prominence of certain growth strategists in a biofilm environment that is both nutrient limiting as well as spatially arranged, *Bacillus subtilis* population was evolved in an emulsion based droplet regime. Each bacillus was enclosed in a separate droplet during the selection process and thus, competition between the linages was minimized. After 48 hours when cells in the droplets grew into aggregates, emulsion was disrupted and the culture was transferred to a new emulsion system with an appropriate dilution that enables confinement of only one cell in a droplet. Following 40 such transfers (~ 350 generations), cell types with delayed growth but higher yield were obtained. When the selected yield strategist strain was competed with the ancestor in a well-mixed environment, it is easily outcompeted by the fast growing competitor. Spatially structured environment like droplets enabled it to grow steadily and ultimately reach higher yield than the ancestor in the same emulsion. Interestingly, biofilm progression shows the early abundance of the fast growing ancestor strain after 24 hours followed by the emergence of yield strategists in the biofilms. These experiments showed that indeed the yield strategist cells appear in the biofilm delayed compared to the ancestors but eventually have increase in biomass confirming that this is an ecologically beneficial strategy in biofilms and other spatially arranged natural environments.

MCV03

SiaABCD coordinates cellular aggregation and virulence of *Pseudomonas aeruginosa* in response to environmental conditions

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Question: We recently identified the c-di-GMP dependent signaling pathway SiaABCD to regulate macroscopic cell aggregation of *Pseudomonas aeruginosa* in response to the non-natural surfactant SDS. The present study was conducted as we anticipated SiaABCD to have a more general role in regulating phenotypic traits in this organism.
Methods: Gene regulation was studied using transcriptional reporter assays, quantitative PCR (qPCR), and surface plasmon resonance (SPR). To visualize and quantify aggregation in response to various conditions, we used photo imaging analysis, scanning electron microscopy (SEM), and a crystal violet staining method. To explore the impact on virulence, we used a macrophage based phagocytosis assay.
Results: We provide compelling evidence that the *cupA* fimbrial genes represent a novel target for RsmA by direct binding and decreasing mRNA stability. Further, we show that the RsmA antagonist *rsmZ* is regulated in a SiaA/D dependent manner during surfactant exposure. We also reveal that the recently reported cellular aggregation during growth with glucose is SiaABCD dependent. Finally, we show that aggregation directly influences phagocytosis of cells by macrophages indicating a possible role for SiaABCD in persistence during host infection.
Conclusion: From these data we conclude that the SiaABCD pathway interconnects the c-di-GMP and RsmA networks to reciprocally coordinate cell aggregation and virulence in response to various external stimuli by shifting the equilibrium in favor of either component.

MCV04

Formation and integrity of multicellular aggregates in *Staphylococcus aureus*

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Multicellular communities or biofilms that can be formed by many bacterial species are the predominant mode of microbial life. The opportunistic pathogen *Staphylococcus aureus* is conventionally used as a model to study biofilm formation because it frequently develops biofilm-associated chronic infections in hospital settings. Biofilm formation has been traditionally studied in *S. aureus* using submerged polystyrene surfaces. Using this approach, extensive knowledge has been generated regarding the complex pathways and regulators involved in the process of biofilm formation as well as the enormous variability for surface colonization that exists between strains.

Recently, our lab has developed an alternative *in vitro* approach to study biofilm formation under conditions as they are found in chronic infections of bones, joints and soft tissues[1]. We propose a macrocolony biofilm-like developmental model in which the microbial community grows on a solid surface and it allows us to dissect biological processes related to the architecture of the community, such as microbial evolution or bacterial cell differentiation. Using this new approach, we aimed to identify the group of genes that play a role in maintaining the architecture of the macrocolony to further address the biological significance of these genes in these particular growing conditions. To do this, we screened a genome-wide mapped transposon library[2] and we selected the mutants that showed significant morphological alterations. Moreover, deletion mutants of these genes were generated and phenotypically compared to the screened transposon mutants. This suite of genes was identified, functionally classified and several genes were selected to investigate their role in maintaining the architecture of these multicellular communities. Three of these genes, previously unknown to participate in biofilm formation will be presented and the underlying genetic mechanisms will be discussed. These genetic mechanisms provided us with important insight in the signaling processes that are involved in multicellular development and the

nutritional requirements for *S. aureus* to form these structured multicellular communities. Overall, our work represents an important contribution to the current understanding of biofilm formation in *S. aureus* and it generates an alternative developmental assay to study biofilm formation in laboratory conditions.

1. Koch G, Yepes A, Forstner KU *et al.* Evolution of Resistance to a Last-Resort Antibiotic in *Staphylococcus aureus* via Bacterial Competition. Cell 2014; 158: 1060-1071.
2. Fey PD, Endres JL, Yajjala VK *et al.* A genetic resource for rapid and comprehensive phenotype screening of nonessential *Staphylococcus aureus* genes. mBio 2013;4:e00537-12.

MCV05

Viability of *Deinococcus geothermalis* in biofilms during desiccation

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Desiccation is a stressor for microbial life resulting in changes of membrane fluidity, protein conformation, and increased generation of harmful radicals [1]. Biofilms offer a lifestyle in which bacteria embedded in extracellular polymeric substances (EPS) can overcome environmental stress, including periods of desiccation [2].
This study investigates the effects of desiccation on the viability of *Deinococcus geothermalis*. We hypothesized that the organism is more tolerant to desiccation when present in a biofilm rather than as planktonic cells. Biofilms of *D. geothermalis* were generated on membranes placed on solid agar media. After incubation for 2 days at 45 °C, confluent multi-layer biofilms formed at the solid-air interface. In order to compare biofilms and planktonic bacteria, cells grown in liquid culture were deposited on membranes. Biofilms and membrane-deposited planktonic cells were desiccated, either in ambient air or in a desiccator resulting in reduced relative humidity, and incubated under the same conditions at room temperature for up to 3 months. At defined time points, the cells were recovered and analyzed for viability in terms of culturability, membrane integrity by Live/Dead® staining (combined with microscopy and flow cytometry), ATP levels, and presence of 16S rRNA (FISH).
Throughout the desiccation period, biofilms showed a reduction in culturability but sustained cultivation-independent viability to a high degree. Planktonic cells, on the other hand, showed significantly reduced viability in terms of culturability, membrane integrity, and ATP levels. The presence of 16S rRNA remained unaffected by desiccation in both life forms. Biofilms were less susceptible to reduced relative humidity than planktonic cells. Both biofilms and planktonic cells exhibited declining plate counts accompanied by stable total cell counts, suggesting that a part of the population entered a viable but non-culturable state as a response to desiccation. Proteins and polysaccharides were identified in the EPS of *D. geothermalis*. These compounds may be hygroscopic and store water during dehydration. It seems that the composition, distribution, and spatial arrangement of the EPS within the biofilm matrix contributed to an increased tolerance of *D. geothermalis* to desiccation.

[1] Potts (1994) Microbiol Rev, 58(4), 755.
[2] Flemming and Wingender (2010) Nat Rev Microbiol, 8(9), 623.

MCV06

Biofilms as a protective niche for non-halophilic sulfur cycling bacteria at groundwater springs in the Dead Sea

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The Dead Sea is one of the most hypersaline aquatic environments on earth with salt concentrations reaching up to 34 %. A recent study had reported extensive biofilm formation on Dead Sea sediments, especially connected to subsurface groundwater discharge[1]. These biofilms harbor a very diverse bacterial community with several bacterial groups involved in sulfur cycling. These observations came as surprise, as the community composition of these biofilms is not characteristic to hyper saline environments and sulfate reducing bacteria reported as abundant in the biofilms (e.g. *Desulfobulbaceae*) are not known to be halo-tolerant. In the present study we try to understand if formation of biofilms conferred halo-tolerance to non-halophilic bacteria. Our study employs a cultivation based approach with emphasis on sulfide-oxidizing and sulfate-reducing biofilm bacteria. Growth of a different isolates both as biofilm and free cells at different salt concentrations was determined. The isolate *FV2* belonging to genus *Exiguobacterium* (neither sulfate reducing nor sulfide oxidising) showed extensive biofilm formation. Further characterization of this bacterium showed a continuous decrease of growth rates of suspended cells till 16 % salt concentration. No growth of free cells was observed at salt concentrations above 16 %. However when grown in biofilms, good growth was observed up to 32 % salt, with optimal biofilm formation observed at 12 %. The other isolates SODS18 (a sulfide oxidizing bacterium) and sulfate-reducing *Desulfobulbaceae* showed no growth either as biofilms or as free cells above 5 and 2 % salt concentrations respectively. However when grown along with *FV2* biofilms these isolates showed good growth till 20 % salt. Based on these results we hypothesize that in the Dead Sea biofilm community, few microorganisms play an important role as halophilic biofilm engineers (e.g., *FV2*), while several other organisms which are not intrinsically halotolerant but involved in Sulphur cycling can overcome salt stress by inhabiting these biofilms. To date all the studies focused on understanding microbial halo-tolerance considered this phenomenon to be an intrinsic property of a cell, to the best of our knowledge we present first evidence that halo-tolerance could also be attained by symbiotic associations at community level.

1 Häusler S, Weber M, Siebert C, Holtappels M, Noriega-Ortega BE, De Beer D & Ionescu D (2014) Sulfate reduction and sulfide oxidation in extremely steep salinity gradients formed by freshwater springs emerging into the Dead Sea. *FEMS Microbiol Ecol* 90: 956-969.

MCV07

Omics in metal resistant *Streptomyces*

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The highly diverse and variable genetic predispositions of *Streptomyces* including their ability to form exospores makes them well suited for survival in soils in high abundancies ─ even under extreme conditions like metal stress. High metal loads prevail especially in areas influenced by mining activities, where mineral oxidation leads to the release of bioavailable metals at strongly acidic pH. Understanding the heavy metal resistance mechanisms of these adapted *Streptomyces* strains will help to establish effective bioremediation strategies.
The strains investigated were isolated from the former uranium mining area at Ronneburg, Germany. While one strain is resistant to a range of different metal ions, the other specifically copes with nickel loads up to 130 mM nickel in minimal media. Both have been shown to possess different metal resistance mechanisms including intracellular sequestration, release of siderophores and biomineralization.
With regard to future use in proteomic and transcriptomic studies, we obtained the genome sequences for two strains isolated from the metal contaminated area. *S. acidiscabies* E13 and *S. mirabilis* P16B-1 were sequenced by long-read real time sequencing and data was supplemented with short-read next-generation sequencing to increase sequence coverage. These investigations generated two high-quality draft genomes, which were annotated furthermore. This genome information together with predicting bioinformatic analyses was then used to identify proteins by gel- and MS-based proteome studies comparing normal and metal stressed conditions. The study was used to identify genes involved in metal resistance and metal regulation on a molecular level. From these, we were able to identify several interesting genes and gene clusters that have to be further analysed.

MCV08

Metagenomic analysis of an acidophilic (pH 3.5) and microaerophilic enrichment culture dominated by iron oxidising strains of the genus *Sideroxydans*

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The bacterial community in samples from a pilot plant for the treatment of acid mine drainage (AMD) water from the lignite mining district in Lusatia (East Germany) has been shown to be dominated by two groups of acidophilic iron oxidizers: the novel putative genus "*Ferrovum*" and a group considered to comprise *Gallionella*-like strains (Heinzel *et al.*, 2009). The family *Gallionellaceae* had previously only been known to consist of the microaerophilic and neutrophilic iron oxidizers *Gallionella* and *Sideroxydans*. Isolation of these acidophilic strains has, in contrast to "*Ferrovum*", so far proven impossible. We therefore employed a metagenomics approach to obtain access to their genetic information. To increase coverage of microaerophilic iron oxidising strains, we combined

this approach with their prior enrichment at acidic pH (3.5) using the gradient tube technique originally proposed by Kucera and Wolfe (1957).
16S rRNA based analysis of the bacterial diversity using both sequence analysis of a clone library (Sanger) and of 16S-tags (Illumina) indicated the presence of presumably three distinct *Gallionellaceae* strains in the enrichment culture that are closely related to the neutrophilic iron oxidizer *Sideroxydans lithotrophicus* ES-1. The availability of the complete genome of strain ES-1 now permits the detailed comparison of the metabolic capacity of neutrophilic and acidophilic members of the same genus and, thus, the detection of biochemical features that have been acquired by the acidophilic strains to support life under acidic conditions. For a start, the genome data of the acidophilic iron oxidising strains indicate the presence of a urease encoding gene cluster which is absent in the genome of *S. lithotrophicus* ES-1. The role of urease in pH homeostasis has originally been shown for the gastric pathogen *Helicobacter pylori*, but has also been suggested for the acidophilic iron oxidiser "*Ferrovum*" strain JA12 (Ullrich *et al.*, submitted). Additionally, the presence of predicted K^+/H^+ antiporters that are thought to prevent H^+ influx *via* formation of a reversed (inside positive) membrane potential (Baker-Austin & Dopson, 2007), seem also to be pronounced in the acidophilic strains.

Baker-Austin C, Dopson M (2007) Life in acid: pH homeostasis in acidophiles. Trends Microbiol 15: 165-171.
Heinzel E, Janneck E, Glombitza F, Schlömann M, Seifert J (2009) Population dynamics of iron-oxidizing communities in pilot plants for the treatment of acid mine waters. Environ Sci Technol 43: 6138-6144.
Kucera S, Wolfe RS (1957) A selective enrichment method for *Gallionella ferruginea*. J Bact 74: 344-349.
Ullrich SR, Poehlein A, Tischler JS, González C, Ossandon FJ, Daniel R, Holmes DS, Schlömann M, Mühling M (*submitted*) Genome analysis of the biotechnologically relevant acidophilic iron oxidising strain JA12 indicates phylogenetic and metabolic diversity within the novel genus "*Ferrovum*".

MCV09
The social amoeba and its opponents – a source of novel small molecules
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The social amoeba *Dictyostelium discoideum* represents one of the earliest branches of the common ancestor of all eukaryotes thus rendering it an ideal model organism in studying general eukaryotic cellular mechanisms. Differentiation and multicellularity in this amoeba, however, have evolved in a setting where *D. discoideum* is surrounded by mutualistic and pathogenic bacteria, as well as predators. Only in light of this ecological context, can a deep understanding of the fundamental molecular communication and signaling processes be gained.
We investigate the interaction between the social amoeba *Dictyostelium discoideum* and other dictyostelids as well as environmental bacteria. We are interested in determining signaling and effector molecules involved in these interspecies interactions. This will allow us both to understand underlying signaling processes, as well as enable us to discover new natural products that would not be produced in the absence of the required environmental cues.

MCV10
Microbial invasion into drinking water-related bacterial communities
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Using microbes for water treatment has a long history. Yet, the underlying processes of their performance are largely unknown. Water pollution and the rising drinking water demand make it necessary to close the Urban Water Cycle (UWC), i.e., water-to-wastewater-to-water cycle, in a sustainable and cost-efficient way. This can be achieved by development and implementation of the Microbial Resource Management & Engineering (MRME) strategy to control and steer microbial communities in water treatment. Microbial communities usually face environmental changes, e.g., temperature and nutrient stress, and by other microbes that invade/leave the resident community.
Working on the drinking water side of the UWC, we hypothesize a link between microbial community co-evolution in an engineered system and invasion by potential pathogens. Therefore, we want to elucidate the relation between community structure and its invasibility by a fecal drinking water contaminant.
To this end, screening of more than 20 sand filter isolates from drinking water plants for growth yielded 10 strains to compose defined bacterial communities. We then developed a model to challenge these synthetic communities with a GFP-expressing *E. coli* invader strain, and to monitor the response to this perturbation over time via turbidity measurement (OD_{600}) and flow cytometry (FCM). In this invasion model system an initially even resident community first co-evolves over four generations, and afterwards the subsequent generations are challenged with the invader to follow-up on the correlation of community evolution and invasibility over time.
We observed that invasibility increased during co-evolution of the initially even resident community over time. Additionally, the invader strain had a negative effect on the growth of the synthetic community. Assuming that a shift in community evenness occurs over time, this observation is consistent with previous findings that uneven communities are easier invaded (1).
Within the framework of MRME we therefore conclude that this relation indicates that the evenness of microbial communities involved in drinking water treatment should be monitored with view to invasion, e.g., by waterborne pathogens. Further research will include biofilm assays to provide more detailed information on invasion, and the impact on community diversity will be investigated by a comprehensive approach using a powerful experimental design, microcosms, FCM, and molecular techniques (e.g., qPCR, Illumina sequencing). Finally, we aim to apply this systematic approach to an undefined microbial community in a pilot reactor build for the treatment of used water to water that is hygienically safe.

(1) de Roy *et al.*, 2013. Nature Communications 4: 1383.

MCV11
***Staphylococcus schleiferi* volatiles inhibit quorum sensing controlled phenotypes in Gram-negative bacteria**
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Bacteria release distinct volatile organic compounds (mVOCs) which are often a source of structurally unique and/or new bioactive compounds [1, 2]. When analysing the VOC emission of *Staphylococcus schleiferi* DSMZ 4807 using GC/MS, we found more than 30 compounds including carboxylic acids, alcohol and ketones. Among the ketones were 3-(phenethylamino)butan-2-one (schleiferon A) and (Z)-3-(phenethylimino)butan-2-one (schleiferon B) of which the structures were elucidated by NMR. They were new compounds which have never been reported to be produced by any organism. To get some insight into the possible biological function of these compounds the volatiles of *S. schleiferi* DSMZ 4807 were tested on several Gram-negative bacteria, e.g. *Vibrio harveyi*, *Serratia marcescens*. The growth of these bacteria were not affected, while interestingly co-cultivation of S. *schleiferi* with *Serratia marcescens* V11649 and *Vibrio harveyi* DSMZ 6904, reduced production of prodigiosin and bioluminescence up to 60 %, respectively. The volatiles of *Staphylococcus warneri* CCM2730 (a schleiferon non-producer) did not inhibit the prodigiosin synthesis and bioluminescence of these two Gram-negative bacteria. Prodigiosin and bioluminescence are known to be controlled by quorum sensing/quorum quenching, which is a cell-to-cell communication system used by some bacteria to coordinate e.g. production of virulent factors and antibiotics, biofilm formation and bioluminescence [3, 4]. In order to find out whether the compounds schleiferon A and B function as quorum quenchers for prodigiosin synthesis and bioluminescence, we applied different concentrations of synthetic schleiferon A and B to bacterial cultures of *S. marcescens* and *V. harveyi.* We observed inhibition of the prodigiosin accumulation and bioluminescence in a concentration dependent manner, while the growth of the bacteria was not affected. We conclude that schleiferon A and B inhibit quorum sensing controlled phenotypes in Gram-negative bacteria.

1 Kai *et al.* Appl. Microbiol. Biothenol. 2010.
2 Weise *et al.* Beilstein J. Org. Chem. 2012.
3 Teasdale *et al.* Appl. Environ. Microbiol. 2009.
4 Chu *et al.* PLOS Pathogens. 2013.

MCV12

Ultrafast alignment and analysis of metagenomic DNA sequence data from the Tyrolean Iceman using MALT

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Question: Modern next generation sequencing technologies have led to the production of vast amounts of DNA sequence data from metagenomic samples, which permits the study of complex microbial communities in unprecedented detail. For these analyses high-throughput computational methods are needed that allow for an extremely fast processing of sequencing data while retaining a high level of sensitivity and precision.
Methods & Results: Here we present MALT (MEGAN Alignment Tool), a program for the fast alignment of DNA sequencing reads to a database of all microbial reference genomes available on GenBank. MALT is able to process hundreds of millions of reads within only a few hours, thus permitting the analysis of a whole metagenomic sequencing run within a single day. Its tight integration with the metagenome analysis software MEGAN (Huson *et al.*, 2011) allows for an assignment of single reads to different taxonomic levels with a precision that facilitates the identification and quantification of specific bacterial species or strains. In addition, MEGAN provides a variety of methods for more detailed analysis and visualization of the data. Performed in a comparative manner, this approach can be employed for studying the dynamics of microbial communities over time, or from different habitats or hosts. The method is particularly well suited to investigations of the human microbiome, which is comprised not only of a large number of commensals, but potentially also pathogens that have evolved with their human host. To gain insights into these evolutionary relationships, the field of paleogenetics aims to study ancient DNA extracted from archaeological remains.
In this context we demonstrate MALT by its application to the metagenomic analysis of two ancient microbiomes from oral cavity and lung samples of the Tyrolean Iceman, a 5,300-year-old Copper Age mummy.
Conclusion: With MALT we present a fast metagenomic DNA sequencing read aligner integrating a taxonomic binning algorithm for in-depth analyses of ancient and modern microbial communities.

D.H. Huson, S. Mitra, N. Weber, H.-J. Ruscheweyh, S.C. Schuster. Integrative analysis of environmental sequences using MEGAN 4. Genome Research 21, 1552-1560 (2011).

MCV13

Auxotrophy and intrapopulation complementary in the 'interactome' of a cultivated freshwater model community

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Microorganisms are usually studied either in highly complex natural communities or in isolation as monoclonal model populations that we manage to grow in the laboratory. However, we uncovered the biology of some of the most common and yet-uncultured bacteria in freshwater environments using a dilution-mixed-culture from Lake Grosse Fuchskuhle. From a single shotgun metagenome of a freshwater mixed culture of low complexity, we recovered four high-quality metagenome-assembled genomes (MAGs) for metabolic reconstruction. This analysis revealed the metabolic interconnectedness and niche partitioning of these naturally dominant bacteria. In particular, vitamin- and amino acid biosynthetic pathways were distributed unequally with a member of Crenarchaeota most likely being the sole producer of vitamin B12 in the mixed culture. Using coverage-based partitioning of the genes recovered from a single MAG intrapopulation metabolic complementarity was revealed pointing to 'social' interactions for the common good of populations dominating freshwater plankton. As such, our study highlights the power of mixed cultures to extract naturally occurring 'interactomes' and to overcome our inability to isolate and grow the microbes dominating in nature.

MCV14

Genome-wide mapping of *Aspergillus nidulans* and *Streptomyces* interaction

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Multispecies microbial communities play important roles in nature and can also lead to the activation of fungal silent secondary metabolite (SM) gene clusters (1). Therefore, the investigation of such interactions provides a very promising approach to discover novel SMs (2). We have been studying the interaction of the fungus *Aspergillus nidulans* and the bacterium *Streptomyces rapamycinicus* (3). Interestingly, co-cultivation of these two organisms led to the activation of the silent orsellinic acid (*ors*) gene cluster. Crucial for this interaction is the activity of the acetyl transferase GcnE (Gcn5 in *Saccharomyces cerevisiae*) of *A. nidulans*, which primarily acetylated lysine 9 and 14 of histone H3 during the co-cultivation (4). In order to demonstrate the relevance of these modifications for gene expression and production of SMs we exchanged several amino acids of histone H3 of *A. nidulans*. Major changes for the penicillin, sterigmatocystin and orsellinic acid biosynthesis in mutants mimicking non-acetylated H3 lysine 9 and 14 were detected (5). Therefore, we initiated genome-wide ChIPseq to analyse the distribution of these acetylations upon co-cultivation. Interestingly, numerous genes associated with the nitrogen metabolism of the fungus were found to be reduced in acetylation during interaction which correlated with a decreased expression of the respective genes.

(1) Brakhage (2013) Nat Rev Microbiol.
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MCV15

Molecular basis of the symbiotic interaction between prokaryotes in phototrophic consortia

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Introduction: Phototrophic consortia are multicellular associations in which green sulfur bacteria have gained the advantage of mobility by entering into symbiosis with motile *Betaproteobacteria*. The consortium "*Chlorochromatium aggregatum*" shows a scotophobic response and chemotaxis towards sulfide, enabling the green sulfur bacterial epibiont to conduct anoxygenic photosynthesis. The partners of the consortium coordinate their division to form two complete consortia. Specific ultrastructures at the cellular contact site of the epibionts are indications of specific adaptations to symbiosis. "*Chlorochromatium aggregatum*" has been obtained in culture and therefore provides the unique opportunity to study the molecular basis of this most highly developed symbiosis between two prokaryotes. Comparative genome studies between the epibiont and free-living relatives revealed unique open reading frames which included three large putative symbiosis genes (Cag_1919, Cag_0614, Cag_0616) resembling virulence factors of pathogenic bacteria (1).

Questions: Are proteins with virulence factors involved in the symbiotic interaction of two partner bacteria? Is it possible to assess a metabolic coupling between the two partners?
Methods: In order to elucidate the role of the corresponding proteins in the symbiotic interaction between the two partner bacteria, recombinant proteins were expressed in *E. coli* and used to produce antibodies for immunogold labelling and high resolution fluorescence microscopy for intracellular localization. The possibility of metabolic coupling between the two partner bacteria was assessed by nanoSIMS, magnetic capture and transcriptome analyses.
Results: The proteins encoded by the three symbiosis genes are transported across the cell envelope of the epibiont into the central bacterium. Cag_1919 contains a RTX domain which is typically found in Gram-negative pathogenic bacteria and was localized in condensed areas of the cell wall of the central bacterium. Cag_0614 and Cag_0616 represent the largest open reading frames (110418 and 61938 bp) known among prokaryotes and are transported across the cell envelope of the epibiont and are localized in the cytoplasm of the central bacterium. The epibiont provides metabolites to the central partner bacterium depending on the external nitrogen source and exogenous carbon substrates. The central bacterium, though not phototrophic, remains metabolically inactive in the dark and transcribed a variety of transporters.
Conclusions: The unexpected transfer of virulence factor-like proteins strongly suggests an involvement in the tight symbiotic interaction. There is also a metabolic coupling between the two partner bacteria which depends on the physiological activity of the epibiont.

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MCV16
Global and local patterns of bacterial communities associated with peatland bryophytes
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Introduction: Northern peatlands are pristine ecosystems that act as important global carbon stocks in which C- and N-cycles are driven by plants (mosses, sedges) and a specialized microbiota. *Bryophytes* like *Sphagnum* and *Amblystegiaceae* ('brown mosses') are typical representatives of these peatlands. Recent studies focusing on *Sphagnum* mosses and other bog plants identified an essential core microbiom[1], but also uncovered *Sphagnum*-species specific bacterial groups[2]. A comprehensive sampling design is lacking which includes brown mosses and aims at elucidating the influence of environmental parameters shaping the moss-associated bacterial (MAB) community at a global scale.
Questions: We aimed at describing the influence of environmental parameters on the MAB community at the local and the global scale.
Methods: In a systematic approach, we sampled mosses and references (sedges and/or sediment) from 26 sites at 4 different locations: Svalbard (High Arctic, Spitsbergen), Samoylov (Arctic, Siberia), Neiden (Subarctic, Norway) and Mueritz (Temperate, Northeast Germany). After separation of endo- and epiphytic bacteria of MAB, Illumina sequencing of the 16S rRNA gene was applied. Cell wall analysis (lignin-like polymers, holocellulose, CEC) and C/N determination of mosses and sedges were carried out. Environmental parameters (pH regime, DOC, ions, organic acids) as well as temperature, CH_4 and O_2 gradients were measured and used for statistical analysis.
Results: The sample sites represent two different ecosystem types: neutral pH peatlands with submerged brown mosses (Svalbard, Samoylov) and low pH peatlands with emerged/submerged *Sphagnum* mosses (Neiden, Mueritz). These types were also reflected in two distinct global clusters of the MAB communities. The low pH clusters generated different MAB subclusters of emerged and submerged *Sphagnum*-associated species and additionally displayed differences on the local scale. Overall, the brown moss sites showed a higher diversity than the *Sphagnum* sites. A more defined analysis on the endophytic MAB showed a high dominance of *Alphaproteobacteria* in the low pH Sphagnum sites in comparison to a high dominance of *Actinobacteria* in the neutral pH brown moss sites.
Conclusions: MAB patterns on the global scale reflect environmental controls driven by pH and hydrology. Within each studied site, highly similar, but distinct MAB communities occupy local environmental niches represented by subsites. Additionally, we identified specific, site-independent, endophytic MAB in *Sphagnum* and brown mosses.

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MCV17
Microbial hub taxa link host and abiotic factors to plant microbiome variation
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Question: Microbiomes critically affect the physiology and performance of plant hosts and are thus subject to selection along with the host genome. Thus, evolution and ecology of plants can only be understood in a holobiont context that includes microbes that colonize the phyllosphere. A variety of factors influence host microbial community structures, but little is known about *how* these factors act on the microbial community and especially what role microbe-microbe interaction dynamics play.
Methods: We addressed this knowledge gap by simultaneously studying three major groups of *Arabidopsis thaliana* symbionts (bacteria, fungi, and oomycetes) using a custom amplicon sequencing protocol and a systems biology approach to analysis. We combined sampling of wild *A. thaliana* populations and field plantings to evaluate effects of location, sampling time and host genotype and isolated phyllosphere microbes to confirm findings in the lab with successive host colonization experiments.
Results: First, we confirmed previous results showing that abiotic factors and host genotype affect plant colonization. We then uncovered a network on inter-kingdom microbe-microbe interactions that affect community structure. A few taxa, which we term microbial 'hubs' were especially interactive in the phyllosphere. *In-planta* lab experiments supported field observations and proved an important mechanism whereby host and abiotic factors control colonization by hubs, which in turn transmit effects to the microbial community, resulting in observed correlations. Importantly, in our experiments with hub microbes including the obligate biotrophic oomycete pathogen *Albugo,* the basidiomycete yeast fungus *Dioszegia*, and the bacteria *Caulobacter*, we found evidence for direct interaction between the hubs in addition to direct effects on the larger microbial communities. Specifically, while *Albugo* generally caused decreased bacterial alpha diversity and stabilized beta diversity compared to uninfected plants, *Dioszegia* inhibited *Caulobacter* growth and promoted *Albugo* virulence.
Conclusion: Our results demonstrate the importance of hubs in host microbiome structuring and in turn the importance of the microbiome on hub fitness. Thus, both plants and microbial hubs are organisms with hologenomes and we hypothesize that the microbial community represents a location of indirect interaction and competition between host and these influential microbes. Thus, the identification of microbial 'hubs' has crucial implications for plant-pathogen and microbe-microbe research and opens new entry points for ecosystem management and future targeted biocontrol.

MCV18
Transcriptional profiling during *in planta* development of the corn smut fungus
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Ustilago maydis is the causative agent of corn smut disease. After penetrating the plant epidermis, the fungus grows as branching mycelium and induces tumors in colonized tissues. The fungal hyphae grow initially intracellularly but at later stages, i.e. during tumor formation, they form huge aggregates in apoplastic caves. Within these aggregates maturation of teliospores takes place. The transcriptional changes during the *in planta* development of this strictly biotrophic fungus are not well documented.
We performed RNAseq based transcriptional profiling of *Ustilago maydis* during seedling infection. The analyzed developmental stages range from plant penetration to induction of tumors and spore maturation. More than half of the 6700 *Ustilago maydis* genes are differentially expressed throughout the life cycle. The most highly expressed genes encode secreted effector proteins, which are needed to establish the biotrophic interaction between fungus and host plant. We categorized the 320

putative effector genes according to their expression profiles and were able to differentiate the following categories: on early and off at later stages; off early and on at later stages; on throughout the life cycle; on throughout the life cycle with expression peak during tumor formation.
Our data demonstrates that each developmental stage of *Ustilago maydis* is characterized by an effector cocktail in part explaining the large number of specific effectors found in this organism.

MCV19
Acclimatization of arbuscular mycorrhizal fungi leads to increased stress tolerance of their host plants
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Arbuscular mycorrhizal (AM) fungal strains differ in their abilities to provide their host plants with mineral nutrients and to confer resistance to root pathogens and tolerance to abiotic stress. For example, mycorrhizal plants perform much better on sites contaminated with heavy metals (HMs) compared to their non-mycorrhizal counterparts. Numerous studies showed that taxonomically-related AM fungal strains differ in their capacity to confer HM tolerance to plants and can even loose this character, if propagated under HM-free conditions. The current study compiles the following hypotheses: (1) AM fungi can acclimatize to HM stress over a few generations. (2) This acclimatization process can be monitored on molecular level. (3) Acclimatized strains show increased abilities to confer heavy metal tolerance to plants.
Rhizophagus irregularis DAOM-197198 was chosen as model AM fungal strain and propagated in root organ cultures in the presence of moderate Zn or Pb concentrations. After five generations, cultures were tested at increased HM concentration for hyphal and spore development and for the expression of a set of HM-regulated genes. The results confirmed the first hypothesis: AM fungi can acclimatize to HM stress. Hyphal density or the number of spores was higher, if the strains grow in the presence of HMs compared to the non-acclimatized control strains. The AM fungal gene expression patterns reflect also the process of acclimatization. RNA of particular genes accumulated earlier or to increased amounts in the acclimatized strain confirming the second hypothesis.
In order to test the third hypothesis, the final generation of newly developing spores from the acclimatized and from the non-acclimatized strain was used for inoculating maize plants in pot cultures, treated with high amounts of Zn and Pb. At harvest, plants inoculated with an acclimatized strain formed higher biomasses and showed increased uptake of phosphate and lower amounts of HMs in their shoots compared to plants colonized by the non-acclimatized strain. We propose that this process of acclimatization accompanied by monitoring RNA accumulation of particular genes can be transferred to a commercial directed inoculum production process with quality control adapted to particular customer needs.

MCV20
The tripartite symbiosis of *Piriformospora indica*, its endofungal bacterium, and plants
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The plant growth-promoting fungus *Piriformospora indica* harbors an endobacterium which is frequently detected in low abundance in hypha and spores of fungal lab cultures by fluorescence *in situ* hybridization and quantitative PCR. Sharma *et al.* (2008) isolated and identified the endobacterium as *Rhizobium radiobacter* (formerly: *Agrobacterium tumefaciens*). While the endobacterium can grow in pure culture (strain *Rr*F4) it has not been possible to cure the fungal host. Thus, the role of the endobacterium in the tripartite symbiosis with plants is still unclear. In contrast to other fungal endobacteria, the genome of strain *Rr*F4 is not reduced in size. Instead, it shows a high degree of similarity to the genome of the plant pathogenic *A. tumefaciens* strain C58, except vibrant differences in the tumor-inducing (pTi) and accessor (pAt) plasmid, explaining the loss of pathogenicity. Similar to its fungal host, *Rr*F4 shows plant growth promotion and induced systemic resistance against fungal and bacterial pathogens. Quantitative real-time PCR data confirmed the proliferation of *Rr*F4 in roots of axenically grown barley, wheat and Arabidopsis plants over the time of incubation. The root colonization patterns of *Rr*F4 and its host *P. indica* were compared by the application of GUS and GFP-tagged *Rr*F4 cells combined with light, confocal laser scanning, scanning and transmission electron microscopy (Glaeser *et al.* 2015). We showed that *Rr*F4 colonizes the rhizodermis and cortex tissue of the root hair zone in a similar manner as its fungal host. But, unlike its fungal host, *Rr*F4 is able to penetrate through the endodermis into the root stele as known for many other plant growth promoting bacteria. Thus, these data, along with the finding that the abundance of endobacterial cells strongly increased during the tripartite symbioses, suggest that *Rr*F4 seems to contribute to the beneficial activity that *P. indica* exerts on plants.

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MCV21
What it takes to be a giant gut bacterium – metabolic flexibility and diel lifestyle of *Epulopiscium*
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Epulopiscium fishelsoni and related bacteria are a large, morphologically diverse group of intestinal symbionts found exclusively in the guts of herbivorous surgeonfishes. Though they are phylogenetically related to *Clostridia*, many of their biological traits including size (up to 600 µm), possession of an unusual mode of reproduction and being highly polyploidy are to date only atypical to *Epulopiscium*. Yet a basic understanding of their metabolism is still lacking to the extent that it is currently unknown how such gigantic bacteria manage their carbon and energy pools especially given the diffusional constraints imposed by their size. Although it is understood that the reproduction of *Epulopiscium* follows a daily rhythm that is intertwied with the feeding behavior of its host, which feeds during the day and rests at night, their role in the mobilization of low-value algal biomass and significance in gut fermentative processes is still obscure. To fill this knowledge gap, we reconstructed the metabolism of different *Epulopiscium* cell morphortypes from single cells and population genomes obtained from three different surgeonfishes. Additionally, temporal profiles of gene expression were obtained by mapping metatranscriptomics datasets derived from the *Epulopiscium*-rich stomach of *Acanthurus sohal* over time against the reconstructed genomes. For the first time, these datasets demonstrate that *Epulopiscium* (1) have the capacity to efficiently turnover algal biomass, albeit with each genotype specialized to degrade different polymers, and (2) play a major role in the nitrogen economy of the host. Reflecting these potential is the additional evidence based on temporal profiles of transcripts involved in the *in situ* turnover of candidate polysaccharides. For instance *Epulopiscium* appear to initially utilize freely available low-molecular weight organic carbon (glycerol and citrate) coupled with sodium-dependent enzymatic pathways in a manner that facilitates the regeneration of reducing equivalents and elevates the net ATP pools but switch to glucose-based fermentation in the afternoon, when cells are actively dividing. Copy numbers of important genes also increase several folds over the course of the day consistent with the assumption that polyploidy serves to redistribute key enzymes throughout the cells thereby facilitating cellular activities and diminishing diffusional constraints. Thus, while the cells appear to be constrained — both energetically and at the level of metabolite transport across the extensive cellular space — they have adaptive mechanisms that help to conserve energy in addition to the canonical fermentative pathways. The metabolic versatility of *Epulopiscium* and the significance of this diel lifestyle will also be discussed in detail during the presentation.

MCV22

Surface modifications of *Escherichia coli* influence ingestion and digestion of the ciliate *Tetrahymena pyriformis*

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Endosymbiosis a common phenomenon in nature, also and especially in single-celled organisms (Görtz, 2010). A logical and mandatory prerequisite for establishment of endosymbiotic relationships in protozoa is evading digestion by food bacteria. Recently, it has been shown that a non-pathogenic strain of *Escherichia coli* K12 is able to evade digestion by *Tetrahymena pyriformis* and additionally escapes from food vacuoles, resulting in persistence in the cytoplasm of the host (Siegmund *et al.*, 2013). To shed some light on possible factors involved in evading digestion and even escaping from food vacuoles of the potential host, we chemically modified the surface of a transformant strain of *E. coli* by covalently binding different substances to the surface by means of carbodiimde. We observed the effects on ingestion and digestion of *T. pyriformis*. The fate of the bacteria was followed by fluorescence microscopy, since the transformant strain expresses DsRed, a red fluorescing protein of the jellyfish *Discosoma*.

Depending on the coupled substance, the ciliates' ingestion rates either decreased (e.g. different amino acids) or increased (e.g. endoporter, an artificially designed hydrophobic oligopeptide). This clearly indicates a recognition site at the cytostome region acting prior to ingestion. Comparable results were obtained by feeding chemically modified microparticles based on bovine serum albumin (Dürichen *et al.*, submitted). Further, by decreasing of fluorescence intensity, the digestive process was followed. Bacteria covered with substances that enhance hydrophobicity of the surface were not completely digested in every case, even surviving bacteria in fecal pellets were observed.

By binding additional substances, double feeding experiments and appropriate staining techniques, some surface alterations of bacterial cells are identified that enable food bacteria to evade digestion or even to escape food vacuoles and, in consequence, allow persistence in the host's cytoplasm. These findings probably improve our understanding of mechanistic prerequisites for establishing endosymbiosis.

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MCV23

Evidence of terpene degradation by pine weevil (*Hylobius abietis*) microbiota and its effect on host fitness

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Nutritional mutualisms between insects and microorganisms are not restricted to the synthesis and provision of essential amino acids or vitamins but also encompass the degradation of noxious compounds in the host's diet. This, although demonstrated in fungal-insect associations, remains poorly described for bacterial-insect interactions.

The pine weevil (*Hylobius abietis*) feeds on bark and cambium of conifers ingesting high amounts of terpenoids, a form of conifer chemical defense, that are toxic or deterrent to herbivores. We are interested in studying whether the pine weevil´s gut microbiota is involved in the degradation of such compounds.

To this aim, we have used a multidisciplinary approach utilizing techniques such as next generation sequencing, analyzed with a variety of bioinformatics tools, as well as phylogenetics, *in vitro* and *in vivo* bioassays and chemical analyses.

Our results show that the pine weevil harbors a geographically stable microbiota across different European populations dominated by *Wolbachia*, Enterobacteriacae and Firmicutes. We have also compared this community to that of other beetles exploiting similar and different ecological niches. We demonstrate that the gut microbial community of the pine weevil is similar to that of conifer-exploiting beetles particularly within the Enterobacteriaceae family. Our predicted bacterial metagenome based on the 16s rRNA gene, contains a complete gene cluster involved in diterpene degradation. *In vitro* assays also demonstrate that the bacterial gut community can degrade diterpenes. Bioassays with beetles that have been treated with antibiotics do not digest terpenes as efficiently as untreated ones, and that the artificial supplementation of the native community recovers digestion efficiency. We also observe a higher fitness in beetles feeding on artificial diet with terpenes. Current analyses will shed light on the breakdown products of the digestion of these compounds, and our efforts to sequence the bacterial metagenome will provide putative candidates for genes and enzymes involved in such process.

Collectively, our results suggest that the bacterial community of the pine weevil is essential for the exploitation of its ecological niche, most likely through the detoxification of plant secondary metabolites.

MCV24

Exploiting *Streptomyces* in agro-ecosystems for biological control and plant growth promotion

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Introduction: Yield losses caused by phytopathogens should be minimized to maintain the food quality and quantity for the demand of massively growing human population. Thus, searching for sustainable solutions to suppress phytopathogens, as well as to increase the yield is gaining high interests. Beneficial plant associated microorganisms can contribute to crucial ecosystem services in agricultural landscapes, including plant growth promotion (PGP) and biological control.

Question: Do *Streptomyces* have promising potentials to be used as biological control agents (BCAs) and PGB bacteria?

Materials and methods: Two *Streptomyces* strains isolated from surface sterilized plant roots, *S. exfoliatus* FT05W and *S. cyaneus* ZEA17I, showed up to 75 % *in vitro* mycelial growth inhibition of *Sclerotinia sclerotiorum*, the causal agent of lettuce drop [1]. We labeled them with the enhanced green fluorescent protein (EGFP) marker to investigate *Streptomyces*' ability to colonize the lettuce roots using confocal laser scanning microscopy (CLSM) [2,3]. Furthermore, we quantified *S. exfoliatus* FT05W and *S. cyaneus* ZEA17I viability and persistence in the rhizosphere and in surface sterilized lettuce root tissues. Additionally, we investigated the PGP activities of the two strains on the germination and growth of different plant species in growth chamber. Finally, we studied the biological control activity of the two strains against *S. sclerotiorum* under both growth chamber and field conditions.

Results: The abundant colonization of young lettuce seedling (2 or 3 days old) by both strains demonstrated *Streptomyces*' capability to interact with the host from early stages of seed germination and root development, and the two strains were detected also on two-week-old roots. On average, after six weeks of growth, we recovered 2×10^4 CFU/g dry weight (DW) from the rhizosphere and 2×10^5 CFU/g DW from the inner root tissues. Plant-strain specific PGP activity was observed; e.g., *S. cyaneus* ZEA17I promoted the growth of lamb lettuce but not that of tomato. When they were applied to *S. sclerotiorum* inoculated substrate in growth chamber, *S. exfoliatus* FT05W and *S. cyaneus* ZEA17I significantly reduced disease incidence by 40 % and 50 %, respectively, compared to the control ($P < 0.05$). Interestingly, under field conditions, *S. exfoliatus* FT05W reduced disease incidence by 54 % ($P = 0.12$), but we did not observe protection of lettuce plants against *S. sclerotiorum* by *S. cyaneus* ZEA17I.

Conclusion: Our results indicated the promising potentials of *Streptomyces* for exploitation in agro-ecosystem applications. Further studies will provide additional knowledge to understand the mechanism of *Streptomyces* mediated biocontrol and PGP.

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NPV01

Biosynthesis of the 6-pentylsalicylate building block in the antibiotic micacocidin

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The hybrid polyketide synthase/non-ribosomal synthetase MicC initiates the biosynthesis of the antibiotic micacocidin. Our previous work confirmed that the polyketide synthase module of MicC acts as a 6-pentylsalicylate synthase (6-PSAS) (1, 2). Further analysis of the 6-PSAS was carried out with site-

directed mutagenesis. This analysis indicated that a β-ketoreductase domain from MicC is crucial for the formation of 6-pentylsalicylate. To determine the exact timing of β-ketoreductase activity, we interrupted 6-pentylsalicylate biosynthesis by chemical chain termination (3). High resolution mass spectrometry analysis of the off-loaded intermediates revealed that the selective β-ketoreduction directly takes place after the second elongation step of the linear polyketide intermediate (4). These results show that the investigated 6-PSAS follows the same logic as the previously characterized 6-methylsalicylate synthase (5), thereby establishing a unified paradigm for the biosynthetic mechanism of these proteins.

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NPV02

NPS2 of *Ceriporiopsis subvermispora* exemplifies the model for the most conserved basidiomycete peptide synthetase

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Question: A gene encoding a nonribosomal peptide synthetase (NPS) which consists of one complete module and a T-C repeat is found in numerous basidiomycete genomes and represents the most conserved peptide synthetase gene in the group of fungi. In *Ceriporiopsis subvermispora* it is represented by nps2. NPS2 resembles siderophore synthetases. To test the hypothesis of NPS2 also serving for siderophore biosynthesis, this study is focused on the identification and characterization of the NPS2 product.
Methods: *Ceriporiopsis subvermispora* nps2 was heterologously expressed in *Aspergillus niger* P2 under the control of the terA promoter (Gressler *et al.*, 2015). The ATP[32P] pyrophosphate exchange assay was used for substrate identification. The secondary metabolite was analyzed via *in vitro* product formation assay, chrome azurol-S (CAS) assay, high pressure liquid chromatography and mass spectrometry (LC-MS).
Results: The adenylation domain of NPS2 activated N5-acetyl-N5-hydroxy-L-ornithine (AHO) and N5-anhydromevalonyl-N5hydroxy-L-ornithine (AMHO). *In vitro* product formation analyses by LC-MS and the CAS assay revealed a trimer of AHO that was active as iron chelator. Under iron-depleted conditions, the secretion of a siderophore by *Ceriporiopsis subvermispora* was detected. In agreement, under these conditions the expression of nps2 and the adjacent monooxygenase gene were upregulated.
Conclusion: In summary, these results characterize a representative of the most conserved basidiomycete NPS. Further investigations on the siderophore produced *in vivo* are envisaged to elucidate its complete biosynthesis.

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NPV03

SimC7 is an unusual angucyclinone ketoreductase essential for antibiotic activity of simocyclinone D8

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Introduction: Simocyclinone D8 (SD8) is an antibiotic produced by *Streptomyces antibioticus* Tü6040 that targets DNA gyrase, an essential bacteria-specific enzyme and validated target for clinically useful antimicrobials [1,2]. SD8 has an unusual hybrid structure composed of an aminocoumarin and an angucyclinone that are linked by a linear tetraene and a deoxysugar. The biosynthetic simocyclinone (*sim*) cluster has been identified and sequenced. Although a hypothetical biosynthetic pathway has been proposed, very few enzymes have been functionally analysed, mainly due to the genetic inaccessibility of the natural producer and enormous size of the gene cluster.
Objectives: In this study we analysed the structure and function of SimC7 involved in simocyclinone biosynthesis.
Methods: The entire 72-kb simocyclinone gene cluster was isolated on a single phage artificial chromosome (PAC) to facilitate genetic manipulations and product analysis. Simocyclinones were extracted, purified, and analysed by high resolution NMR spectroscopy and UV-vis LCMS, and their biological activity was determined against DNA gyrase. The *de novo* protein structure of SimC7 in complex with simocyclinones was defined by X-ray crystallography.
Results: We revealed that SimC7 is an NAD(P)H-dependent angucyclinone ketoreductase that acts on the C-7 carbonyl group of the angucyclinone, revising the previously proposed dehydratase activity on the tetraene linker [1]. Importantly, SimC7 is essential for the biological activity of simocyclinone because deletion of *simC7* results in production of a novel and inactive simocyclinone intermediate (7-oxo-SD8) with a normal tetraene linker but a modified angucyclinone. Several high-resolution protein structures of SimC7 revealed an unusual catalytic mechanism without involvement of a catalytic tyrosine characteristic for SDR proteins. In addition, SimC7 shares homology to epimerases and other sugar-modifying enzymes but surprisingly little homology to known ketoreductases.
Conclusions: Our study demonstrates the essential biosynthetic role of SimC7 for the inhibitory activity of SD8 and provides novel insights into the molecular basis of alternative mechanisms for unusual SDR proteins. The structural homology of SimC7 to sugar-modifying enzymes implies an evolutionary adapted function driven by an improved biological activity of SD8. These results indicate that the biosynthetic pathway of simocyclinone is not entirely understood.

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2 Galm, U. *et al.* Cloning and analysis of the simocyclinone biosynthetic gene cluster of *Streptomyces antibioticus* Tü 6040. *Arch Microbiol* 178, 102-114 (2002).

NPV04

Elucidation of the biosynthetic gene cluster involved in the biosynthesis of the natural compound sodorifen in *S. plymuthica* 4Rx13

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Microorganisms are fascinating because they intrude our lives without noticing or any possibility of avoiding them. The mold on the bread we forgot over the weekend, the antibiotic we take against an infection, the glass of wine we enjoy after a long day of work. They are omnipresent and produce a variety of natural products, which are often unique. In many cases the biosynthesis is unknown. However, for a long time it was overlooked that microorganisms release a plethora of volatile compounds as well. Recently the database mVOC (1) was setup compiling about 1200 VOCs released by so far 350 bacterial and 100 fungal species.
The rhizobacterium *Serratia plymuthica* 4Rx13 is able to produce the unique volatile compound sodorifen, which is produced by only a few isolates. The structure was elucidated and revealed an extraordinary bicyclic ring structure consisting only of carbon and hydrogen with multiple methyl groups (2). It harbors no other heteroatoms, which is peculiar for a natural compound. However, the biosynthesis was for a long time a mystery due to the assumption that novel enzymes are involved.
To unravel the biosynthesis of sodorifen a comparative genome and transcriptome analysis was applied and a list of potential candidate genes was generated. A cluster of four genes was found. Their involvement in the sodorifen biosynthesis was verified by mutagenesis. Indeed, an altered phenotype was observed for the mutants. Three genes within the cluster demonstrated a sodorifen negative phenotype, while the fourth gene showed a significantly reduced emission. Thus, it was speculated that a second gene in the genome of *S. plymuthica* 4Rx13 is able to compensate the function.
Especially interesting was the change of the volatile blend of the terpene cyclase present in the cluster. The knockout resulted in the disappearance of sodorifen but a new dominant compound appeared within the spectrum. The compound has to be examined to clarify whether it is a direct intermediate in the biosynthesis of sodorifen or a byproduct. Therefore, investigations of the

new compound could be achieved by the incorporation of isotope labeled precursors along with NMR analysis.

1) Lemfack *et al.*; 2013, Nucl. Acid Res., 1-5
2) Reuss *et al.*, 2010, Angew. Chem. Int. Ed. 49, 2009-2010

NPV05

The role of short-lived intermediates in the *Pseudomonas aeruginosa* alkylquinolone biosynthesis pathway

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Question: The *pqs* biosynthetic operon of *Pseudomonas aeruginosa* codes for the synthesis of an arsenal of secondary metabolites, the most prominent one being the quorum sensing signal molecule PQS. However, several other physiologically important compounds such as 2-heptyl-4(1*H*)-quinolone (HHQ), 2-heptyl-4-hydroxyquinoline-*N*-oxide (HQNO), 2,4-dihydroxyquinoline (DHQ) and 2-aminoacetophenone (2-AA) derive from the same pathway. We addressed the question of how these molecules are synthesized and how the balance between the diverse products is achieved.
Methods: Biosynthetic enzymes were purified, reaction intermediates and analogs chemically synthesized and individual reactions were characterized using chromatographic and spectroscopic methods. Theoretical approaches to multi-step reactions were applied to provide complementary insight. Metabolite levels of wild-type and different knockout mutants of *P. aeruginosa* were compared.
Results: The "PQS response protein" PqsE, which has regulatory functions, is additionally involved in alkyquinolone (AQ) biosynthesis, acting as thioesterase on the labile intermediate 2-aminobenzoylacetyl-CoA (2-ABA-CoA) (1). The released 2-ABA is the substrate of PqsBC for formation of HHQ (2), but it is also found extracellularly and, depending on environmental conditions, decomposes to either DHQ or 2-AA, a physiological inhibitor of PqsBC. The monooxygenase PqsL is required for formation of HQNO (3). Remarkably, its reaction product is highly unstable, affecting the balance between HQNO and HHQ. The monooxygenase PqsH is essential for the hydroxylation of HHQ to form PQS but has a side activity on HQNO. The product, PQS-*N*-oxide, is unstable, rendering a "backdoor pathway" to PQS.
Conclusion: The apparent diversity of alkylquinolone-related secondary metabolites is the result of a finely tuned balance between enzymatic reactions and abiotic decomposition processes. Because labile pathway intermediates can react to different products, *P. aeruginosa* may exploit their chemical properties as a means of responding to environmental influences and the own metabolic condition.

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(3) Lépine *et al.*, *J. Am. Soc. Mass Spectrom.*, 15 (2004), 862-869.

NPV06

The cyclochlorotine mycotoxin is produced by the nonribosomal peptide synthetase CctN in *Talaromyces islandicus* ("*Penicillium islandicum*")

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Talaromyces islandicus ("*Penicillium islandicum*") is a widespread foodborne mold that produces numerous secondary metabolites, among them potent mycotoxins belonging to different chemical classes. A notable metabolite is the hepatotoxic and carcinogenic pentapeptide cyclochlorotine. It contains the unique 3,4-dichloroproline as well as the amino acids β-phenylalanine and 2-aminobutyric, which are rarely found in nature. Although the chemical structure has been known for over five decades, nothing is known about the biosynthetic pathway of cyclochlorotine.
Bioinformatic analysis of the recently sequenced genome of *T. islandicus* identified a wealth of gene clusters potentially coding for the synthesis of secondary metabolites. Here we show by RNA interference-mediated gene silencing that a nonribosomal peptide synthetase, CctN, is responsible for the synthesis of cyclochlorotine. Moreover, we identified novel cyclochlorotine chemical variants, whose production also depended on CctN expression. Surprisingly, the halogenase required for cyclochlorotine biosynthesis is not encoded in the *cct* cluster. Nonetheless, our findings enabled us to propose a detailed model for cyclochlorotine biosynthesis. The work is the first description of an NRPS gene cluster in the genus *Talaromyces*. The NRPS pathway could serve as a prototype for the biosynthesis of cyclic and chlorinated pentapeptide toxins in the genus *Talaromyces* as we identified similar gene clusters are prevalent among all available genomes of *Talaromyces* species, including those with significant impact in medicine and food industry.

NPV07

A yersiniabactin-like siderophore/virulence factor of entomopathogenic bacteria

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Entomopathogenic bacteria of the genera *Photorhabdus* and *Xenorhabdus* live in symbiosis with *Heterorhabditis* or *Steinernema* nematodes, respectively, which infect insect larvae. After infection, the bacteria are released into the insect haemolymph where they kill the insect by producing a wide range of bioactive compounds, including siderophores. Siderophores are small molecule virulence factors that are able to bind iron from the environment and transport it into the cell.[1] Analysis of sequenced *Photorhabdus* and *Xenorhabdus* genomes revealed a widespread gene cluster with high similarity to the yersiniabactin biosynthetic gene cluster of *Yersinia enterocolitica*. Yersiniabactin is a catecholate siderophore that is biosynthesised by a hybrid nonribosomal peptide synthetase (NRPS) - polyketide synthase (PKS) enzyme complex.[2] To investigate whether *Photorhabdus* and *Xenorhabdus* also produce related siderophore metabolites, a promoter exchange mutant was constructed in *X. szentirmaii*. Subsequent HPLC-MS analysis revealed the presence of a new metabolite, which differed from yersiniabactin by 14 mass units and was highly unstable against nucleophiles like methanol. High resolution MALDI MS along with labelling experiments confirmed an unusual sum formula of the molecule. Differential analysis by 2D NMR spectroscopy (DANS) performed directly on the crude cell extract provided support for the proposed structure.[3] The regulation and biological function of this yersiniabactin-like metabolite were also investigated. Construction of a ferric uptake regulator (Fur) mutant of *X. szentirmaii* showed that expression of this biosynthetic gene cluster is under the regulatory control of Fur as well as other genes involved in iron metabolism. In addition to functioning as a siderophore, this compound might also act as a virulence factor in the lifestyle of these entomopathogenic bacteria.[4]

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[3] Forseth, R. R. and Schroeder, F. C., Methods Mol Biol, 2012, 944: 207-219.
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NPV08

Construction of a *Corynebacterium glutamicum* platform strain for the production of high-value plant secondary metabolites

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Question: The soil-bacterium *Corynebacterium glutamicum* is an important microorganism in industrial biotechnology for the production of bulk chemicals, especially amino acids. Due to the presence of a complex catabolic network for aromatic compounds, there was minor effort for production of aromatic compounds using *C. glutamicum*. We aim to exploit the yet untapped potential of *C. glutamicum* to produce naturally occurring polyphenolic compounds by abolishment of competing degradation pathways for aromatic precursor metabolites, *e.g.* phenylpropanoids.
Methods: The *C. glutamicum* genome was modified by in-frame deletions of relevant gene clusters coding for enzymes of the catabolic network for aromatic compounds. Plasmid-based expression of heterologous genes was optimized for the production of distinct polyphenols, namely stilbenes and flavanones, in the newly constructed strain. Product titers were quantified employing LC-MS analysis after whole cell extraction using organic solvents.
Results: Very recently, we identified the Phd-pathway essential for phenylpropanoid degradation allowing growth of *C. glutamicum* with phenylpropanoids as sole carbon and energy source [1]. Phenylpropanoids represent the precursors of nearly all plant polyphenols [2]. By deleting three gene clusters comprising altogether 20 genes essential for degradation of phenylpropanoids and other aromatic compounds, we obtained a strain that was subsequently used for the production of polyphenols (stilbenes and flavanones). Heterologous expression of

codon-optimized genes coding for 4-coumarate: CoA-ligase (4CL) and stilbene synthase (STS) in this strain led to the production of three different stilbenes starting from phenylpropanoid precursors. Several cultivation parameters influencing growth and gene expression were optimized with respect to improved product titers. Under optimal conditions, stilbene concentrations ranging from 60 to 150 mg/L could be obtained. In the same manner, heterologous expression of chalcone synthase (CHS) and chalcone isomerase (CHI) led to production of 20-25 mg/L of corresponding (2S)-flavanones from two tested phenylpropanoids.

Conclusions: While these results are the first demonstration of the polyphenol production capabilities of *C. glutamicum*, the strain also offers huge potential for high-yield production of other high-value aromatic compounds.

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NMV01

Plant pathogenic anaerobic bacteria use aromatic polyketides to access aerobic territory

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Worldwide, over 25 % of food crop is lost because of infectious plant diseases, including microbe-induced decay of harvested crops[1]. In wet seasons and under humid storage conditions, potato tuber are readily infected and decomposed by *Clostridium puniceum* that grow in the absence of oxygen[2]. Although the association of these anaerobic bacteria with rotting vegetables has been described over a century ago, to date nothing is known about any factors promoting the devastating plant disease. In particular, the central question by which means an air-sensitive plant pathogen can enter and claim an oxygen-rich plant environment has remained unanswered. We demonstrate that these anaerobic plant-pathogenic clostridia harbor a gene locus (type II polyketide synthase) to produce unusual polyketide metabolites (clostrubins) with dual functions[3,4]. The clostrubins, which act as antibiotics against other microbial plant pathogens, enable the anaerobic bacteria to survive an oxygen-rich plant environment. Genome mining revealed a type II PKS gene locus for clostrubin biosynthesis that is unique among all hitherto sequenced genomes (>200) non-plant pathogenic clostridia. Quantification of tuber degradation, live/dead FACS analysis and electron microscopy show dramatically different phenotypes of wild type and targeted clostrubin-negative mutant under aerobic conditions. Clostrubin synthase may be a useful target for development of antibacterial therapeutics and plant-protective agents.

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NMV02

Treasures of the submarine rain forests – Kelp-associated Planctomycetes as a novel source for powerful bioactive compounds

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Planctomycetes are a group of budding bacteria widely and ubiquitously distributed in both, aquatic and terrestrial environments. Enabled by their two staged life cycle, these bacteria form biofilms and attach to the surface of higher organisms such as kelp. These brown alga form large 'forests' in shallow coastal marine waters, resembling the submarine equivalent of tropical rain forests. In some of these habitats Planctomycetes were found to be the major players in remineralization of carbon produced by kelp. Playing a key role in these highly competitive organismic interactions, the slow-growing Planctomycetes must produce a wide variety of complex chemical molecules to succeed and outcompete other organisms. However, efforts to cultivate novel planctomycetal strains in order to exploit their potential as novel bioactive compound producers are still scarce.

We here present a targeted cultivation and screening strategy, which has led to the successful isolation of more than 250 strains including 12 novel planctomycetal genera associated with brown- (kelp) and other algal species. Following isolation and identification, we yet sequenced the genomes of five of our novel isolates and investigated their potential as bioactive compound producers through bioinformatic prediction and bioactivity screening. Employing a minimal inhibition concentration assay, crude extracts of our strains showed effective growth inhibition properties against a palette of diverse bacterial and fungal target strains with relevance in human or general pathogenicity.

Taken together, we are encouraged to further investigate the potential of Planctomycetes associated with higher organisms as secondary metabolite and novel bioactive compound producers by specifically enriching and isolating novel strains. We aim to employ them in our activity and bioinformatic screening, tapping the powerful resources of the planctomycetal secondary metabolome yet to be discovered.

NMV03

Diversity and metabolite profiles of Actinobacteria from the Atacama Desert

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Atacama Desert is well known as the driest place and as the oldest desert on Earth which comprises a number of extreme environments, from hyperarid soils, various types of salt lakes (Salares) with different composition of mineral salts and its concentration as well as also hot springs and geysers. These habitats are exposed constantly to very high UV radiation and strong fluctuations in temperature. These conditions make the Atacama Desert a hot spot of microbial biodiversity, in which adaptation plays an important role in microbial life, leading to a great diversity of unique microorganisms.

Question: Molecular studies based on 16S rRNA gene libraries and high throughput sequencing have shown a high diversity of Actinobacteria along the Atacama Desert, but only few studies have been done in the isolation of new strains and their exploration for biotechnological applications.

Results: The focus of our studies is on the isolation, identification and characterization of Actinobacteria from rhizosphere samples of endemic plants from the Atacama Desert (near Socaire) and their potential as producers of new antibiotics. A total of 102 strains were isolated and identified using the 16S rRNA gene sequences. The isolates belong to the genera *Streptomyces*, *Nocardia*, *Rhodococcus*, *Micromonospora*, *Arthrobacter*, *Kribella* and *Kocuria*. Preliminary experiments of crude extracts showed a high diversity of compounds and antibiotic activity against *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus lentus*.

Conclusion: The isolation of Actinobacteria from extreme environments is presenting a great diversity of these bacteria which are expected to represent a promising source of novel antibiotic active natural products. We expect to find new bioactive compounds in the isolated Actinobacteria.

NMV04

Discovery of the trypacidin gene cluster in the human-pathogenic fungus *Aspergillus fumigatus*

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Question: The opportunistic human pathogen, *Aspergillus fumigatus* produces a myriad of different natural products (NPs). Pertaining to the different biosynthetic gene cluster of *A. fumigatus*, many have been identified and correlated to their respective metabolites, but there still remain several unidentified. Understanding the complete metabolome of such a major pathogenic fungus is crucial in establishing proper techniques of treatment and identification in patients. Here we assigned the anthraquinone derivative, trypacidin, its respective gene cluster. Since trypacidin was shown to be located in the spores, the main route of distribution in the environment by the fungus, its role in the interaction with amoebae and macrophages was studied.

Methods: The genome of *A. fumigatus* was mined for NP gene clusters based on comparison of clusters of known NPs similar in structure with *A. fumigatus* and other Aspergilli. The polyketide synthase (PKS) of the identified cluster was deleted and confirmed by southern blot and LCMS analysis. Both, mutant and parental strain were studied in phagocytosis assays with the amoeba *Dictyostelium discoideum* and human macrophages.

Results: Here we have pin-pointed the known NP trypacidin to its biosynthetic gene cluster in *A. fumigatus*. This metabolite, in particular, is of interest because of its biological activity and possible role in the infection process. It was shown that macrophages would phagocytize the spores of the knock-out mutant more frequently than the parental strain. This indicates that this spore-related metabolite could play a role in the interaction of the fungus with the macrophage in one of the first lines of defence for the host during the infection process. Additionally, when confronted with the phagocytic amoeba, *D. discoideum*, they also showed to be sensitive when introduced to the A. fumigatus metabolite.
Conclusion: This shows from an ecological perspective that the fungus protects its spores by arming them with NPs to not only avoid from being killed in the environment, but also in its potential host.

Mattern *et al.* Appl Microbiol Biotechnol. 2015 Dec;99(23):10151-61

NMV05
Experimental evolution of metabolic dependency in bacteria
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Bacteria that adapt to specialized environments frequently lose biosynthetic genes, which render them dependent on an environmental uptake of the corresponding metabolites. However, it remains generally unclear whether selection or drift is driving this genome-streamlining. Here we experimentally determined the propensity of bacteria to lose biosynthetic functions when the metabolite is environmentally available. For this, we serially propagated replicate lines of the bacterium *Escherichia coli* for 2,000 generations in an amino acid-containing environment. A subsequent quantification of auxotrophic mutants revealed that genotypes that essentially required amino acids to grow evolved already after 1000 generations and were highly abundant (up to 20 %) in all replicate populations after 2,000 generations. Derived auxotrophic mutants were significantly more productive and fitter than the prototrophic ancestor in the presence of amino acids. However, this growth advantage was lost in amino acid-deficient environments, suggesting environmental compensation of amino acids contributed to the observed gains in fitness. The adaptive loss of biosynthetic functions resulted from mutations in both structural and regulatory genes. Interestingly, the auxotrophic genotypes derived amino acids via an environmental uptake as well as by cross-feeding from co-existing prototrophic strains. Our study provides quantitative evidence that nutrient-containing environments exert a strong selection pressure for a loss of metabolic functions in bacteria and has significant implications for the evolution of microbial genomes and communities.

NMV06
Virulence in smut fungi – insights from evolutionary comparative genomics
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Smut fungi comprise a large group of about 1,400 species, which all need to establish fine tuned biotrophic interactions with their hosts to complete sexual reproduction. Host plants are typically grasses, including important crops like maize, sorghum, barley, wheat, oat and sugar cane. Five smut genomes of different species parasitizing specific hosts are available: *Ustilago hordei* parasitizing barley [1], *U. maydis* infecting maize and its wild ancestor teosinte [2], *Sporisorium scitamineum* growing on sugar cane, *S. reilianum* f. sp. *zeae* parasitizing maize [3] and *S. reilianum* f. sp. *sorghi* growing on sorghum grass. Based on their typically narrow host range and the availability of molecular tools enabling genetic manipulations, smut fungi are a particularly interesting model to uncover genes contributing to virulence and potentially host specificity.
We used a computational approach to identify genes in each species with an interesting evolutionary history: genes showing signatures of positive selection and orphan genes. To detect genes showing signs of positive selection, we reconstructed families of homologues using clustering techniques. We employed this data set to search for genes with elevated rates of non-synonymous substitutions by applying non-homogenous models of sequence evolution without *a priori* assumptions. Families of homologous sequences containing only members of one species were considered to be orphan genes and further confirmed by a tblastn search against the non-redundant nucleotide data base of NCBI.
A deletion mutant of a gene under positive selection in *S. reilianum* f. sp. *zeae* (*sr10529*) showed drastically reduced virulence symptoms, indicating an important contribution to pathogenicity. Since the orthologous gene in *U. maydis*, *um01375* (*pit2*), was previously characterized as inhibitor of a group salicylic acid-induced cysteine proteases [4], we currently assess whether the allele of *S. reilianum* f. sp. *zeae* shows differences in inhibition of cysteine proteases compared to the allele of *S. reilianum* f. sp. *sorghi* in apoplasic fluids. To obtain clues of a potential role in host specificity, we additionally exchange *pit2* alleles exchanges between both variants of *S. reilianum* and monitor the influence on virulence. In this way, clues of a potential role in host specificity for Pti2 can be obtained.
Whether orphan genes play a role in virulence of *U. maydis* is currently investigated by creating deletion mutants.

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NMV07
Ancient *Yersinia pestis* genome from a post-Black Death outbreak in Southwestern Germany
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Question: *Yersinia pestis* has been implicated in three pandemics in human history. Of the three, the most deadly was the second plague pandemic, which began with the infamous Black Death in 1347 and continued with recurrent outbreaks in Europe until the late 18th century. Little is known about the distribution of plague lineages in Europe during and after the Black Death. Current hypotheses suggest that post-Black Death plague outbreaks were either attributed to recurrent plague introductions from the East, or to a local European focus, which persisted after the Black Death. To test these hypotheses we attempt genome reconstruction of a *Yersinia pestis* strain involved in a 16th century plague outbreak in Ellwangen, in Southwestern Germany.
Methods: We tested 79 tooth remains from three mass graves in the site of Ellwangen, for the presence of a *Y. pestis*-specific gene using a previously established qPCR-screening assay (Schuenemann, 2011). Samples yielding amplification products were used for whole genome hybridization capture and subsequent phylogenetic analysis.
Results: We were able to reconstruct a complete *Yersinia pestis* genome from a plague victim from Ellwangen. The genome-wide data obtained allowed us to compare the bacterial strain from the 16th century to both modern and other ancient *Y. pestis* strains in order to make inferences on the spread of this historically important pathogen. Phylogenetic reconstruction identified this 16th century strain as a descendant of a previously described Black Death strain from London, 1348 - 1350 (Bos, 2011). Of those sampled to date, no extant lineages of *Y. pestis* belong to the Ellwangen lineage.
Conclusion: We were able to reconstruct a *Yersinia pestis* genome from a 16th century plague outbreak in South Germany. Since this lineage has a previously described Black Death strain as its ancestor, our data add legitimacy to the existence of a historical European plague focus, which is now likely extinct.

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NMV08

Impact of the extent of pyoverdine production in *Pseudomonas* populations on the development of cooperation

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Question: A central question in evolutionary biology is the development of cooperativity in cultures of cooperators and free-riders [1, 2]. There are several solutions to this problem, one comes from theoretical models combining internal evolution and growth dynamics of a population [2, 3]. Here we analyze how the extent of pyoverdine production of *Pseudomonas putida* strains influences development, extent and duration of cooperation.
Methods: Our experimental model is the soil bacterium *P. putida* KT2440 and its production of the iron scavenging siderophore pyoverdine. For our experiments we used cooperator strains which either have a wildtype regulated expression of the pyoverdine master regulator PfrI and/or a constitutive one. To determine the selection pressure we measured the relative fitness. Furthermore we examined the development of subpopulations composed of a free-rider and a producer (cooperator). After forming groups with random distribution of initial cell number and producer fraction, growth for different periods of time and merging, producer fraction and cell number were determined.
Results: The extent of pyoverdine production by cooperators increases the relative fitness advantage of free-riders. Regarding the development of cooperativity in merged groups we obtain a constant increase with the wildtype, and a transient one using strains which have a constitutive pyoverdine production. The results are in agreement with theoretical models predicting a transient development of cooperativity under our experimental conditions. The extent and duration of cooperativity depends on the initial cell number, producer fraction and environmental conditions.
Conclusion: A higher extent of pyoverdine production leads to higher metabolic costs for the cooperator, and increases the availability of pyoverdine for free-riders. As a consequence we only observed a stable increase of cooperativity under our experimental conditions when pyoverdine production is controlled by the regulatory system of the wildtype.

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OTV01

Two regulatory RNA elements affect toxin-driven depolarization and persister formation in *Escherichia coli*

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Bacteria generate subpopulations with distinct phenotypic characteristics in order to guarantee survival under a variety of environmental conditions. This strategy is often referred to as bet-hedging. Formation of persister cells represents one of these survival strategies. Persisters are viable but non-growing (dormant) cells that appear at very low rates (~ 0.01 %) in exponentially growing populations. They are tolerant (not resistant) to environmental cues, such as antibiotics, and their generation mainly depends on toxin-antitoxin (TA) systems.
Escherichia coli contains several TA systems, among these the TisB/IstR-1 system that drives persister formation under DNA-damaging conditions [1]. Transcription of the toxin gene *tisB* is repressed by LexA, the master regulator of the SOS response. When DNA damage occurs, e.g. through the action of antibiotics, *tisB* transcription is induced due to self-cleavage of LexA. However, translation of *tisB* is inhibited by two regulatory RNA elements: the RNA antitoxin IstR-1 [2] and an inhibitory structure in the 5'UTR of *tisB* mRNA [3]. Once TisB toxin is produced, it depolarizes the inner membrane [4], which is believed to generate non-growing cells.
Here, we used cell staining combined with flow cytometry to investigate dynamics of depolarization on the single-cell level. Our data provide evidence that only a subset of cells undergoes TisB-dependent depolarization (~ 50 % in wild-type cultures) and that both regulatory RNA elements contribute to this phenotypic heterogeneity. Furthermore, automated imaging of persister assays revealed that both the level and the duration of persistence are increased when the inhibitory action of the two RNA elements is abolished. Our data provide important insights of how regulatory RNAs shape bacterial subpopulations.

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OTV02

IscR of *Rhodobacter sphaeroides* functions as repressor of genes for iron-sulfur metabolism and represents a new type of iron-sulfur-binding protein

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All aerobic living organisms have to face oxidative stress caused by reactive oxygen species (ROS) which are generated during respiration. While iron and iron-sulfur (Fe-S) clusters are essential for virtually all forms of life, free iron favours ROS generation in the iron catalysed reduction of H_2O_2, known as Fenton reaction. Furthermore, in photosynthetic organisms like *Rhodobacter sphaeroides*, iron limitation results in a 2- to 10-fold increase in ROS levels compared with those found in cells grown in iron-replete conditions. Thus, organisms have to coordinate and balance their responses to oxidative stress and iron availability.
E. coli IscR is an important regulator of iron metabolism and acts as a sensor of the cellular demands for Fe-S cluster biogenesis by binding a Fe-S cluster with $(Cys)_3(His)_1$ ligation. The three essential cysteine residues are conserved in many proteobacteria, but only a single cysteine residue is present in all *Rhodobacteraceae* IscR. Using a global transcriptome analysis in the background of a Δ*iscR* strain we showed that *R. sphaeroides* IscR functions as transcriptional repressor of genes preceded by a specific DNA binding motif (Iron-Rhodo-box). Furthermore, we confirmed that IscR from *R. sphaeroides* coordinates a Fe-S centre despite the missing cysteine residues and provide first hints to amino acids involved in this ligation. These results contribute to understand the sensing of oxidative stress and iron starvation in *Rhodobacteraceae*.

OTV03

Formation of polyphosphate by polyphosphate kinases and its relationship to Poly(3-Hydroxybutyrate) accumulation in *Ralstonia eutropha*

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A protein (PhaX) that interacted with poly(3-hydroxybutyrate) (PHB) depolymerase PhaZa1 and with PHB granule-associated phasin protein PhaP2 was identified by two-hybrid analysis. Deletion of *phaX* resulted in an increase in the level of polyphosphate (polyP) granule formation and in impairment of PHB utilization in nutrient broth-gluconate cultures. A procedure for enrichment of polyP granules from cell extracts was developed. Twenty-seven proteins that were absent in other cell fractions were identified in the polyP granule fraction by proteome analysis. One protein (A2437) harbored motifs characteristic of type 1 polyphosphate kinases (PPK1s), and two proteins (A1212, A1271) had PPK2 motifs. *In vivo* colocalization with polyP granules was confirmed by expression of C- and N-terminal fusions of enhanced yellow fluorescent protein (eYFP) with the three polyphosphate kinases (PPKs). Screening of the genome DNA sequence for additional proteins with PPK motifs revealed one protein with PPK1 motifs and three proteins with PPK2 motifs. Construction and subsequent expression of C- and N-terminal fusions of the four new PPK candidates with eYFP showed that only A1979 (PPK2 motif) colocalized with polyP granules. The other three proteins formed fluorescent foci near the cell pole (apart from polyP) (A0997, B1019) or were soluble (A0226). Expression of the *Ralstonia eutropha ppk* (*ppkReu*) genes in an *Escherichia coli* Δ*ppk* background and construction of a set of single and multiple chromosomal deletions revealed that both A2437 (PPK1a) and A1212 (PPK2c) contributed to polyP granule formation. Mutants with deletion of both genes were unable to produce polyP granules. The formation and utilization of PHB and polyP granules were investigated in different chromosomal backgrounds.

Tumlirsch T, Sznajder A, Jendrossek D. 2015. Formation of polyphosphate by polyphosphate kinases and its relationship to poly(3-hydroxybutyrate) accumulation in *Ralstonia eutropha* strain H16. Appl Environ Microbiol 81:8277-8293

OTV04

Identification of the key enzyme of roseoflavin biosynthesis

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The rising number of multi-resistant pathogens demands the development of new antibiotics in order to reduce the lethal risk of infections (Pedrolli, *et al.*[1]).
Here, we investigate roseoflavin, a vitamin B_2 analogue which is produced by *Streptomyces davawensis* and *Streptomyces cinnabarinus*. We consider roseoflavin to be a "Trojan horse" compound. Its chemical structure is very similar to riboflavin but in fact it is a toxin. Furthermore, it is a clever strategy with regard to the delivery of an antibiotic to its site of action but also with regard to the production of this chemical: The producer cell has only to convert a vitamin (which is already present in the cytoplasm) into a vitamin analog. Roseoflavin inhibits the activity of Flavin depending proteins (Langer, *et al.*[2]), which makes up to 3.5 % of predicted proteins in organisms sequenced so far (Macheroux, *et al.*[3]).
We sequentially knocked out gene clusters and later on single genes in order to find the ones which are involved in the roseoflavin biosynthesis. Consequently, we identified the gene *rosB*, coding for the protein carrying out the first step of roseoflavin biosynthesis, starting form Flavin mononucleotide.
Here we show, that the protein RosB has so far unknown features. It is per se an oxidoreductase, a decarboxylase and an aminotransferase, all rolled into one enzyme. A screen of cofactors revealed needs of oxygen, NAD^+, thiamine and glutamic acid to carry out its function. Surprisingly, thiamine is not only needed for the decaboxylation step, but also for the oxidation of 8-demethyl-8-formyl Flavin mononucleotide. We had managed to isolate three different Flavin intermediates with different oxidation states, which gave us a mechanistic insight of RosB functionality. Our work points to a so far new function of thiamine in *S. davawensis*. Additionally, RosB could be extremely useful for chemical synthesis. Careful engineering of RosB may allow the site-specific replacement of methyl groups by amino groups in polyaromatic compounds of commercial interest. Finally, the complete clarification of the roseoflavin biosynthesis opens the possibility of engineering cost-effective roseoflavin producing strains.

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OTV05

The cell cycle of *Corynebacterium glutamicum*

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The bacterial cell cycle has been studied for decades in classical model organisms such as *Escherichia coli* or *Bacillus subtilis*, where overlapping replication cycles allow for rapid growth. Other model bacteria like *Caulobacter crescentus* complete only a single round of replication per division cycle. However, in *Corynebacterium glutamicum*, which is of economic importance as an amino acid producer and closely related to pathogens like *Mycobacterium tuberculosis*, cell cycle parameters are largely unknown. Therefore, we set out to elucidate how the origin of replication and the replisome are spatiotemporally localized and how replication is coordinated in this organism.
Allelic replacements were used to produce fluorescently tagged protein versions of the origin-binding protein ParB and the replisome sliding clamp DnaN and studied *in vivo* using fluorescence microscopy and single cell tracking via time lapse analysis. Origin (*oriC*) to terminus (*terC*) ratios were quantified by marker frequency analysis and whole genome sequencing. Chromosome numbers were estimated by flow cytometry using the DNA dye SYBR GreenI.
Predivisional *C. glutamicum* cells have two chromosomes as judged by two initial *oriC's* labeled by ParB-eYFP fusion protein at cell pole positions. Replication initiation gives rise to 2-3 further *oriC's* that dynamically localize to newly formed septa. Up to six dynamic DnaN-mCherry foci were determined per cell; ParB and DnaN foci number correlate with cell length and increase over time. Coexpression of fluorescently tagged ParB and DnaN hint to highly variable cohesion times of sister *oriC's*. During fast growth in complex medium *oriC*/*terC* ratios are significantly higher than during the stationary phase or slow growth in minimal medium. Analyzing the DNA content after replication runout revealed up to eight chomosomes per cell, depending on the growth medium applied. Reassuringly, flow cytometry analysis confirmed remarkably high chromosome numbers per cell, which is supported by microscopy data suggesting up to 5 ParB-eYFP and up to six DnaN-mCherry foci per cell.
In summary, our data give further insights in the spatiotemporal chromosome organization of *C. glutamicum* and allow proposing complete cell cycle models under different growth conditions.

OTV06

The heterododecameric, membrane-associated bacterioferritin of *Magnetospirillum gryphiswaldense* is not involved in magnetite biosynthesis

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Iron is essential for almost all organisms. Because of its versatile biochemical properties it serves as a cofactor in a multitude of biochemical reactions including respiration, photosynthesis or DNA synthesis. Excess iron, however is toxic due to its ability to increase the formation of reactive oxygen species via the Fenton reaction. To avoid iron-mediated toxicity, bacteria use iron storage proteins of the ferritin-like superfamily to store excess iron as a ferrihydrite-like inert iron mineral phase and, thus, regulate cytosolic iron levels. A rather unusual role of ferritin-like proteins was proposed for magnetotactic bacteria (MTB). In species of the *Magnetospirillum* genus a so far uncharacterized ferritin-like component was found to play a major role in the intracellular biomineralization pathway of membrane-enclosed ferrimagnetic magnetite $[Fe^{2+}(Fe^{3+})_2O_4]$ crystals, called magnetosomes. These unique prokaryotic organelles enable magnetospirilla and other MTB to orient themselves along the Earth's magnetic field lines to find growth-favoring an- or microoxic zones within their aquatic habitats.
In order to identify the ferritin-like iron metabolite and its role for magnetite biomineralization we studied ferritin-like proteins of *M. gryphiswaldense*. Using deletion mutagenesis, transcomplementation assays, as well as biochemical analyses we show that the ferritin-like proteins Bfr and Dps are not required for magnetite biomineralization but involved in oxidative stress resistance. We also provide evidence that Bfr of *M. gryphiswaldense* is a membrane-associated, heterododecameric protein complex able to bind DNA and thereby protect it against oxidative damage.

OTV07

The early immune response by human monocytes to *Candida albicans*

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Question: Circulating blood monocytes 'patrol' along the lumen of blood vessel and are recruited to peripheral tissues during inflammation where they differentiate either into macrophages or dendritic cell. Monocyte recruitment to sites of infection is crucial in restricting growth and invasion of various microorganisms including pathogenic fungi *C. albicans*. Although monocytes can exert bactericidal activity via phagocytosis and production of reactive oxygen and nitrogen species, their immediate response to pathogenic fungi is poorly characterized.
Methods: Monocytes were isolated using magnetic beads technique and tracked for their interaction with *C. albicans* by live cell imaging with confocal laser scanning microscopy. To visualize extracellular traps and protein binding fixed cell imaging and immunofluorescence staining was used. To quantify extracellular trap formation Fluorescence assay was used. Extracellular traps were isolated with enzymatic digestion of DNA and *C. albicans* growth was monitored with optical density measurement.
Results: Monocytes react on *C. albicans* by immediate phagocytosis and by release of decondensed DNA to form extracellular traps (called monocytic extracellular traps: MoETs). These traps contain citrullinated histones and myeloperoxidase, which are specific markers for extracellular traps. Both types of monocytes (CD14++CD16-/CD14+CD16++) form MoETs within the first three hours upon contact with *C. albicans*. MoETs immobilize and kill *C. albicans*, as demonstrated by microscopy and killing assays. Interestingly we found complement regulator protein factor H attached to the surface of MoETs and also NETs. Whether recruitment of factor H restricts complement activation on the extracellular traps and/or mediates additional functions is so far unclear.
Conclusion: Altogether human monocytes recognize *C. albicans* and immediately start strong defense mechanisms by phagocytosis and by releasing DNA traps similar to neutrophils.

OTV08

Biological agents in sight – danger avoided: the GESTIS-database on biological agents combines expertise

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Objectives: Which risks do arise from microorganisms? What are the potential infections and pathways of transmission of microorganisms? And more important, how can employees be protected? What does the company do for the occupational safety and health? A network of expertise is the answer: the GESTIS-database on biological agents.
Methods: Figuratively in biotechnology all "colors" are represented: red, white and green biotechnology is established in all areas of everyday life. In red biotechnology microorganisms are used to prepare new therapeutic and diagnostic procedures. With the white biotechnology detergents and cosmetics are produced more efficiently in industry. New plant species are grown in the green biotechnology. There are a lot of specific technical applications of biological agents that are developed in many companies and successfully used in production. Also many biological agents are applied in science, research and in the laboratory animal facility. Without any risks?
Employees come into contact with biological agents, for example in cleaning and restoration work or in (veterinary) medicine, in agriculture and forestry, waste water treatment or waste management, in biogas plants and slaughterhouses. Hereby hazards occur, that need to be controlled safely. Therefore, biohazards have to be considered in the risk assessment in many work places in various industries and also in public institutions such as colleges and universities. The degree of risk has to be considered as a function of the duration of the potential exposure, the occupational activities and *classified biological agents, which are categorised into four groups according to their level of risk of infection. T*he appropriate safety measures have to be derived, which requires a high level of knowledge. So far all the information needed can be found widespread in various media only.
Results: Hence, an internet based database on biological agents has been developed by a collaboration between the Ministry of Labour and Social Affairs (BMAS), the German Social Accident Insurance Institution for the raw materials and chemical industry (BG RCI) and the German Social Accident Insurance (DGUV). Maintained by the Institute for Occupational Safety and Health (IFA) of the German Social Accident Insurance (DGUV) the database containsfreely accessible information on biological agents.
Conclusion: Risks arising from activities involving biological agents must be safely assessed and controlled. The additional value of this new database is that occupational safety and health aspects are considered in addition to scientific facts about potential infection, disease patterns and pathways of transmission of microorganisms. Hereby a database has been achieved not only for specialists and scientists but for employers, employees, contractors and other interested parties, such as experts on occupational safety and occupational physicians.

STV01

Cross-talk between the Kdp and Pho two-component systems interconnects K^+ and PO_4^{3-} homeostasis in *Escherichia coli*

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Potassium is the most abundant cation in bacteria and is crucial for several cellular processes like maintenance of turgor, regulation of the intracellular pH and activation of enzymes. *Escherichia coli* maintains high intracellular K^+ levels by uptake through the low affinity transporters Trk and Kup and the high affinity transporter KdpFABC. Synthesis of KdpFABC is regulated by the KdpD/KdpE two-component system in response to K^+ limitation or osmotic stress (1). The bifunctional histidine kinase KdpD senses intra- and extracellular K^+ levels to regulate its kinase and phosphatase activities (2, 3). Cells lacking the low affinity transporters Trk and Kup and the histidine kinase KdpD are unable to grow under extreme K^+ limitation because KdpFABC cannot be synthesized. In this study we found that an additional deletion within the *pstSCAB-phoU* operon encoding the phosphate-specific transporter Pst can rescue this growth defect. The Pst transporter forms a signaling complex together with the PhoR/PhoB two-component system to induce gene expression of the Pho regulon under phosphate starvation, however, deletion of any of the *pst* genes causes constitutive activation. In these mutants we observed *kdpFABC* expression in the absence of KdpD, which was found to be dependent on PhoR. Our data suggest that cross-talk between the KdpDE and PhoRB two-component systems mediates the balance between positively and negatively charged ions in the cell.

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STV02

Coping with stress – convergence of cell cycle and stress signaling pathways by a bifunctional histidine kinase

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Free-living bacteria are frequently exposed to various environmental stress conditions, including heat, changes in osmolarity or nutrient depletion. To survive under such adverse conditions, cells must induce pathways that prevent and alleviate cellular damages, but they must also adjust their cell cycle to guarantee cellular integrity. Although past work provided important insight into bacterial stress responses, the molecular mechanisms adjusting cell cycle progression in response to stress conditions remain poorly understood.
Here, we analyzed the impact of various stress conditions on the regulation of the cell cycle in the model bacterium *Caulobacter crescentus* by using a combination of genetics and cell biology techniques.
We observed that different stress conditions result in drastic changes in cellular morphology resulting from altered regulation of cell cycle processes. Most conspicuously, increased external salt or ethanol concentrations lead to strong cell filamentation and an accumulation of multiple chromosomes, indicating that cells grow and replicate their DNA but stop dividing. Upon stress release cells resume cell division within a few hours, suggesting that the observed filamentous phenotype is due to transient changes in cell cycle regulation. Global gene expression analysis by RNA-sequencing technology revealed that most genes belonging to the regulon of the key cell cycle and cell division regulator CtrA are differentially expressed under these conditions. Consistently, we observed a rapid drop in CtrA abundance upon exposure to salt, ethanol or heat stress, which we found results from remarkably fast CtrA proteolysis by the protease ClpXP. Our genetic studies suggest that the increased rate of CtrA degradation stems from a stimulation of the phosphatase activity of the bifunctional histidine kinase CckA that is normally required to phosphorylate and thus activate and stabilize CtrA in pre-divisional cells. The stimulation of CckA's phosphatase activity under stress conditions leads to rapid inactivation and destabilization of CtrA and consequently to a cell division block.
Based on our results we hypothesize that the membrane-bound kinase CckA directly or indirectly responds to membrane stress upon salt or ethanol exposure, and thereby signals CtrA to block cell division and cell cycle completion. A specific block of cell division under adverse conditions might provide an important strategy that helps cells to maintain cellular integrity and to survive.

STV03

Genetic analysis of competence development in *Micrococcus luteus*

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The current knowledge of the process of natural transformation in bacteria is derived from only a few model organisms. Although natural transformation was discovered in members of the *Actinobacteria* (high G+C Gram-positive bacteria) already more than four decades ago, the structural components or the regulation of the competence system have not been studied in any representative of the entire phylum.
In this report, we show that a distinct type of pilus biogenesis genes (*tad* genes), which so far had been connected only with biofilm formation, adherence and virulence traits, are required for genetic transformation in the actinobacterial species *Micrococcus luteus*. By expressing a tagged variant of the putative major prepilin subunit and immunofluorescence microscopy, filamentous structures extending from the cell surface could be visualized. Genetic analysis indicated that two *tad* gene islands

complementarily contribute to the formation of a functional competence pilus in this organism.
We also confirm previous observations that *M. luteus* competence is a transient state and depends on the growth phase and nutrient availability. By using eYFP transcriptional reporter strains we show that under inducing conditions competence genes are expressed in only a fraction of the cells of an isogenic population. In order to further elucidate the mechanisms governing competence development, we generated a large set of regulatory knockout mutants (~ 70 genes) and screened them for their ability to undergo transformation. This screen led to the identification of key factors, probably part of a stringent response regulatory circuit, which links nutritional signals to competence induction. Our data provides first insights into the components and the regulation of natural transformation in a member of the *Actinobacteria* phylum and may also prove to be relevant to a number of important pathogens belonging to the same taxonomic group.

STV04
Regulation of phenotypically heterogeneous anthraquinone production in *Photorhabdus luminescens* via the novel transcriptional activator AntJ
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Photorhabdus luminescens, a bioluminescent Gram-negative bacterium, contains a complex life cycle, which involves a symbiotic interaction with nematodes as well as a pathogenic association with insect larvae. *P. luminescens* exists in two distinct phenotypic forms, designated as primary and secondary cells. Individual primary cells undergo phenotypic switching after prolonged cultivation and convert to secondary cells. Compared to primary cells, secondaries are less bioluminescent, unable to live in symbiosis with nematodes and non-pigmented due to the absence of anthraquinones. The biological role of the anthraquinone pigments has not yet been elucidated, but a weak antimicrobial activity as well as a feeding deterrence against birds has been hypothesized. Anthraquinone biosynthesis is achieved via a type II polyketide synthase and several modifying enzymes (AntA-I) (1).
Via chromosomally integrated P_{antA}-*mCherry* fusions a heterogeneous promoter activity of the anthraquinone operon could be observed in wild-type primary cells (2). Additionally, the regulator AntJ has been identified as activator of *antABCDEFGHI* expression by binding within the respective promoter region. Overproduction of AntJ resulted in a homogeneous activation of P_{antA} in primary cells. Interestingly, a basal homogeneous distribution of P_{antA}-activity was observed also in the non-pigmented secondary cells, revealing that a yet unknown ligand of AntJ might be the key to heterogeneous anthraquinone production. Overproduction of AntJ was sufficient to restore the pigmentation in secondary cells. This demonstrates that the loss of pigmentation in secondaries is not caused by a global change of the overall metabolic state, but rather due to solely phenotypic heterogeneity of P_{antA} activity.

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STV05
The *Aspergillus fumigatus* DHN-melanin production is regulated by MEF2-like (RlmA) and bHLH (DevR) transcription factors
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Melanins play a crucial role in defending organism from external hazards. In many pathogenic fungi, melanin production is also essential for virulence. The human pathogenic fungus *Aspergillus fumigatus* produces two different types of melanins. One of them, the dihydroxynaphthalene (DHN)-melanin, is classified as secondary metabolite, and is mainly produced during sporulation. It plays a crucial role in protecting conidia from being recognized by the host immune system. The DHN-melanin biosynthesis pathway contains six genes grouped in a cluster. The central gene in the cluster is *pksP*, which codes for a polyketide synthase. Promoter analyses identified specific DNA binding sites in the *pksP* promoter region that can be potentially recognised by basic helix-loop-helix (bHLH) and myocyte enhancer factor-2 (MEF2-like) transcriptional regulators. Independent and combined deletion of two genes coding for the transcription factors DevR and RlmA disturbed sporulation and melanisation. Furthermore, both transcription factors were proven to control the expression of the DHN-melanin gene cluster. *In vitro* surface plasmon resonance interaction analysis indicated that the computationally predicted binding sites were recognized by the respective transcriptional regulators with high affinity and specificity. Moreover, *in vivo* experiments made by targeted mutations of the *pksP* promoter, combined with either deletion or over-expression of the transcriptional regulators, confirmed that both factors cooperatively regulate melanin biosynthetic genes. Additionally, these experiments revealed that DevR and RlmA act both as repressors and activators, depending on the recognised DNA binding motif, suggesting a highly regulated mechanism for DHN-melanin production. Taken together, the presented results revealed a novel mechanism in gene regulation for bHLH and MEF2-like transcriptional regulators.

STV06
Regulation by the nitrogen PTS^{Ntr} in *Pseudomonas putida* – metabolism rules
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The nitrogen branch of the phosphotransferase system (PTS^{Ntr}) of *Pseudomonas putida* is a multicomponent regulatory device, which computes extracellular (e.g. sugars) and intracellular (e.g. PEP/Pyr ratio) signals and translates them into post-translational regulation of target activities through protein-protein interactions [1]. *P. putida* is an ideal organism to study the function of the distinct PTS proteins, as it possesses only two PTS entities. The genes *fruA* and *fruB* encode a sugar-specific PTS for fructose transport (PTS^{Fru}), and *ptsP*, *ptsO*, and *ptsN* are coding for the PTS^{Ntr}. One major target of regulation by PtsN/PtsN-P is the sensor kinase KdpD of the KdpD/KdpE two component system, regulating transcription of the K^+-transporter KdpFABC. We aim to unravel the underling mechanism and to elucidate the physiological role of such a regulation.
To this end, we constructed a *kdpF*-promoter (*kdpFp*) reporter plasmid by fusing the promoter region of the *kdpFABC* operon to the *lux* gene reporter system. This was employed to thoroughly analyse the activity of this promoter in various *pts* mutant strains and under different metabolic conditions in a highly time resolved manner. This allowed us to calculate the mRNA synthesis rate from the *kdpF* promoter, taking into account the degradation rate of the Lux proteins as well as the growth rate.
We observed that the absence of PtsN or the presence of only non-phosphorylated PtsN led to increased *kdpFp* activity [2]. Thus, the lower *kdpFp* activity in the wildtpe strain is a consequence of the presence of PtsN-P. We could show that the regulation of *kdpFp* activity takes place via direct protein-protein interaction between the sensor kinase KdpD and the PtsN protein and seems to be a result rather of the absolute amount of PtsN-P than of the ratio of PtsN/PtsN-P. Increase in the osmolarity of the culture condition had no effect, neither in the wildtype nor in any of the *pts* mutatnt strains, making an involvement of the KdpFABC system in osmoadaptation unlikely.
There is increasing evidence, that the PTS^{Ntr} acts as a complex actuator device in which diverse physiological inputs (intra- and extracellular) are ultimately translated into phosphorylation or not of PtsN, which, in turn, checks the activity of key metabolic and regulatory proteins. Such a control of bacterial physiology is a good example for the prominence of biochemical homeostasis over genetic ruling – and not vice versa.

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STV07
Phosphorylation and thiol-redox modifications as molecular switches in host-microbe interactions
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The respiratory airways, including bronchial epithelial cells and specialized immune cells, constitute a frontline barrier against airborne bacterial pathogens such as the pneumonia causative agent *Staphylococcus aureus*. Proteinogenic regulatory switches, in particular phosphorylation or cysteine thiol oxidation, are essential components of cell signaling networks ensuring rapid modulation of cellular responses. We hypothesize that interaction of bacterial pathogens with cells of the airway epithelium is accompanied by characteristic alterations in posttranslational modifications (PTM) that are either associated with defense mechanisms of the host or establishment and progression of the infection. Characterization of the regulatory switches can therefore lead to a greater understanding of the molecular pathomechanisms of infections and the establishment of novel host-centric targets for anti-infectives.
We utilize models of *S. aureus*-infected human bronchial epithelial cells and a monocyte differentiation model in combination with enrichment techniques for protein kinases, phosphopeptides and thiol-redox modifications to comprehensively characterize changes in phosphorylation and thiol-redox-modifications under infection-relevant conditions.
Functional data analysis on the investigated PTMomes not only identified affected biological processes and molecular functions, our studies also highlight key regulatory proteins. For example, in human bronchial epithelial cells the activity of the epidermal growth factor receptor was found to be critical for sensitivity towards staphylococcal alpha toxin. In addition, temporal phophosphorylation signatures indicate a key role of cycline and calcium-dependent kinases during the early phase of infection and pharmacological inhibition of relevant kinases significantly diminished the bacterial load. TAK1 kinase in THP-1 monocytic cells, on the other hand, was found to be implicated in monocyte-to-macrophage transition, chemokine production and bacterial killing. Eventually, our studies indicate thiol-redox and phosphorylation cross-talk and emphasize the important role of hydrogen peroxide as a second messenger.
In conclusion, mass spectrometry-based PTMomics can reveal alterations in cellular signaling during host-microbe interactions and highlight relevant regulatory hubs that might be suitable for pharmacological intervention.

STV08
Regulation of the C_4-dicarboxylate sensor kinase DcuS by the transporters DcuB and DctA
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Question: The sensor histidine kinase DcuS of *Escherichia coli* forms a sensor complex with the C_4-dicarboxylate transporters DcuB or DctA under anaerobic or aerobic conditions, respectively [1]. It was determined wether DcuS or the anaerobic transporter DcuB are responsible for substrate recognition in the DcuB/DcuS sensor complex. The role of the transporter in the sensor complex and in sensing was analyzed.
Methods: The substrate spectrum of DcuB was determined by a competition uptake assay and was compared to the substrates inducing expression of a *dcuB-lacZ* reporter gene. Substrate specificity of the sensor was modified by mutation in the periplasmic PAS domain (PAS_P) of DcuS. DcuS was titrated with DcuB expressed from an arabinose inducible plasmid.
Results: The substrate spectrum of the transporter DcuB differs from the substrate spectrum for induction of *dcuB-lacZ* expression. Additionaly, the substrate spectrum of the sensor complex could be modified by introducing point mutations in PAS_P. Titration of DcuS with increasing levels of DcuB lead to formation of increasing levels of C_4-dicarboxylate responsive sensor DcuS
Conclusion: The PAS_P domain of DcuS is responsible for signal perception in the DcuB/DcuS complex. The transporter DcuB is required for transfer of DcuS to the C_4-dicarboxylate responsive form, without having a sensory function. Signal transduction in DcuS involves a piston type movement of transmembrane helix 2 (TM2) [2]. It is suggested that the cytoplasmic PAS domain (PAS_C) is a silencer of DcuS [3] that requires DcuB as a cosilencer. The silenced form is able to respond to C4-dicarboxylate stimulation by translocation of TM2.

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SMV01
The *Bacillus* BioBrick Box 2.0 – generation and evaluation of new essential genetic building blocks for standardized work with *Bacillus subtilis*
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Standardized and well-characterized genetic building blocks allow the convenient assembly of novel genetic modules and devices, ensuring reusability of parts and reproducibility of experiments. The initial *Bacillus* BioBrick Box contained integrative vectors, promoters, reporter genes and epitope tags for the Gram-positive model bacterium *Bacillus subtilis*. [1]
In the next generation of the *Bacillus* BioBrick Box, we provide tools for fine-tuning protein expression, evaluate the whole spectrum of fluorescence proteins (FP) in *B. subtilis*, investigate properties of linkers for fusion-proteins, and introduce new vectors designed to evaluate promoter activity.
We developed six new expression vectors which either replicate or integrate into the genome. These vectors are equipped with xylose- or bacitracin-inducible promoters followed by a multiple cloning site in BioBrick RFC10 standard. In addition, we evaluated a set of ribosome binding sites (RBS) that cover a range of 2.5 orders of magnitude in expression level using six different RBS.
For the evaluation of the FPs, we tested different strategies of codon adaptation specific for *B. subtilis* to achieve best translation rates and showed their functionality. With two FPs we demonstrate functional fusions via linkers of different lengths.
We also introduce new vectors carrying the chloramphenicol resistance gene and *luxABCDE* in one operon, for screening functional promoters qualitatively via antibiotic selection and quantitatively via luminescence.
We believe that the new parts of our well-evaluated *Bacillus* BioBrick Box 2.0 are a valuable extension to the existing BioBrick Box and expand the range of standardized genetic work with *B. subtilis*.

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SMV02
CRISPy-web – design sgRNAs for CRISPR applications in microbes using an easy online tool
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Question: Originally evolved in prokaryotes as an adaptive immune system against bacteriophages, the CRISPR/endonuclease systems now see widespread application in genome editing workflows. The most popular system at the moment uses the *Streptococcus pyogenes* endonuclease Cas9. To direct Cas9 to the right target, so-called single guide RNAs (sgRNAs) need to be designed for each targeted editing site. While there are many tools available to design sgRNAs for the popular model organisms, only few tools exist that allow designing sgRNAs for non-model strains.
Methods: sgRNAs are identified if they fulfill two conditions: (i) the 20 bp target sequence needs to be immediately upstream of a "protospacer adjacent motif" (PAM). The *S. pyogenes* PAM sequence is "NGG". (ii) The target sequence should be unique within the genome of the target organism to prevent off-target activity, i.e.the cleavage of the chromosome in the wrong position.
CRISPy searches for the "NGG" PAM on both strands of the selected target region, and then checks for off-target hits with 0, 1, 2 and 3 bp mismatches for a 13 bp core sequence upstream of the PAM [1]. Hits with a high number of off-target matches are discarded, the remaining results are stored.
A microservice web architecture provides a responsive graphical interface to (i) upload the genome of interest or directly load it from an antiSMASH [2] result, (ii) select a region of interest, (iii) view and download desired sgRNA sequences. The service can also be accessed via a REST API.
Results: We have developed CRISPy-web (http://crispy.secondarymetabolites.org), an easy to use web tool to design sgRNAs for any user-provided microbial genome.
Conclusions: CRISPy-web allows researchers to interactively select a region of their genome of interest to scan for possible sgRNA. After checks for potential off-target matches, the resulting sgRNA sequences are

displayed graphically and can be exported to text files. All steps and information are accessible from a web browser without the requirement to install and use command line scripts.

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SMV03

Synthetic secondary chromosomes to study chromosome maintenance in *Escherichia coli*

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Advances in *de novo* synthesis of DNA and assembly methodology make construction of synthetic chromosomes a reachable goal [1]. Considering a potential design leads almost certainly to the question what the essential parts of a chromosome are. Investigations on this question have mainly focused on the minimal set of genes needed to allow cells to live. However, chromosomes are more than arrays of genes. Chromosomes need systems to replicate, segregate and organize the encoded genetic information. We explore such chromosome maintenance systems by application of synthetic biology approaches and have designed and constructed the small synthetic secondary chromosome synVicII [2]. The replication origin stems from the secondary chromosome of the human pathogen *Vibrio cholerae* which carries a natural secondary chromosome. Application of a new assay for the assessment of replicon stability based on flow-cytometric analysis of an unstable GFP variant revealed an improved stability of synVicII compared to a secondary replicon based on *E. coli oriC*. Cell cycle analysis and determination of cellular copy numbers of synVicII indicate that replication timing of synVicII in *E. coli* is comparable to the natural chromosome II in *V. cholerae*. Now, synVicII is used as backbone to construct bigger replicons (~ 100kbp) with variations in the distribution of DNA motifs relevant for chromosome maintenance. Downstream functional characterization should allow deeper understanding of chromosome maintenance. Our long term goal is to establish synthetic secondary chromosomes as experimental system to study chromosome maintenance and to provide chromosome construction rules for biotechnology applications.

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SMV04

Employing photocaged carbohydrates in light-controlled cell factories for synthetic bio(techno)logy and single cell applications

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Optogenetic tools are light-responsive components that allow for a simple triggering of cellular functions with unprecedented spatiotemporal resolution and in a non-invasive fashion. In particular, light-regulated gene expression exhibits an enormous potential for various biotechnological and synthetic biology applications.[1]
Here, we report on the development and evaluation of light-responsive microbial expression systems based on photocaged compounds such as caged IPTG[2] or caged arabinose. To this end, the photocaged carbohydrates have been used for accurately controlling target gene expression in different biotechnologically relevant production hosts.
Short UV-A light exposure is sufficient for the photo-cleavage of light-sensitive caged inducers resulting in rapid and efficient protein production in *E. coli* as initially demonstrated *in vivo* with different reporter proteins. Furthermore, expression output was gradually varied by means of both light-intensity and exposure times. Microfluidic single cell analysis[3] revealed expression heterogeneity which could be abrogated by using photocaged carbohydrates as inducers. Apparently, their increased membrane-permeability superseded specific inducer uptake systems. Finally, the biotechnological applicability of light-responsive inducers was demonstrated by distinct improvements of production yields for terpenoids and antibiotics produced in different industrially relevant Gram-positive and Gram-negative expression hosts.
In vivo expression analyses demonstrated that photocaged carbohydrates together with their corresponding transcriptional regulator/promoter system can be employed as optogenetic "plug-and-play" modules for synthetic biology approaches. These expression modules can be applied in novel photomicrobioreactors and single cell cultivation platforms to precisely control expression of target genes and thereby fully automatize the optimization of microbial production processes[4]. Especially for closed (e.g. anaerobic) systems and increasing numbers of parallelized expression cultures, non-invasive and spatiotemporal light induction will provide a higher-order control.

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SMV05

Investigation of the anaerobic propionate metabolism in *Escherichia coli* K12

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Propionate is a very abundant volatile fatty acid (VFA) in nature as well as in biotechnological processes. So far anaerobic oxidation of propionate was studied in syntrophic organisms. Here, the Gibbs free energy under state conditions is positive. Thus oxidation by propionate degraders is only possible when the concentrations of the products, H_2 and/or formate, are kept low by H_2 and/or formate depleting organisms such as methanogens. The enteric bacterium *Escherichia coli* is also exposed to high concentrations of propionate in its environment due to the fact that it is a typical fermentation by- or endproduct of other microorganisms. These organisms are also located in gastrointestinal tracts of their mammalian hosts. To overcome the negative effects of the VFA-accumulation and use it as carbon and electron source, *E. coli* evolved the 2-methylcitrate pathway to metabolize propionate to pyruvate. The cluster of genes which enable the propionate degradation are referred to as *prp*-genes and are arranged in an operon with two divergently transcribed units. The first unit consists of the transcriptional activation regulator gene *prpR*. The *prpBCDE* gene cluster, the second transcriptional unit, is composed of the genes *prpB*, *prpC*, *prpD* and *prpE* which encode the enzymes for propionate degradation.
In this work the ability of *E. coli* K12 to grow solely with propionate as carbon-source under oxic and anoxic conditions was investigated. Experiments with the wildtype strain of *E. coli* K12 showed only growth with O_2 as electron acceptor whereas with NO_3^- and DMSO neither growth nor propionate consumption could be detected. Transcriptome analysis revealed an additional anaerobic posttranscriptional regulation of the *prp*-operon as the $\log_2$-fold change of the normalized reads per kilo basepair (nRPK) decreased drastically along the operon. In the meantime transcription of the ribonuclease R (Rnr) was significantly higher under anaerobic conditions, assigning it a key role in the regulation of the operon. In fact the deletion of the *rnr*-gene enabled *E. coli* to utilize propionate also under anoxic conditions. These results encourage further research on propionate degrading organisms for the regulation of VFA accumulation in anaerobic digestors.

SMV06

Metabolic engineering of *Escherichia coli* for the biosynthesis of *para*-amino-L-phenylalanine

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Introduction: Aromatic amino acids like L-phenylalanine (L-Phe), L-tyrosine (L-tyr) and L-tryptophan (L-trp) are used in different industrial fields (3, 5). These amino acids can be produced in large scale of several metric tons by fermentations with the genetically modified microorganisms like *E. coli* or *Corynebacterium glutamicum* (5). Another non proteinogenic aromatic amino acid, *para*-amino-L-phenylalanine (L-PAPA), is a building block of antibiotics like chloramphenicol or pristinamycin (1, 4). L-PAPA is of special interest because of its broad platform for technical and pharmaceutical applications (2, 6). The aim of

this study was to construct and establish a *de novo* L-PAPA pathway in *E. coli*.
Methods: We modified genetically *E. coli* LJ110 by heterologous overexpressing of selected genes from *C. glutamicum* and *Streptomyces venezuelae* to produce L-PAPA.
Results: By heterologous gene overexpression from *C. glutamicum* para-aminobenzoate synthase (*pabAB*) and *S. venezuelae* 4-amino-4-deoxychorismate mutase (*papB*) and 4-amino-4-deoxyprephenate dehydrogenase (*papC*) L-PAPA was identified in the supernatant of *E. coli*. Furthermore we increased the l-PAPA titer to 0.7g/L by additional modification of the shikimate pathway in *E. coli.*
Conclusion: We demonstrated that *E. coli* can be genetically engineered to produce L-PAPA from a renewable source like glucose.

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SMV07
Introduction of the Calvin-Benson-Bassham cycle to create synthetic autotrophy in *Methylobacterium extorquens*, a heterotrophic Alphaproteobacterium
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Question: The problem of steadily rising atmospheric CO_2 concentrations challenges society to find novel, creative ideas to control this greenhouse gas. The emerging field of synthetic biology provides new options to address this question. A promising approach is the engineering of organisms that are heterotrophs by equipping them with autotrophic carbon-fixing pathways. This would create novel synthetic CO_2-fixing strains for biotechnology or bioremediation.
We are investigating the alphaproteobacterial model organism *Methylobacterium extorquens* as a host for the implementation of heterologous CO_2-fixation pathways. First, we will answer the question whether the successful introduction of a heterologous Calvin-Benson-Bassham (CBB) cycle into *M. extorquens* can sustain autotrophic growth of this heterotrophic species. Then, we will address the challenge whether this chassis is also able to host other, non-natural synthetic carbon fixation pathways.
Methods: To introduce genetic elements necessary for the implementation of a heterologous CBB cycle, state-of-the-art DNA manipulation techniques for Alphaproteobacteria have been developed and were used for the generation of engineered strains. These strains were subsequently evaluated by combining data from enzyme assays, ^{13}C isotope labeling experiments and automated high-throughput growth tests in comparison to naturally occurring autotrophs. Promising candidate strains were subjected to experimental evolution to further select for and improve their desired traits.
Results: The enzymes necessary for creating the CBB cycle in *M. extorquens* were successfully overexpressed and demonstrated to be active. Several mutant strains defective in growth were complemented with the heterologous CBB cycle. The interesting growth phenotype of a strain deficient in methanol assimilation was further characterized to determine whether the newly introduced CBB cycle can sustain substantial growth. The ^{13}C isotope labeling patterns in the central carbon metabolism of the engineered strain were investigated and compared to data from *Paracoccus denitrificans*, an autotrophic Alphaproteobacterium.
Conclusion: The genetic engineering of *M. extorquens* towards a CO_2-fixing organism using the CBB cycle is an important step towards the implementation of synthetic carbon fixation pathways. The engineering process revealed interesting new details about the physiology and evolution of the fundamental biological process of autotrophy and resulted in a clear phenotype that enables further characterization and evolution.

SMV08
Metabolic engineering of syngas fermenting *Clostridium ljungdahlii* for jet fuel production using an efficient genomic delivery system
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Currently, transportation fuels represent the foremost cause for consumption of fossil energy resources. Due to their limiting character and increasing demand in this sector, sustainable alternative feed stocks are needed. Furthermore, the combustion of fossil fuels lead to an increase of CO_2 in the atmosphere which is regarded as the major reason for global warming.
Microbial biosynthesis based on industrial waste gases could serve as a worthy substitute for providing the necessary bulk chemicals that can be further processed by oligomerisation.
The genus Clostridium has a broad product spectrum comprising e.g. several acids, solvents and short to medium chained alcohols. Despite the versatile substrate use of different clostridial species, sugar based feed stocks are frequently used as carbon source for synthesis in industrial scale. A by far more attractive feedstock (especially concerning the 'land use' issue) would be the use of synthesis gas. This mixture of carbon monoxide, carbon dioxide and hydrogen is emitted in large amounts by the steel industry or by gasification of municipal waste. Several strains like e.g. *Clostridium ljungdahlii* are able to grow autotrophically on various syngas compositions using it as sole carbon and energy source.
Here we use *C. ljungdahlii* as a host organism to introduce foreign biochemical pathways to link the favorable substrate metabolism with the desired product synthesis. Until today the metabolic engineering of Clostridia in general and of syngas fermenting Clostridia strains in particular has proven rather challenging. Hence, we developed an efficient genomic delivery system allowing the introduction of complex and large gene clusters into syngas-fermenting Clostridia cells and the stable integration into their genome. We cloned 13 genes derived from *C. kluyveri* and *C. acetobutylicum* in a shuttle vector resulting in a 25 kb construct, introduced it into the cells by conjugation and successfully detected synthesis of butanol and hexanol. This strategy will not only allow for syngas-based fermentation for diverse bulk chemicals for biofuels but will also foster the development of further fermentation processes using other feed stocks and products in the future.

CBV-FG01
Orthogonal natural product studies of the jamaican marine cyanobacterium *Moorea producens* JHB
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Marine cyanobacteria have been one of the richest marine sources of novel and highly bioactive natural products. For the most part, they derive from the assembly of amino acids via the non-ribosomal peptide synthetase pathway, acetate units from the polyketide synthase pathway, and the inter-digitation of these two pathways to form 'hybrid' natural products. While these metabolites possess diverse biological properties, many are toxic to cells and therefore have potential applications in cancer. Indeed, one marine cyanobacterial inspired product, monomethyl auristatin E, is the warhead of an antibody-drug conjugate (ADC) which is FDA approved for the treatment of cancer. Our research laboratory has been studying the unique natural products of marine cyanobacteria for 30 years. For example, one collection of *Moorea producens* JHB (formerly *Lyngbya majuscula*) from Hector's Bay, Jamaica in August 1996, has been an exceptional source of novel bioactive compounds, such as the jamaicamides[1] and hectochlorin.[2] Continued study of this organism using orthogonal approaches, such as isotope feeding experiments, new methods in mass spectrometry, genome sequencing, and alternative culture conditions, have broadened our appreciation of its biosynthetic capacities.[3] In total, we have isolated and characterized two additional classes of natural products from cultures of this cyanobacterium, as well as several new analogs in both of the previously characterized natural product classes. These in depth and alternative natural product investigations of *M. producens* JHB will be presented, and considerably expand our knowledge of the exceptional biosynthetic capacities of this marine cyanobacterium.

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CBV-FG02

The role of carbon-polymer biosyntheses of both glycogen and poly-β-hydroxybutyrate in non-diazotrophic cyanobacteria

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Carbon polymers such as glycogen and polyhydroxybutyrate (PHB) have a crucial impact under macronutrient stress conditions, especially those favoring high C to N ratios. Most cyanobacteria exclusively synthesize and degrade glycogen, which is in agreement with the metabolic principle that only one polymer acts as both a sink and a reserve in one species. A few cyanobacteria such as *Synechocystis* sp. PCC 6803 additionally produce the physico-chemically different PHB. For the first time, the function and interrelation of both carbon polymers are analyzed in a comparative physiological study of single- and double-knockout mutants (Δ*glgC*; Δ*phaC*; Δ*glgC*/Δ*phaC*), respectively. Most of the observed phenotypes are explicitly related to the knockout of glycogen synthesis, highlighting the metabolic, energetic, and structural impact of this process whenever cells switch from an active, photosynthetic 'protein status' to a dormant 'glycogen status'. The carbon flux regulation into glycogen granules is apparently crucial for both phycobilisome degradation and thylakoid layer disassembly in the presence of light. In contrast, PHB synthesis is definitely not involved in this primary acclimation response. Moreover, the very weak interrelations between the two carbon-polymer syntheses indicate that the regulation and role of PHB synthesis in *Synechocystis* sp. PCC 6803 is different from glycogen synthesis.

CBV-FG03

Current and potential exploitation of cyanobacterial natural products in health care and biotechnology industry

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Cyanobacteria have adapted to a remarkable variety of ecological niches over all climate zones of Earth, resulting in an outstanding variety of species. On a molecular level, many cyanobacterial strains are producers of secondary metabolites which show a wide diversity in their chemical structures and bioactivities[1-2]. But how can we access this intriguingly rich natural resource for the discovery and development of novel lead structures for active ingredients in pharmaceuticals, cosmetics and agrochemicals? In order to make cyanobacterial natural products accessible for lead discovery, we at Cyano Biotech make use of the standardized cultivation of Cyanobacteria in photo bioreactors from millilitre to hundreds of litres scale, the ability of analytical high performance chromatography in combination with photo diode and mass selective detectors to monitor natural product formation in cell and medium extracts from newly isolated cyanobacterial strains and an isolation process based on preparative centrifugal partition chromatography to isolate cyanobacterial natural products. By continuously applying this combination of methods in a standardized process, we produced about ten thousand of concentration-normalized and with regard to their complexity classified pre-fractionated biomass and medium extracts (PFEs) from strains of our in-house collection. Thus, bioactivities of previously uncharacterized natural products from newly isolated cyanobacterial strains can be quickly identified by activity assays. Our screening for antimicrobial activity showed that 13 % of all PFE are active, indicating a remarkable presence of bioactive molecules. In summary, we developed and established a process from the environmental sample over unialgal cultures to standardized pre-fractionated extracts containing potentially novel natural products that can be assayed for bioactivities of commercial interest.

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CBV-FG04

Metabolic pathway engineering using the central signal processor P_{II}

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Question: P_{II} signal processor proteins are widespread in prokaryotes and plants where they control a multitude of anabolic reactions. Efficient over production of metabolites requires relaxing the tight cellular control circuits. Here we demonstrate that a single point mutation in the P_{II} signaling protein in the cyanobacterium *Synechocystis* sp. PCC 6803 and *Anabaena* sp. PCC 7120 is sufficient to cause drastic changes in the amino acid metabolism. This work exemplifies a novel approach of pathway engineering by designing custom-tailored P_{II} signaling proteins.
Methods: We constructed genomic mutants of *Synechocystis* sp. PCC 6803 and *Anabaena* sp. PCC 7120 in which the *glnB* gene was replaced by a *glnB* gene carrying the mutation for I86N.To get a deeper insight of the behavior of the generated production strain, microscopic and physiologically studies were performed. To characterize metabolic changes, caused by the P_{II} (I86N) an untargeted metabolomics approach was chosen.
Results: The P_{II} (I86N) variant in *Synechocystis* strongly increases the *in vivo* activity of *N*-acetylglutamate kinase, the rate limiting enzyme of the arginine biosynthesis, leading to a more than 10 fold higher arginine content compared to the wild type. Due to the high intracellular arginine level, *Synechocystis* P_{II} (I86N) is able to accumulate up to 50-60 % cyanophycin (multi-l-arginyl-poly-l-aspartate) per cell dry mass. This product is of biotechnological interest as a source of amino acids and polyaspartic acid.
The P_{II} (I86N) variant in *Anabaena* causes drastic changes in the primary metabolism. Remarkably, *Anabaena* P_{II} (I86N) accumulated on average 10-fold more glutamine with the consequence of a more than 10-folde reduced glutamate-level compared to the wild-type. Moreover, the arginine level as well as the cyanophycin level is not influenced.
Conclusions: In this work, we demonstrat the feasibility of engineering the efficiency of a metabolic pathway by using a variant of the central regulatory P_{II} signal transduction protein. The P_{II} (I86N) variant of *Synechocystis* is the most potent cyanophycin producer described so far and a promising option for photoautotrophic production of arginine as well as cyanophycin. The P_{II} (I86N) variant in *Anabaena* leads to a strongly increased glutamine production. This behavior is a promising mean for photoautotrophic production of glutamine and indicate a novel aspect of P_{II} regulation in filamentous cyanobacteria.

CBV-FG05

GC-MS based profiling of primary metabolism in Cyanobacteria

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Cyanobacteria evolved more than 2.5 billion years ago and shaped the global atmosphere by decreasing the atmospheric carbon dioxide (CO_2) concentration while increasing the proportion of molecular oxygen (O_2). Ribulose-1,5-bisphosphate carboxylase/ oxygenase (RubisCO) is key to the evolution of photosynthesis and catalyzes the central reaction of Ci-fixation where ribulose-1,5-bisphosphate (RuBP) reacts with CO_2 to produce two molecules of 3-phosphoglycerate (3PGA). High levels of atmospheric CO_2 in Earth´s early history favored the carboxylation reaction. But RubisCO also accepts O_2 as a substrate. The oxygenase reaction competes with Ci fixation and produces equimolar amounts of 3PGA and the toxic product 2-phosphoglycolate (2PG). Cyanobacteria adapted to increasing atmospheric O_2 both, by largely avoiding 2PG production via the evolution of an efficient CO_2 concentrating mechanism (CCM) and by evolving mechanisms for 2PG degradation through photorespiratory 2PG metabolism. GC-MS based profiling of primary metabolism applied to *Synechocystis* sp. PCC 6803 wild type (WT) shifted from high to low inorganic carbon (Ci)-availability in comparison to mutants that are defective for 2PG metabolism, Ci-regulation, and the Ci-uptake and carboxysome components of the CCM reveals activation of photorespiration under Ci-limitation and how *Synechocystis* acclimates metabolism, physiology and growth to intracellular Ci-limitation.

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CBV-FG06
Physiological aspects of microcystin production in *Microcystis aeruginosa* PCC 7806
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Cyanobacteria can produce a variety of substances with non-obvious physiological functions such as the toxic peptide microcystin. Mass developments or blooms of a few genera regularly affect the drinking water supply in many parts of the world. The bloom habitat is characterized by sharp gradients and diurnal shifts of e.g. oxygen concentration, available inorganic carbon and ambient pH. This talk addresses a putative role of microcystin production in establishing mass developments by the bloom-forming cyanobacterium *Microcystis aeruginosa*.
A rarely discussed feature of microcystin is its ability to form protein conjugates. Analysis of lab strains and field samples revealed that significant amounts of the total cellular microcystin were bound to proteins. One major binding target of microcystin was identified as the key enzyme for primary carbon fixation, RubisCO. Exposition of *Microcystis* cultures to high light strongly stimulated the microcystin-protein interaction. The covalent binding of microcystin to cysteine thiols was strongly supported at pH > 9 *in vitro* and microcystinylation of the Calvin cycle enzymes PRK, CP12 and RbcL resulted in lower susceptibility towards enzymatic proteolysis.
In a comprehensive GC/MS-based analysis 85 (17 percent) of 501 unambiguously detected metabolites accumulated significantly different in the wild-type and the microcystin deficient Δ*mcyB* mutant strain of *Microcystis aeruginosa* PCC 7806, which suggests a role of microcystin in fine-tuning the metabolic flow. Prolonged exposition to high light induced higher levels of compatible solutes in the Δ*mcyB* mutant indicating the potential of microcystin to prevent of cell stress in the wild type. First experiments with enriched RubisCO indicated that microcystinylation could indeed have an impact on enzyme activity.
The here described mode of microcystin-protein interaction along with the change of stability and activity of microcystinylated enzymes could pose a competitive advantage over non-producing genotypes during episodes of high photosynthetic activity and carbon limitation.

EMV-FG01
How do *Anabaena* cells communicate?
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Introduction: The filamentous nitrogen-fixing cyanobacterium *Anabaena* sp. PCC 7120 differentiates specialized cells, heterocysts, that fix atmospheric nitrogen and transfer the fixed nitrogen to adjacent vegetative cells. Reciprocally, vegetative cells transfer fixed carbon to heterocysts. Several routes have been described for metabolite exchange within the filament, one of which involves communicating channels that penetrate the septum between adjacent cells. Several *fra* gene mutants were isolated 25 years ago on the basis of their phenotypes: inability to fix nitrogen and fragmentation of filaments upon transfer from N+ to N- media.
Methods: Cryopreservation combined with electron tomography and immunoelectron tomography, were used to characterize channels between cells as well as investigate the role of three *fra* gene products in channel formation.
Results: We were able to visualize and measure the dimensions of channels that breach the peptidoglycan between vegetative cells and between heterocysts and vegetative cells. The channels appear to be straight tubes, 21 nm long and 14 nm in diameter for the latter and 12 nm long and 12 nm in diameter for the former[1]. We also show that, FraC and FraG are clearly involved in channel formation while FraD has a minor part. Additionally, FraG was located close to the cytoplasmic membrane and in the heterocyst neck, using immunogold labeling with antibody raised to the N-terminal domain of the FraG protein[2].

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EMV-FG02
Determinants of heterogeneous cell development of *Dinoroseobacter shibae*
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Introduction: *Dinoroseobacter shibae*, a marine alphaproteobacterium, develops under suitable growth conditions into cells of highly heterogeneous size and shape. This behavior is controlled by Quorum sensing via a conserved cell cycle regulatory system [1,2]. The physiological role of this differentiation is so far unknown, but might be an adaptation strategy to cope with varying environmental conditions.
Objectives: Here we tested if heterogeneous wild-type cells and homogeneous QS mutant cells differ in their response to fluctuating nutrient availability with regards to PHB maintenance, cell size and survival rate.
Results: Both, wild-type and QS mutant, use excess substrate to form PHB storage bodies. Several distinct PHB bodies are visible in enlarged wild-type cells. When nutrients get scarce, both strains use accumulated PHB to undergo reductive cell division. The QS mutant cell number almost doubles. However, the daughter cells contain no PHB and cell numbers drop fast again. In contrast, the wild-type divides less, but maintains a higher PHB-content and stable cell number under starvation. When nutrients become available again, both strains produce PHB before starting to divide again.
Conclusion: QS might have a dampening effect on reductive cell division and might be advantageous in fluctuating environments. To further investigate the dynamics of cell development and division, live-staining methods and time-lapse microscopy should be employed.

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EMV-FG03
A microscopic perspective of the planctomycetal ecology
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Planctomycetes are ubiquitous environmentally important bacteria. Several conspicuous cell biological traits, such as FtsZ independent yeast-like budding, putative subcellular compartments, endocytosis-like uptake of proteins and lack of a peptidoglycan (PG) cell wall, were postulated. This led to the hypothesis that Planctomycetes represent a 'missing link' between pro- and eukaryotes.
We aimed towards a holistic perspective on the phylum Planctomycetes and hypothesized that their interaction with phototrophs is key to understand both, their ecology and cell biology. In a first attempt we sampled various environments and obtained literally hundreds of novel planctomycetal strains, species, genera and families. After a brief light microscopic pre-screening, we selected the most interesting strains in terms of cellbiological features for in depth microscopic characterization.
Employing various (superresolution) microscopic methods, we demonstrate that Planctomycets are Gram-negative bacteria that comprise a PG cell wall, lack eukaryotic endocytosis but posses an unobserved uptake system to feed on complex polysaccharides. In the light of our microscopic results we raised the hypothesis that the unusual planctomycetal cell division through budding evolved with intermediate stages. Further more, the unseen planctomycetal uptake of complex polysaccharides might represent a novel ecological strategy to feed on various different complex carbon substrates. This makes Planctomycetes the Swiss army knife of environmental microorganisms in terms of carbon remineralization. Thus Planctomycetes are unlikely the 'missing link' between pro- and eukaryotes, but their conspicuous cellbiological features set them apart from other 'typical' Gram-negative bacteria.

EMV-FG04
Towards applications of superresolution microscopy in environmental microbiology
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Superresolution microscopy encompasses a suite of cutting edge microscopy methods able to surpass the resolution limits of light microscopy. Amongs these methods, Structured Illumination Fluorescence Microscopy (SIM) has a resolution of 100-130 nm, while STED and "Blink Microscopy" (e.g. Photoactivated Localization Microcopy - PALM, and direct Stochastical Optical Reconstruction Microscopy - dSTORM) can achieve as low as 20-50 nm. The recent commercial availability of superresolution microscopy has allowed the spread of these techniques in microbiological research, especially to the study of microorganisms amenable to genetic engineering and *in vivo* expression of autofluorescent fusion proteins, e.g. GFP. For example, superresolution microscopy was used to study the structure and segregation of nucleoids and sub-cellular distribution of ribosomes. Studies on model bacteria have shown the existence of two opposing types of organization, one in which the nucleoid occupies a central position, while the ribosomes are concentrated in the periphery of the cell, and the other in which the ribosomes co-localize with the nucleoid and occupy the entire cellular space. The application of these methods to the study of environmental microbes is limited, because require genetic manipulation and the vast majority of the microorganisms are still not cultivable. Therefore, culture-independent methods are needed. rRNA-FISH is an excellent tool for single cell identification in environmental samples. In the same time, by targeting rRNA, it serves as proxy for ribosome localization. We present here methodological improvements for rRNA-FISH to study the sub-cellular localization of ribosomes and nucleoids in environmental microorganisms.

EMV-FG05
Simple generation of stereoscopic 3D images with any light- or scanning electron microscope
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One of the major issues of macro- and microphotography is the low depth of focus. Using sequential focus series of images (z-stacks) and software to extract sharp structures from the image stack helps to overcome this problem [(1)]. Furthermore, the generation of a depth map describing the z-positions of the sharpest pixels can be used to generate virtual stereoscopic 3D images. Here we describe the freeware program PICOLAY [(2)] and its use with light- and scanning electron microscopy (SEM). We compare various approaches to generate stereoscopic 3D images: (1) focus stacking as described above; (2) 'hologram stacking', which not only shows the sharpest, but any structure that exceeds a certain sharpness score; (3) use of a single SEM image as both sharp image and depth map; and (4) how to get a stereoscopic image by sideways moving the specimen and thus simply changing its lateral distance to the secondary-electron detector. Some results will be demonstrated in a stereoscopic slide show for which anaglyph glasses will be provided.

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FBV-FG01
The Zn_2Cys_6 transcription factor BcGaaR regulates D-galacturonic acid utilization in *Botrytis cinerea*
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D-galacturonic acid (GalA) is the most abundant monosaccharide component of pectin. Previous transcriptome analysis in the plant pathogenic fungus *Botrytis cinerea* identified eight GalA-inducible genes involved in pectin decomposition, GalA transport and utilization. Co-expression of these genes indicates that a specific regulatory mechanism occurs in *B. cinerea*. In this study, promoter regions of these genes were analysed and eight conserved sequence motifs identified. The *Bclga*1 promoter, containing all these motifs, was functionally analysed and the motif designated GalA-Responsive Element (GARE) identified as the crucial *cis*-regulatory element in regulation of GalA utilization in *B. cinerea*. Yeast one-hybrid screening with the GARE motif led to identification of a novel Zn_2Cys_6 transcription factor, designated BcGaaR. Targeted knockout analysis revealed that BcGaaR is required for induction of GalA-inducible genes and growth of *B. cinerea* on GalA. A BcGaaR-GFP fusion protein was predominantly localised in nuclei in mycelium grown in GalA. Fluorescence in nuclei was much stronger in mycelium grown in GalA, as compared to fructose and glucose. This study provides the first report of a GalA-specific transcription factor in filamentous fungi. Orthologs of BcGaaR are present in other ascomycete fungi that are able to utilize GalA, including *Aspergillus spp., Trichoderma reesei* and *Neurospora crassa*.

FBV-FG02
Characterization of novel regulators for pectin degradation in *Neurospora crassa*
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To minimize economic losses during the conversion of plant biomass to fermentable sugars all cell wall components - cellulose, hemicellulose and pectin - will have to be degraded with similar efficiency. This necessitates a flexible utilization of cell wall depolymerizing enzymes that can be dynamically tailored to the composition of the biomass at hand. However, a targeted manipulation of pectinase production is not possible due to a lack of knowledge regarding the underlying regulatory mechanisms of pectin perception and degradation. Gaining a better understanding of these processes is therefore important to achieve the best and most profitable conversion of this plant cell wall polysaccharide into its constituent monosaccharides.
Through a phenotypic screening of all *Neurospora crassa* strains with a putative transcription factor knock out and the analysis of the large scale RNA-Seq database provided by The Fungal Nutritional ENCODE Project, three transcription factors involved in the regulation of pectin degradation, as well as one transcriptional regulator could be identified. These novel regulators were further characterized in this work and their putative role in the pectin degradation network elucidated. The gained knowledge about the regulatory network for pectin degradation could help to greatly accelerate the development of efficient strains which can produce more pectinolytic enzymes or completely utilize all cell wall polysaccharides for biorefinery processes.

FBV-FG03
Regulatory networks of the gibberellin cluster in *Fusarium fujikuroi*
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The filamentous fungus *Fusarium fujikuroi* is known to produce a variety of structurally diverse secondary metabolites such as the plant hormones gibberellins (GAs), pigments and mycotoxins. The GAs are a virulence factor of its producer and causer of the so-called 'Bakanae' (foolish seedling) disease but otherwise they are also commonly used in agri-, horti- and viticulture as plant growth regulators. Thus, the understanding of the regulation of the GAs is of great biotechnological interest.
During the past decade of years, we identified a complex regulation network for GA biosynthetic genes. They are repressed by high amounts of nitrogen and were the first secondary metabolites for which a strict dependency on the GATA-type transcription factor AreA was shown. Recently, we revealed that a second GATA-type transcription factor, AreB, is also essential for expression of the GA genes. Besides these two transcription factors the fungal-specific Velvet complex is also involved in the regulation of the GA cluster. Deletion of FfVel1 and the putative methyltransferase FfLae1 led to downregulation of the respective biosynthetic genes while overexpression of FfLae1 resulted in significant upregulation. Furthermore chromatin modification, e.g. deletion of the histone deacetylase FfHda1, strongly affects GA gene expression and production. In addition, the active histone mark H3K9Ac (acetylation of lysine 9 at histone 3) at the GA cluster is enriched under nitrogen-limiting conditions and this is consistent with gene

expression of this cluster. This study will give an overview of the complex regulation of the GA cluster.

FBV-FG04

Aspects in microbial interactions and intracellular regulation of *Schizophyllum commune*

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The basidiomycete *Schizophyllum commune* belongs to the white rot fungi and is relevant for wood degradation worldwide. Its high competitive ability is based on the recognition of other fungi and bacteria, the production of specific extracellular metabolites and a strategy of fast growth. As early colonizer of tree wounds and after forest fire, the fungus has also phytopathogenic importance.
In contact with other wood degrading fungi growing in proximity, different interactions are established. Depending on media composition and competitor, they can be classified as intermingling, inhibition and overgrowing. Final results are developmental changes with re-directed growth and fruiting body production. Secondary metabolites, either released to the environment from the Spitzenkörper, or enriched in fungal tip cells, include pigments. This results in pigmented demarcation lines between competitors. In interactions of *S. commune*, indigo is formed as final product of toxic precursors secreted as a barrier towards competiting fungi and in stress reactions. The multicopper oxidase enzymes laccases are involved in pigment formation, pathogenesis, fungal morphogenesis and stress response in fungus-fungus interactions. Therefore, fast transport processes within the hyphae by different motor proteins and an efficient cytoskeleton are essential.
The fungal cytoskeleton, built of microtubules and actin, was investigated by fluorescence microscopy with regard to regulation *via* Ras GTPases. The *S. commune* genome encodes more than 30 putative signal transduction proteins of the Ras GTPase superfamily, containing the Ras, Rho, Rab, Ran and Arf subfamilies. Their different functions are postulated and need to be confirmed. The function of the *S. commune* small G-proteins Ras1 and Ras2 have been postulated in MAPK signalling, in addition to pheromone response, morphology, asexual development and mating. Constitutively active Ras1 mutant strains show phenotypes like reduced growth rates, hyperbranching and abnormal growth pattern. Within the cell, the activity of protein kinase A is enhanced. Furthermore, the mutants produce abnormal fruiting bodies with weakly developed subhymenium and aberrant spore formation. Thus, both Ras GTPases are major regulators of cellular development.

FBV-FG05

Comparative genomics and transcriptomics to study fruiting body development in ascomycetes

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Filamentous ascomycetes develop several major types of fruiting bodies that share a common ancestor, and a set of common core genes most likely controls this process. One way to identify such genes is to search for conserved genes and expression patterns. In a genome and transcriptome mining approach, we are using data from the Sordariomycete *Sordaria macrospora* and the Pezizomycete *Pyronema confluens* to identify evolutionary trends in fruiting body morphogenesis as well as genes that might play conserved roles in this process. Among the genes with conserved expression patterns that we identified were the histone chaperone gene *asf1*, the transcription factor gene *pro44*, and the SNARE protein gene *sec22*. *asf1* and *pro44* were shown to be essential for fruiting body development in *S. macrospora*, whereas *sec22* is involved in ascospore maturation. Furthermore, the *P. confluens* orthologs of *asf1* and *pro44* can complement the corresponding *S. macrospora* mutants, showing that the genes have an evolutionary conserved function. In addition to the identification of target genes, comparative studies can be used to determine genome-wide transcription patterns during development. Based on the genome and development-dependent transcriptomes of *P. confluens*, we analyzed if genes with different levels of evolutionary conservation differ in their expression patterns. Interestingly, the highest percentage of genes upregulated during sexual development was found among the *P. confluens* orphan genes and Pezizales-specific genes (20 and 15 %, respectively), whereas it was less than 2 % in more conserved gene groups, a finding consistent with the idea of rapid evolution of sex-associated genes. A similar analysis for the *S. macrospora* wild type and the sterile mutant pro1 carrying a mutation in a transcription factor gene showed that upregulated genes in the pro1 mutant tend to Sordariomycete-specific, suggesting that PRO1 acts as repressor of lineage-specific, development-associated genes.

FBV-FG06

Regulation dynamics in the HOG signaling pathway in filamentous fungi

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Detection of environmental signals and signal transfer within the cell to give an appropriate response are vital for microorganisms in order to adapt to varying external conditions during their life cycles. For this purpose, signaling pathways play a crucial role and one example is the high osmolarity glycerol (HOG) signaling pathway responsible for osmoregulation of fungi. This signal cascade comprises a phosphorelay system linked to a MAPK cascade and is extensively studied in the model yeast *Saccharomyces cerevisiae*. In its phosphorelay system only the histidine kinase Sln1p was found as an osmosensor and inactivation of the corresponding gene *SLN1* is lethal in yeast. In filamentous pathogenic fungi osmoregulation is still not well understood and differs accordingly from yeast, e.g. the phosphorelay system comprises more sensor components and filamentous fungal specific elements. Thus, this signaling cascade is worth to be studied in these pathogens.
We studied the HOG-pathway intensively in the rice blast fungus *Magnaporthe oryzae* and found among others the signaling cascade sensing environmental signals such as salt and sugar stress by different sensor kinases MoSln1p and MoHik1p. These signals both were identified to be transferred over the MAPK MoHog1p by means of phosphorylation resulting in an osmotic stress response. However, nothing was known about how MoHog1 is regulating the genetic program of this cellular response to osmotic stress. Therefore, we conducted time course RNAseq (NGS) analysis of the *M. oryzae* wildtype strain compared to a mutant strain with inactivated HOG signaling cascade in order to obtain genes contributing to the transcriptional regulation. With the resulting data, we were able to present a set of strong regulated genes with putative function in osmoregulation which never had been linked to the HOG pathway before. Furthermore, we will demonstrate initial findings concerning the regulatory network and crosstalk of the HOG pathway in filamentous fungi.

FBV-FG07

A gene co-expression network as a tool to predict functional modules in *Aspergillus niger*

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Aspergillus niger is a well-established cell factory in biotechnology used for the production of organic acids and enzymes since almost hundred years and with a published genome sequence since 2008. However, the function of only 2 % of its predicted 14,000 genes has been studied so far and about 50 % of the annotated ORFs encode hypothetical proteins.
Gene co-expression network analysis is a powerful approach for the functional annotation of uncharacterized genes. It aims to find genes with a consistent, correlated expression pattern across phenotypically diverse samples or experimental conditions. Genes within shared expression profiles are tightly connected and are predicted to function in the same regulatory and/or functional pathway ("guilt-by-association" approach). In order to identify tightly connected genes in *A. niger*, we have established a transcriptomics database using Affymetrix microarray data published for *A. niger*. The database includes 158 different cultivation conditions reflecting different carbon and nitrogen sources, starvation and stress conditions, conditions related to temporal and spatial stages during its life cycle, different cultivation concepts and many more. Using Bioconductor, pairwise correlation coefficients were calculated and pairs with a Spearman score higher than 0.7 were considered to have significant co-expression relationship and were connected by an edge in the network. The resulting gene co-expression network was furthermore enriched with a network created for shared putative transcription factor binding sites, a network predicting protein-protein interactions based on orthology to *Saccharomyces cerevisiae* and a network considering physical chromosomal co-localization. The functional modules predicted by the final network were investigated for gene content and validated based on

published data for the function of known secretory pathway genes. These analyses supported the biological relevance of these modules, suggesting that the co-expression network obtained presents a valuable predictive tool for functional annotation of *A. niger* genes.
Our analysis using this tool makes it now for the very first time feasible to zoom into the gene expression networks and physiological processes under which the predicted 78 secondary metabolite gene clusters are embedded in utmost detail.

MPV-FG01
Phase-Locked Mutants elucidate novel functions and differential virulence of variable surface lipoproteins encoded by mycoplasma multigene families
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Mycoplasmas possess complex virulence determinants that are largely unknown. This is inspite the progress in genomic and proteomic studies witnessed in the last decade. Presence of large multigene families encoding phase- and/or size-variable immunodominant surface antigens is a common theme that is often implicated, but never directly proven, to play a role in mycoplasma infection and pathogenesis. We focused on understanding the same using a model system based on *Mycoplasma agalactiae* that exhibits phenotypic diversity through the high-frequency variable expression of a family of immunodominant surface lipoproteins called Vpmas. Having elucidated the genetic mechanism governing Vpma variations, we had earlier constructed the first 'Phase-Locked Mutants' (PLMs) that served as breakthrough tools to assess their role during *in vitro* and *in vivo* infection studies as they steadily expressed a single steady Vpma product (1). Comparing them with a WT strain in an experimental sheep infection model it was demonstrated that Vpma phase variation is not necessary for establishing an infection although it might critically influence the survival and persistence of this pathogen under natural field conditions (2).
Initial attachment to host cells is regarded as one of the most important steps for colonisation and infection. PLMs served as ideal tools to study the role of each of the six Vpmas in cytadhesion, which was otherwise not possible due to the high-frequency switching of Vpmas in WT strain. Using *in vitro* adhesion assay with HeLa and sheep primary cells (3), we could demonstrate significant differences in the adhesion capabilities of each of the six PLMs compared to the WT strain. PLM-U mutant, which expresses VpmaU protein, showed the least adhesion (18 %) where as PLM-V mutant expressing VpmaV protein exhibited the highest adhesion (62 %). To further confirm the role of Vpmas in *M. agalactiae* cytadhesion, we performed adhesion inhibition assays by pre-incubating the PLMs with the respective Vpma-specific polyclonal antisera raised in rabbits. This led to a significant decrease ($p<0.05$) in the adhesion percentage of each of the PLMs. Furthermore, using immunofluorescence staining, Vpma proteins extracted using TX-114 were shown to bind to HeLa and sheep cells proving the direct role of Vpmas in cell adhesion. This is the first report that describes cytadhesion as a novel function of Vpmas. Combined with the antigenic variability of these proteins, the newly identified phenotype is likely to play a critical role in the pathogenicity of *M. agalactiae*, and also provides important clues to the role of similar phase variable lipoprotein families in mycoplasmas.

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MPV-FG02
Skin-specific unsaturated fatty acids were taken by *Staphyloccocus aureus* and their incorporation into lipoprotein boosts innate immune response
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The skin is the largest and most exposed interface of our body with the environment. It has therefore a central role in host defense not only by its physical barrier but also by its innate and adaptive immune systems. We investigated the impact of human unsaturated skin fatty acids on *S. aureus* growth and immune stimulation. The (phospho)lipids of *S. aureus* only contained saturated fatty acids suggesting that only these fatty acids can be synthesized. The question was therefore, whether *S. aureus* can also take up and incorporate unsaturated skin fatty acids into (phospho)lipids and lipoproteins. Although *S. aureus* is unable to synthesize unsaturated fatty acids we show that long-chain unsaturated fatty acids (e.g. linoleic acid) of human skin can be incorporated into its lipoproteins with the effect that *S. aureus* is better recognized by the innate immune system. This is an additional mechanism how our skin controls bacterial colonization and infection.

Minh Thu Nguyen, Dennis Hanzelmann, Thomas Härtner, Andreas Peschel, Friedrich Götz. Skin-specific unsaturated fatty acid boost *Staphylococcus aureus* innate immune response. *Infection and Immunity;* online 26 October 2015, doi: 10.1128/IAI.00822-15.

MPV-FG03
Immunogenicity of lipoproteins and other classical pneumococcal surface proteins
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Question. Despite the availability of effective vaccines *Streptococcus pneumoniae* is responsible for about 3-5 million deaths each year, mostly in children under 5 years of age and in the elderly population. The ideal vaccine would be serotype independent and based on highly and immunogenic proteins. The pneumococcal cell surface is decorated with several clusters of proteins. Namely these are choline-binding proteins (CBPs), sortase anchored proteins, lipoproteins, (trans)membrane linked proteins and so called non-classical surface proteins like enolase. Several proteins from these different clusters, that are highly conserved among pneumococcal serotypes and may therefore represent potential candidates for an innovative vaccine, were analyzed regarding their immunogenicity and abundance on the pneumococcal surface.
Methods. Protein specific polyclonal IgGs were isolated from mouse serum obtained post-immunization with various lipoproteins and other pneumococcal proteins. The relative antibody titers of the polyclonal mouse IgGs were determined by ELISA. The mouse sera and corresponding IgGs were also analyzed with the Luminex® FlexMap3D® technique. In addition, convalescent patient sera obtained from patients suffering from infections with different pneumococcal serotypes were assessed to demonstrate the immunogenicity of selected surface proteins during infections. The cell-surface abundance of the proteins in *S. pneumoniae* D39 was examined by flow cytometry.
Results. The Luminex-based immunoproteomics approach showed that nearly all proteins are immunogenic when administered to mice. Using the polyclonal mouse antibodies the protein abundance on the pneumococcal cell surface differed among the candidates. The most abundant proteins were the CBP and adhesin PspC, SP_0845, the foldase PpmA and PsaA. Interestingly these antibodies also bound to the encapsulated strain D39. In addition, the analysis of convalescent patient sera revealed 5 proteins from different protein clusters (PsaA, SP_0845, PspA, PspC, PavB) inducing high antibody titers during pneumococcal infections. The fact that immunogenicity of some proteins correlates with their cell-surface abundance (PspC, PsaA, SP_0845) turns them into potential candidates for a protein-based vaccine. Further experiments will indicate the efficacy of the specific antibodies to opsonize pneumococci and therefore trigger recognition by the immune system.
Conclusion. The analyzed pneumococcal surface proteins are highly conserved and immunogenic. Besides, the surface abundance of some proteins is high and partly correlates with the observed immunogenicity. Therefore, these proteins represent promising candidates for a protein-based conjugate or subunit vaccine, which are urgently needed to combat pneumococcal infections in a serotype-independent manner.

MPV-FG04
Plasminogen interaction to *Helicobacter pylori* confers serum resistance
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The gastrointestinal pathogen *H. pylori* colonizes the gastric mucosa of more than half of the world's population. Colonization and subsequent infection with *Helicobacter* can result in chronic gastritis, an inflammation in the stomach causing abdominal pain that untreated might lead to ulcers and gastric adenocarcinoma. *H. pylori* interaction with plasminogen has been described previously and some surface proteins has been identified

(1, 2). The mechanism of interaction and its role in colonization of *H. pylori* is not fully understood. In this study, we observed that several clinical isolates of *H. pylori* for can bind human plasminogen at their surface. Therefore, outer membrane proteome of *H. pylori* was analysed in order to find plasminogen binding proteins by using 2D-PAGE proteomics approach. The identified proteins included; 19 kDa Hpg1, Hpg2 (18 kDa), Hpg3 (60 kDa), Hpg4 (16 kDa), Hpg5 (40 kDa), Hpg6 (50 kDa), and a 55 kDa catalase A. PgbA was included as positive control in our study (2). These identified proteins were recombinantly expressed in *E. coli* and purified by Ni-NTA chromatography. Plasmingen interaction to these proteins were verified by using ELISA and Biolayer Interferometry (Octet Red96). Hpg1, Hpg5, PgbA, and catalase bound to plasminogen in a range of K_d =0.5-2.5 μM. Plasminogen bound to the surface of *H. pylori* was accessible to host urokinase plasminogen activator that converted plasminogen into plasmin. The active plasmin bound to the surface of *H. pylori* degraded human fibrinogen, complement components C3 and C5. Our study showed new plasminogen binding proteins of *H. pylori* that may contribute to the virulence of this pathogen.

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MPV-FG05

Pra1, the *Candida* immune evasion protein is a protease that cleaves complement C3 and also blocks the effector components C3a and C3b

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Question: The human opportunistic fungal pathogen, *Candida albicans,* causes superficial and life-threatening systemic infections; particularly immunocompromised humans are at high risk for infections. The human pathogenic yeast *Candida albicans* activates the alternative and the classical pathway of complement. *Candida* has developed sophisticated means to evade host complement attack by recruiting host complement regulators and by blocking effector functions of activation products. *Candida* pH regulated antigen 1 (Pra1) is a multifunctional fungal immune evasive protein. Pra1 binds and complexes native C3 and blocks C3 conversion by the host C3 convertase[1]. Here we asked whether *Candida* Pra1 also binds to the C3 activation fragments and further interferes with their effector functions of complement activation fragments, and importantly how exactly Pra1 blocks/inactivates C3 and it activation fragments.
Methods: ELISA and bio layer interferometry was performed to investigate the binding of Pra1 to C3 activation fragments. C3 cleavage, cofactor assays, C3b deposition, phagocytosis, live cell image analysis and candida killing assays were performed. To investigate C3a antifungal activity, flocytometry, confocal microscopy, and colony forming unit assays was studied.
Results: Here we define *Candida* Pra1 the first fungal C3 cleaving protease. Pra1 cleaves C3 at a unique site. Pra1 directly targets C3, the central human complement protein and thereby blocks complement activation at the earliest time. The resulting C3a-like peptide lacks the C-terminal Arginine needed for C3a-receptor activation and Pra1 itself blocks C3a-like antifungal activity. The C3b-like molecule is degraded by the host protease Factor I. Pra1 also binds to C3a and C3b generated by human convertase and inhibits their effector functions. Pra1 inhibits C3a antifungal activity, C3aR binding and cell activation. Pra1 blocks C3b deposition on the fungal surface, inhibits opsonophagocytosis and *Candida* killing by human neutrophils.
Conclusion: *Candida* Pra1 is a fungal master regulator of innate immunity thatblocks complement activation and action at multiple levels and sites. Furthermore Pra1 blocks the effector function of the host generated effector components C3a and C3b.

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MPV-FG06

Stoichiometry of the bacterial type III secretion export apparatus

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Bacterial type III secretion systems are cell envelope-spanning effector protein-delivery machines essential for colonization and survival of many Gram-negative pathogens and symbionts. The membrane-embedded core unit of these secretion systems is termed needle complex. The needle complex is composed of a base that anchors the machinery to the inner and outer membranes, a hollow filament formed by inner rod and needle subunits that serves as conduit for substrate proteins, and a membrane-embedded export apparatus facilitating substrate translocation. While the stoichiometry of the base and of the major export apparatus protein have been revealed by structural analyses, the stoichiometries of the other export apparatus components and of the inner rod remain unknown.
We employed peptide concatenated standard and absolute quantification-based strategies to analyze the stoichiometry of the entire needle complex by mass spectrometry. Here we provide evidence that the export apparatus of the type III secretion system encoded on *Salmonella* pathogenicity island 1 contains 5 SpaP, 1 SpaQ, 1 SpaR, and 1 SpaS. We have corroborated the previously suggested stoichiometry of 9 InvA per needle complex and describe a loose association of InvA with other needle complex components that may reflect its function. These numbers indicate that the inner membrane patch of the needle complex base houses 104 transmembrane domains in total, a dense assembly whose function in the secretion process we merely understand. Furthermore, we present evidence that not more than 6 PrgJ form the inner rod of the needle complex.

MPV-FG07

Essential role of the SepF mycobacterial cell division protein

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Question: Cell division requires the sequential assembly at mid-cell of several essential proteins into a complex, called the divisome. Central to divisome formation is the FtsZ protein, as in its absence the divisome fails to assemble altogether (1). Early FtsZ-interacting proteins are crucial for the process, because they anchor the FtsZ-ring to the cytoplasmic membrane and act as structural modulators. In model organisms as *Escherichia coli* or *Bacillus subtilis*, these functions are provided by the joint action of two proteins that form together with FtsZ the so-called proto-ring. *Mycobacterium tuberculosis* has a homolog to only one of them - SepF - whose functions we have characterised *in vivo*.
Methods: Mycobacterial SepF-GFP variants were localised in *M. tuberculosis* and its non-pathogenic surrogate *Mycobacterium smegmatis* and the phenotype resulting from conditional overexpression and repression of the *sepF* gene investigated in *M. smegmatis*. Diversity of *sepF* was analysed in 1.500 strains of the *M. tuberculosis* complex (MTBC) in addition to *Mycobacterium leprae* and *Mycobacterium lepromatosis*. SepF variants were tested for complementation in a *sepF* conditional mutant background.
Results: In *M. tuberculosis* and *M. smegmatis* cells, SepF-GFP fusions localised in ring-like structures at potential division sites in a FtsZ-dependent way. Both, SepF overproduction and depletion resulted in a complete block of division prior to septation. Together with the high conservation of the gene in the MTBC, these results suggested that SepF is a key proto-ring protein for mycobacterial proliferation (2).
Toxicity of altered cellular SepF levels *in vivo* required the establishment of a single-copy complementation assay to enable testing *sepF* mutants in detail. This assay allowed us to find that the $SepF_{CtS}$ variant (2), in contrast to the $SepF_{CtS}$-GFP fusion, is functional *in vivo* and to find a subtle effect of the GFP fusion to the C-terminus of the wildtype SepF. Moreover, our results point at the α-helices located in the N- and C-termini as being important for mycobacterial SepF activity.

Conclusion: We show that SepF is an essential divisome component of the bacterial *Mycobacterium* genus (2, 3). It is then an additional inhibitable target to block proliferation of the pathogenic mycobacteria. Our new complementation assay provides the basis to fully characterise SepF functions in the mycobacterial divisome *in vivo*.

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QDV-FG01
Molecular diagnostic in the era of MRGN bacteria
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Currently, the spread of multiresistant Gram-negative bacteria (MRGN) producing extended-spectrum β-lactamases (ESBL) and carbapenemases is a global thread to public health. Bloodstream infections with MRGN bacteria are associated with increased mortality, which is primarily due to delayed appropriate treatment resulting in clinical failure. As most sepsis guidelines, the German guideline suggest an empiric treatment with a β-lactam with antipseudomonal activity (i.e. piperacillin/tazobactam, ceftazidim, cefepim or a carbapenem) in an optional combination with an aminoglycoside or a fluoroquinolone, which does not cover ESBL-producers except for carbapenems (last resort antibiotics). With up to 72 hours to result, the standard blood culture-based routine diagnostic is highly sensitive for detection of bloodstream infections, but takes excessively long.
In attempts to improve and accelerate diagnostic procedures, numerous molecular methods have been developed. Commercially available molecular approaches focus on species identification but cover often only a limited number of resistance genes and are therefore of minor use for clinical decisions regarding antibiotic treatment. Whereas a simple PCR based approach maybe suitable for multi-drug resistant Gram-positives like MRSA and VRE with 'monogenetic' resistance, most of these tests fail to accurately detect MRGN bacteria. This is explained by the highly genetically and phenotypically diverse group of β-lactamases conferring resistance to penicillins, monobactams, cephalosporins (ESBL) and/or carbapenemases. The β-lactamase superfamily counts more than 1500 variants subdivided in four phylogenetic classes (A, B, C and D) with numerous subgroups; some of them are highly conserved requiring a diagnostic resolution on a single nucleotide level, others share less than 40 % similarity challenging the identification of appropriate primer pairs. Finally, dozens of novel β-lactamases are annually discovered. Thus, an one-fits-all assay remains unlikely to be developed covering the species, all β-lactamases and other resistance and toxin factors.
We are going to discuss the diagnostics hurdles and present our running projects that aim to improve the molecular diagnostic in face of the increasing MRGN-problem.

QDV-FG02
Acceleration of microbiological diagnostics of sepsis
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Sepsis is an acute condition with high mortality rates. Immediate antimicrobial therapy is essential while the appropriateness of administered antibiotics is critical for the clinical outcome. One of the main tasks of clinical microbiology is identification and antimicrobial susceptibility testing of pathogens. Particularly in case of sepsis, diagnostics has to be performed rapidly to provide clinicians with the findings allowing early adaptation of antimicrobial therapy. Rapid sepsis diagnostics has been shown to guide antimicrobial therapy, which leads to improved patients' outcome.
Several methodological improvements have recently become available for accelerated microbiological diagnostics of sepsis. The advent of matrix-assisted laser desorption ionization/time-of-flight mass spectrometry (MALDI-TOF MS) has revolutionized clinical microbiology. The immediate (few minutes) species identification has become available from colonies after overnight sub-cultivation from positive blood culture broth. Furthermore, MALDI-TOF MS can directly be performed from positive blood cultures using lysis-centrifugation procedure. It allows species identification in 78.6 % of cases for Gram-negative rods, 41.9 % of cases for Gram-positive cocci, and in 62.5 % of cases for yeasts. Alternatively to this direct method, which takes approximately 20-30 minutes to perform, MALDI-TOF MS identification can be accomplished from the very short-term cultures, sub-cultivated onto solid medium from positive blood culture broth. The average incubation time needed for successful species identification from such "young" cultures is only 2.0 hours for Gram-negative rods, and 3.1 hours (with a short extraction procedure) for Gram-positive cocci. The same biomass can be inoculated into the automated susceptibility testing system at the early time point, i.e. after 2.4 hours or 3.8 hours for Gram-negative rods and Gram-positive cocci, respectively. Thus, the results of susceptibility testing are provided one day earlier. This short incubation method appears to be the best suitable for routine processing of positive blood cultures because it provides early result without any additional cost or time expenditure.
Direct blood culturing on solid medium has been investigated as an alternative to the liquid-based automated blood culture systems which are currently broadly used. Species identification from directly incubated agar plates was always achieved earlier than the growth detection by the automated system. At the positivity detection with automated system, only Gram stain is possible. The average time difference between bacterial growth detection in the automated system and species identification from microcolonies shortly incubated on solid medium was 4.6 hours.
In conclusion, a considerable acceleration of sepsis diagnostics is now available for use in clinical laboratories.

QDV-FG03
DiAL-FISH for the rapid detection and identification of bacterial agents
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Fluorescence *in situ* hybridization (FISH) is a powerful method for the cultivation-independent *in situ* detection and identification of microorganisms. Since its establishment over two decades ago it has become an essential tool in microbial ecology. As it is an easy, robust, cheap and rapid method, FISH is also occasionally used in clinical settings. However, until recently only three bacterial species could be identified simultaneously in one hybridization step, making the identification of larger sets of bacterial species laborious and time-consuming, thereby impairing the use of FISH in many diagnostic approaches. Here we report on the development of an rRNA-targeted FISH baseddiagnosticalgorithm (DiAL-FISH) allowing for the cultivation-independent rapid detection, identification and quantification of up to thirteen bacterial pathogens in clinical samples.
Novel group-specific probes targeting members of the *Rickettsiaceae*, the *Bacillus cereus* group and *Leptospiraceae* as well as species-specific probes targeting *Vibrio cholerae, Yersinia pestis, Escherichia coli* and *Coxiella burnetii* were designed using the arb-software package. The specificity of the probes was evaluated using formamide-series and the daime software. By combing these probes with previously published probes for *Brucella* spp, *Burkholderia mallei*, *Burkholderia pseudomallei, Neisseria meningitidis,* and *Francisella tularensis* the diagnostic assay now targets thirteen bacterial pathogens. Using multicolored double-labeled oligonucleotide probes, these species can now be identified by only two hybridizations in less than four hours.
We applied our set of probes successfully for the identification of infectious agents in various sample materials, including paraffin sections of lymph nodes, powder samples and skin surfaces. To overcome the need for a fume hood and allow for broader application of this method, we currently aim to substitute for toxic formamide, which is used in conventional FISH to adjust the stringency of the probe, by non-toxic urea. Interestingly, usage of urea so far not only results in the specific detection of bacteria, but also in brighter fluorescence signals for some probes.
In summary, DiAL-FISH allows the fast and direct visualization of rarely occurring, but nevertheless important bacterial pathogens in a straightforward and robust manner. In addition, it provides a different and independent laboratory method that supplements PCR-based detection methods for these bacteria.

QDV-FG04

Effect of nisin on the survival of *Listeria monocytogenes* in sour curd cheese after artificial contamination

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To protect cheese products against foodborne pathogens, food additives such as nisin are added. The influence of common nisin concentrations to various *Listeria* species has been well discussed *in vitro*. However, the aim of this study was to evaluate the bioavailability and the effect of nisin on the survival of *Listeria monocytogenes* in sour curd cheese (SCC). Different nisin concentrations were tested *in vivo* with a mix of eight different strains of lactic acid bacteria (LAB) and one *L. monocytogenes* strain, respectively. Therefore, SCC were supplemented with or without 150 units nisin per g cheese. SCC produced in our laboratory were contaminated with 10^5 CFU g^{-1} *L. monocytogenes* and incubated for two days at 30 °C and 99 % humidity. After incubation growth of LAB and *L. monocytogenes* were determined with conventional cultivation techniques and fluorescence *in situ* hybridization (FISH). The cultivation dependent approach revealed that 150 U g^{-1} nisin did not inhibited growth of LAB but 2000 U g^{-1} nisin reduced LAB for one log CFU g^{-1}. However, growth of *L. monocytogenes* was reduced 2.6 log CFU g^{-1} at 150 U g^{-1} nisin. The aerobic most probable number was 10^8 CFU g^{-1} after cheese ripening, regardless if *L. monocytogenes*, nisin or both were present. FISH based results were in line with cultivation based results for both LAB and *L. monocytogenes*. Furthermore, FISH analyses showed that *L. monocytogenes* did not proliferated over incubation time at nisin addition of 150 U g^{-1}. In conclusion, nisin addition was bioavailable and anti-listerial active in SCC, therefore reducing cell counts of *L. monocytogenes* but not LAB after ripening time usually applied in dairy. Our results were a successful challenging test and upcoming research will be carried out with typical *Listeria* cell amount found in cheese.

QDV-FG05

A view to a kill? – ambient bacterial load of frames and lenses of spectacles and evaluation of different cleaning methods

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Surfaces with regular contact to the human body are usually contaminated with a variety of microorganisms and might be considered as fomites [1, 2]. Although widespread in the population, little is known about the ambient microbial contamination of spectacles, particularly in non-clinical environments. We investigated 11 worn spectacles of students and employees from a university environment and 10 worn spectacles of inhabitants of a nursing home for elderly people. Each spectacle was swab sampled at 7 sampling sites following DIN ISO 10113. The microbial load was determined by serial dilutions and aerobic cultivation on TSA agar. Dominant bacterial morphotypes were identified by MALDI biotyping. All investigated spectacles were found to be contaminated with bacteria, most densely at the nose pads and the inner sides of the ear clips, i.e. at sites with direct skin contact. Averaged over all sites, the microbial load of the university spectacles ($9.6 \pm 29.7 \times 10^3$ CFU cm^{-2}) was not significantly different compared to the spectacles of the nursing home people ($4.3 \pm 6.0 \times 10^3$ CFU cm^{-2}). 213 bacterial isolates were obtained from all spectacles and assigned to 11 genera, with *Staphylococcus* being by far the most dominant one. On genus-level, bacterial diversity was significantly higher on spectacles from the nursing home (10 genera) compared to the university environment (2 genera). Five cleaning methods were investigated using lenses artificially contaminated with *Escherichia coli*, *Micrococcus luteus*, a mixture of *E. coli* and *M. luteus*, and *Staphylococcus epidermidis*, the dominant isolate in our study, respectively. Best cleaning results (96 % - 100 % median germ reduction) were obtained with impregnated wipes; dry cleaning was less effective (74 - 95 % median germ reduction). Finally, 10 worn university spectacles were cleaned with wipes impregnated with an alcohol-free cleaning solution before sampling. The average bacterial load was significantly lower ($0.08 \pm 0.18 \times 10^3$ CFU cm^{-2}) compared to the uncleaned university spectacles investigated before (see above). Clearly, spectacles are contaminated with bacteria of mostly human skin origin, including significant shares of potentially pathogenic ones, and might play a role in eye infections [2].

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RSV-FG01

Engineering synthetic regulatory systems for enhanced chemical production

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Engineering microbial metabolic pathways offers the opportunity to produce renewable chemicals and advanced biofuels. In order for this technology to be economically viable, heterologous production systems must be optimized to have high titers, yields, and productivities. Microbes evolved sophisticated regulatory network to adapt to various environments for cell growth, but not to produce chemicals in large quantities and with high efficiencies. To improve heterologous chemical production, we employed sensor-regulators to dynamically control the expression of pathway genes, which balance the metabolism of heterologous pathways and prevent the accumulation of intermediates to toxic levels. We also develop sensor-selectors to continuously select for high-performing, non-genetic variants within iso-genetic populations. Using the designed synthetic regulatory systems, we have demonstrated significantly enhanced product titers, yields, productivities and genetic stability on multiple biosynthetic pathways. Design principles of these synthetic regulatory systems should be useful in other areas of synthetic biology, enabling new avenues of research and applications.

RSV-FG02

Hunting for new genetic targets – biosensor-based FACS screening of microorganisms

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The engineering of microbial strains for the production of small molecules is a time-consuming, laborious and expensive process. This can be mostly attributed to the fact that good producers cannot be readily obtained by high-throughput (HT) screening approaches since increased product formation usually does not confer a clear phenotype to producing strain variants.

Recently, advances were made in the design and construction of genetically encoded biosensors for detecting small molecules at the single-cell level [1]. At the IBG-1, we focus on transcription factor-based biosensors. In these sensor systems a transcriptional regulator binds to its target promoter in response to the presence of the small metabolite of interest and subsequently activates expression of a fluorescent reporter protein. In combination with fluorescent-activated cell sorting (FACS) we could already demonstrate the value and potential of such biosensors by screening large libraries of chemically mutagenized *Corynebacterium glutamicum* cells for L-lysine producers [2].

Motivated by the assumption that other amino acid substitutions in MurE might lead to even higher L-lysine titers, we developed RecFACS for the site-directed saturation mutagenesis of microbial genomes. RecFACS combines targeted genome mutagenesis by recombineering with biosensor-guided HT screening [3]. We successfully used RecFACS to generate and screen a site-saturation library of *murE* of *C. glutamicum* via FACS and identified 12 different amino acid substitutions causing different L-lysine titers.

Currently, we are engineering the L-lysine sensor and other biosensors to alter their individual ligand specificities. Goal is the design of custom-made biosensors for the detection of compounds of biotechnological interest.

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RSV-FG03
Engineered riboswitches – convenient building blocks for the construction of synthetic genetic circuits
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One of the most interesting areas of Synthetic Biology is the control of cellular behaviour using engineered circuits. Genes with selected features are combined in a building block manner and transferred to organisms of interest to achieve the desired biological functions. However, the expression level of the corresponding genes must be regulated and fine-tuned to avoid unbalanced gene expression and the accumulation of toxic intermediates. In order to achieve this, a versatile set of RNA-based control devices, so called engineered riboswitches, have been developed.
We make use of *in vitro* selected, small molecule binding aptamers inserted into untranslated regions of several mRNAs. Thereby we exploit the fact that upon ligand binding the aptamer structure interferes with cellular functions such transcription, translation or splicing. The striking advantage of such regulator is that in principle they can be designed to sense any ligand of choice. In addition, the direct RNA-ligand interaction renders auxiliary factors unnecessary.
We will present engineered riboswitches developed for all three domains of life and targeting different cellular processes. In addition, we will give a mechanistic insight into these regulators.

RSV-FG04
Synthetic RNA-based control units for balanced triterpene biosynthesis in cyanobacteria
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As oxygenic phototrophs with a versatile metabolism, cyanobacteria constitute an economically advantageous platform for the production of a diversity of high-value compounds including plant triterpenes. Concretely, the natural occurrence of squalene as an intermediate of the hopanoid pathway in the model cyanobacterium *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*) provides a new vantage point for bioengineered triterpene production. Cloning of a triterpene biosynthesis cassette from *Arabidopsis thaliana* for expression in *Synechocystis* together with engineering the cyanobacterial host allows us to synthetically produce a variety of interesting precursors, e.g. marneral, thalianol, β-Amyrin. However, accumulation of high levels of intermediates appears to be a challenging problem of synthetic pathways that can become toxic resulting in reduced host growth. Thus, we are programming metabolic pathways using newly designed logical gates that transmit and process information in an innovative way, namely via RNA-based control units. For example, an extended RNA-based comparator unit has been established for self-adjustment and balancing the gene expression of two genes. Accumulation of the first intermediate induces specific, synthetic RNA regulators that on the one hand results in repression of its own synthesis, and on the other hand activate expression of the second gene encoding for the downstream enzyme in the pathway. A second internal feedback-loop measures accumulation of the second intermediate and represses/activates vice versa. In that way, a smart, automated and dynamically adapting control can be implemented (i) sensing changes in metabolite concentrations and (ii) using this information to control transcriptional regulation of genes encoding enzymes in metabolic pathways with a high degree of flux control. Our toolbox of synthetic RNA control units will expand the field of synthetic biology within the research area of phototrophic organisms and necessarily improve metabolic engineering strategies in cyanobacteria and other hosts.

SIV-FG01
Cryptic Pathways at the Host-Microbe Interface
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Small molecules play important roles in microbial interactions as they may support the mutualism or serve as pathogenicity factors. In many cases, however, the molecular basis of the chemical crosstalk remains unknown. Thus, it is often useful to combine analytical and genetic tools to gain insight into the molecules that are produced at the host-microbe interface. This talk focuses on our progress in exploring molecular assembly lines in less investigated bacterial genera such as *Burkholderia* spp.

SIV-FG02
How indole-3-acetic acid is modulating *Tricholoma vaccinum* ectomycorrhiza?
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The auxin indole-3-acetic acid (IAA) is a long time known phytohormone affecting the growth of plants and its roots. Here, we report on fungus-derived IAA, causing morphological changes in the development of ectomycorrhiza. Both partners, tree and fungus are affected. The biosynthesis in fungal hyphae, excretion, induced ramification in fungal cultures, and enhanced Hartig' net formation in mycorrhiza were observed and gene expression, labeled IAA precursors, aldehyde dehydrogenase *ald1* overexpressing *Tricholoma vaccinum* and heterologous expression of a transporter were used to study the effects in molecular detail.
In *T. vaccinum,* IAA is produced from tryptophan via indole-3-pyruvate and indole-3-acetaldehyde finally oxidized by an aldehyde dehydrogenase. Upregulation of *ald1* was found in ectomycorrhiza and by external supplementation with indole-3-acetaldehyde. In mycorrhization studies, *ald1* overexpressing *T. vaccinum* showed an increased width of the apoplast between the cortical cells of the Hartig' net, as well as upregulation of the multidrug and toxic extrusion (MATE) transporter Mte1, involved in the export of IAA from fungal cells. External supply of IAA and its precursors induced elongation and increased branching in mycorrhizal fungi, whereas no morphogenetic changes were observed in saprobic fungi like *Schizophyllum commune.* These findings indicate a crucial role for IAA in the regulation of ectomycorrhiza formation and morphology.

SIV-FG03
Bacterial-macroalgal interactions – the symbiotic tripartite community of *Ulva* (Chlorophyta)
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The interactions between marine macroalgae *Ulva mutabilis* Føyn (Chlorophyta) and their associated bacteria depend strongly on chemical stimuli. Many studies have attempted to extract external bacterial compounds with growth regulatory activities motivated by the evidences that marine bacteria produce auxin- and cytokinin-like regulators. A pre-requisite for the investigation of these signal mediated interactions is the availability of axenic cultures. To produce axenic cultures gametophytes of *U. mutabilis* were induced to form gametangia by removal of sporulation inhibitors. Released gametes were separated from their accompanying bacteria by taking advantage of the gametes fast movement towards light [1]. These axenic cultures develop into callus-like colonies. The combination of specific bacteria such as the *Roseobacter* sp. (MS2) (among other alpha and gamma- proteobacteria) and the *Cytophaga* sp. (MS6) can recover growth, development and morphogenesis via diffusible morphogenetic compounds and replaces thus completely the bacterial microflora of *U. mutabilis* [2,3]. This well-defined unialgal/bi-bacterial system allowed us to study the activity of novel signal molecules and to determine the mechanism by which bacteria and *U. mutabilis* interact with each other. To prove the ecological significance, axenic algae were also used to survey morphogenetic activities in seawater of the Ria Formosa Lagoon (Faro, Portugal). The tripartite community of *U. mutabilis, Cytophaga* sp., and *Roseobacter* sp. is an ideal system to investigate, for example, (i) symbiotic interactions, (ii) the chemotactic attraction of bacteria (iii) the quorum sensing systems between bacteria and the host, and (iv) the interactions between the macroalga and opportunistic bacteria/pathogens. The bio-analytical and microbiological approach will be supported by a newly developed transformation system for *U. mutabilis* [4]. The approaches in chemistry and genetics along with the short generation time of a naturally occurring developmental mutant of *U. mutabilis* make this tripartite community well suited to model studies in chemical ecology and developmental biology.

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SIV-FG04
Host and symbiont jointly control gut microbiota during complete metamorphosis
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Holometabolous insects undergo a radical anatomical re-organisation during metamorphosis. This poses a developmental challenge: the host must replace the larval gut but at the same time retain symbiotic gut microbes and avoid infection by opportunistic pathogens. By manipulating host immunity and bacterial competitive ability, we study how the host immune system interacts with symbiotic bacteria to manage the composition of the microbiota during metamorphosis. Disenabling one or both symbiotic partners alters the composition of the gut microbiota, which incurs fitness costs: adult hosts with a gut microbiota dominated by pathogens such as *Serratia* and *Staphylococcus* die early. Our results reveal an interaction that guarantees the safe passage of the symbiont through metamorphosis and benefits the resulting adult host. Moreover, the data also suggest that the risk of opportunistic infection during the destruction of the larval gut is countered by the host through up-regulation of immune function. These findings may be broadly applicable to insects with complete metamorphosis, including many disease vectors.

SIV-FG05
Nitric oxide is an ambivalent mediator of microbial interactions in beewolves
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The selective pressure to protect themselves against competing microbes drove beewolves to evolve several sophisticated defense mechanisms. They engage in a symbiosis with *Streptomyces* bacteria that protect the beewolf offspring with an antibiotic cocktail against opportunistic mold fungi in the surrounding soil. Further, the beewolf egg releases a strong burst of nitric oxide into the brood cell only a few hours after oviposition. On the one hand side, beewolves thereby initiate a radical brood cell sterilization that significantly delays the onset of fungal growth. However, as a potent antimicrobial agent, nitric oxide also poses a threat to the defensive symbiosis. Besides damaging proteins and lipid, nitric oxide also induces severe DNA damage. The repeated exposition over millions of years left an evolutionary signature in the *Streptomyces* symbionts. They not only show an exceptional strong induction of radical defense mechanisms upon nitric oxide exposure, but also suffer from advanced genome erosion. Beewolves probably need to maintain a delicate equilibrium. Releasing to much nitric oxide into a brood cell cell might damage their symbiont beyond repair, releasing not enough could allow a fatal infestation with opportunistic mold fungi. Thus, the fate of this unique symbiosis depends upon one simple, small molecule.

SIV-FG06
Pleasant guests restrain – Can selective advantages explain the AT-bias of endosymbiotic genomes?
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The genomic base composition of bacteria is highly diverse. Strikingly, intracellular bacterial symbionts as well as plasmids and viruses which all share a host-dependent lifestyle generally exhibit higher AT-contents. However, the driving force towards high AT-contents remains unclear. The loss of nucleotide biosynthesis genes as well as a nucleotide uptake from the host's cytoplasm has been hypothesized to reduce the metabolic burden an endosymbiont imposes on its host. Since dATP and dTTP are the most abundant nucleoside triphosphates in a cell, low GC-contents of endosymbiotic genomes are expected to be selectively favoured. However, an experimental test of this hypothesis is currently lacking, since the endosymbiont-host-relationship is difficult to disentangle experimentally.
This project aims at unravelling the molecular explanation for the observed decrease in genomic GC-content by the use of a tractable model system. For that, *Escherichia coli* strains which differ in their demand for AT- and GC-nucleotides were generated by introducing plasmids of high AT- or high GC-contents. Experimentally increasing GC-contents caused a drastic decrease of the cells' fitness, which is in line with the proposed hypothesis. Furthermore, determining the plasmid copy number via quantitative real-time-PCR revealed lower copy numbers of all GC-rich plasmids compared to AT-rich plasmids. Providing the cells with GC-nucleotides resulted in a fitness increase of cells with higher demands for GC nucleotides, indicating that GC nucleotides being a scarce resource that limit growth. Taken together, our results provide strong experimental evidence for a selective advantage of high AT-contents in the genomes of intracellular bacteria.

WAV-FG01
***Legionella* in evaporative cooling systems – new federal immission control act**
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Beim Betrieb von Verdunstungskühlanlagen kann es zur Entstehung von Legionellen-haltigen Aerosolen kommen. Diese Aerosole stellen ein gesundheitliches Risiko dar, da sie bei Inhalation zu schweren Lungenentzündungen führen können. Da sich Legionellen mit den Aerosolen über mehrere Kilometer ausbreiten können, kann es im Umkreis einer Anlage zu einer großen Anzahl von erkrankten Personen kommen. In Deutschland gab es bisher drei größere Legionellose-Ausbrüche mit insgesamt ca. 300 Erkrankungsfällen und 7 Todesfällen, bei denen Verdunstungskühlanlagen als Infektionsquelle nachgewiesen oder vermutet werden.
Zur Gewährleistung eines hygienisch einwandfreien Betriebs von Verdunstungskühlanlagen wurde die VDI Richtlinie VDI 2047-2 erarbeitet. Sie bildet die Grundlage für eine neue Immissionsschutzverordnung für Verdunstungskühlanlagen. Im Vortrag wird der aktuelle Stand dieser Verordnung vorgestellt.

WAV-FG02
Occurrence, frequency and distribution of *Legionella pneumophila* strains isolated from environmental sources in Germany
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Legionella bacteria are a commonly distributed in man-made water systems. After transmission via aerosols to humans these bacteria can cause pneumonia. Currently, 59 species exist within the genus *Legionella*. The most relevant species *L. pneumophila* can be divided into at least 16 serogroups. Serogroup 1 can be further subtyped by monoclonal antibodies into so called monoclonal subgroups. Further subtyping of *L. pneumophila* strains is performed by so called SBT, a seven gene sequence based typing assay, which is currently the gold standard for *Legionella pneumophila*.
More than 83 % of the community-acquired and travel-associated cases were causes by *L. pneumophla,* serogroup 1, MAb 3-1 positive strains. Only 43 % of nosocomial cases did so. In contrast only 11 % of environmental isolates not related to human infections reacted with Mab 3-1. Albeit, the molecular basis for this phenomenon is not fully understood it is a useful marker to distinguish between highly virulent and less virulent *L. pneumophila* strains*. L. pneumophila* strains belonging to serogroups 2 to 15 cause seldom infections in healthy persons, but account for 45 % of nosocomial infections. They are found at a similar frequency in environmental samples. Thus, in immunocompromised hosts less virulent strains might cause infections*. L. non-pneumophila* species are seldom isolated from patients, but commonly found in routine water samples. Most common environmental species is *L. anisa.*
The clinical isolates could be classified into 24 different sequence types (ST). In Germany, ST1 (24 %) and ST9 (7 %), ST 23 (5 %), ST 62 (11 %) and ST182 (18 %) were the most frequently ones. The latter one is almost exclusively detected in the Berlin area.
The detected ST profiles were a combination of both frequently isolated and unique STs, and of both worldwide distributed and more local strains (ST 332, 425, 334). Environmental isolates are much more heterogeneous (276 ST) arguing for the assumption that only few strains cause the majority of infection especially in not immunocompromised hosts. Beside serogroup 1 strain some ST of serogroups 2, 3, and 6 seems to have a higher virulence since they are often associated with human infection.

WAV-FG03

Legionella species diversity and dynamics from surface reservoirs to cold and hot tap water: from a cold adapted to a thermophilic community

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Members of the genus Legionella are found around the globe in a variety of natural and man-made freshwater environments. However, very little is known about their specific abundances in these environments on a species level. This is primarily due to their Viable-But-Non-Culturable (VBNC) state and their inability to grow outside their protozoan hosts. Best studied is *L. pneumophila* which has been found in natural freshwater environments such as lakes, streams and Drinking Water Supply System (DWSS). It also contaminates air conditioning systems, cooling towers and condensers. We analysed water samples from a complete DWSS in Northern Germany for its Legionella species composition using genus-specific PCR amplicons and SSCP fingerprints analyses based on 16S rRNA genes. These analyses comprised the whole supply chain including the two raw waters, treatment process and large scale storage, and a seasonal study of finished drinking water sampled monthly from cold and hot tap water. Treatment of the raw water had a major impact on Legionella species by reducing their diversity and abundances. The Legionella species composition of the tap water was highly distinct from that of both source waters. In cold water 7 to 14 different phylotypes of Legionella (PTLs) were observed per sample with relative abundances ranging from >1 % to 53 %. In hot water, *L. pneumophila* was present during all seasons at high relative abundances (8 % to 40 %) accompanied by 5 to 11 other PTLs of which 6 PTLs were in common with cold water. Cold and hot water did not show a distinct seasonal pattern. Many of the observed PTLs are considered to represent new Legionella species indicating a large diversity of uncultured Legionella species in tap water. This study demonstrated the establishment of a thermophilic Legionella community, including *L. pneumophila*, able to grow in the hot water above 50 °C. An increase of the *L. pneumophila* population from 50 °C to 58 °C was observed with the molecular approach used and could be confirmed with independent real-time PCR measurements. If a part of this community is infectious to humans has to be assessed in future studies. This thermophilic community is of general relevance with respect to hot water management and public health, but also with respect to the ecology and evolution of the genus Legionella.

Lesnik, R., Brettar, I and M. G. Höfle (2015) Legionella species diversity and dynamics from surface reservoir to tap water: from cold adaptation to thermophily. ISME J. published online, DOI: 10.1038/ismej.2015.199

WAV-FG04

Detection of system-wide Legionella contaminations in drinking water plumbing systems – risk factors, temporal-spatial variability, strategies

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Introduction: It is necessary to access the hygienic-microbiological situation of drinking water plumping systems (DWPS) by use of limited information. This in turn has important implications for the design and implementation of monitoring and remediation measures.
Objectives: The aim of the study was to gain deeper insight into Legionella contamination of DWPS. The dimension of German-wide Legionella contamination of DWPS should be evaluated. Risk factors and parameters that could help to detect contaminations had to be identified.
Methods: All German public health authorities were interviewed by a questionnaire about monitoring. Acquired data of water samples of the period 2003-2009 were merged into a relational database. In a field study, long-time monitoring of water quality in eight Legionella contaminated buildings was done over half-a-year according to German drinking water ordinance (GDWO; ISO 19458:2006). Additionally, short-term variability in the course of a day was monitored twice in one building by sampling every two hours. *Legionella* spp. was analysed by culture methods following GDWO and DIN EN ISO 11731-2:2008 and by qPCR using the *L. pneumophila* mip gene (Shannon *et al.* 2007, modified for higher heat stability) for amplification after sample filtration and DNA extraction with Chelex Resin 100 and proteinase K (Walsh *et al.* 1991).
Results: In Germany, 12.2 % (n=22,124) of drinking water samples from public buildings showed Legionella contamination exceeding the technical threshold limit (TTL). Detailed data showed that hospitals were at highest risk. Nursing homes (n=438), schools (n=703) and kindergartens (n=928) exceeded the TTL of Legionella at least one time in the period 2003-2009 in more than 20 % of the objects.
Incidences of *Legionella* contamination showed a strong spatial and temporal (short- and long-term) variability. Sampling points where cultural or qPCR results continuously exceeded the GDWO technical threshold level at the outlet were rarely detected. Statistical analysis and modelling showed that the parameters, temperature at constancy', stagnation and pipe length seem to be the best predictors of *Legionella* contamination at single outlets.
Conclusions: The observed long and short term variation of *L. pneumophila* should result in a re-evaluation of guidelines concerning sampling and monitoring strategies. Identified risk factors should be taken into account for detection at an early stage.

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WAV-FG05

New detection methods for *Legionella* in water and air

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Legionellosis outbreaks have occurred consistently during the last years worldwide. Aerosolized *L. pneumophila* from evaporative cooling towers were found frequently as source. Regarding the high risk of infection, rapid detection methods for *Legionella* have a great impact on environmental health and diagnostics. We have developed a combined analytical process that uses monolithic adsorption filtration (MAF) and centrifugal ultrafiltration (CeUF) to concentrate viable *Legionella* from 10 L to 1 mL in 45 min. Bioaerosols are collected by cyclone impinger Coriolis µ. Multiplexed quantification of *Legionella* is performed by DNA and antibody microarrays on the analysis platform MCR 3. A sandwich microarray immunoassay (SMIA) is developed for monoclonal subtyping of *L. pneumophila*. Isothermal amplification on DNA microarrays is established for rapid quantification of *L. pneumophila* and Legionella ssp.
A monoclonal antibody (mAb) against *L. pneumophila* Sg 1 Bellingham and a polyclonal antibody (pAb) against *L. pneumophila* Sg 1 was immobilized as capture antibodies on microarray chips. Surface water samples were processed by MAF-CeUF prior to SMIA. A limit of detection of 8.7 CFU/mL and 0.39 CFU/mL was achieved for pAb and mAb, respectively. A recovery of 99.8 ± 15.9 % was determined for concentrations between 1 CFU/mL and 1,000 CFU/mL [1]. In a first study, sampling sites along the river Isar were chosen with increasing anthropogenic influence by e.g. wastewater treatment plants, industrial plants, and agriculture. With increasing anthropogenic influence along the river, the pAb has shown significantly higher CL signals compared to the source. At a reservoir we have identified subtype Bellingham.
Bioaerosols and treated waste water from factories that produce waste water with an increased temperature (e.g. breweries, diaries, and paper production) were studied by usage of an antibody microarray that contains a panel of monoclonal antibodies for subtyping of L. pneumophila. First results have shown that *L. pneumophila* could be identified in treated waste water after concentration by MAF and CeUF and in bioaerosol samples.
Finally, we have established the first microarray-based isothermal amplification method to quantify *L. pneumophila* and *Legionella* ssp. The detection limit was 1.8 × 101 GU/mL and 1.2 × 104 GU/mL, respectively.

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WAV-FG06

Detection of Legionella in aerosols from cooling towers

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Cooling towers are used since many years to dissipate heat loads from industrial processes. Their cooling effect is based on the principle of evaporation of water. In order to make this process as effective as possible in most cases a trickling or spraying of the cooling water is done to create a large evaporation surface. This can also contribute to formation of water droplets (aerosols) which can be discharged into the ambient air. Due to

favorable growth conditions (moisture, nutrients, temperature) for microorganisms in the water and in particular in biofilms in the circuits as well as on the evaporation surfaces, the water droplets may also contain *Legionella*. *Legionella*-containing aerosols represent a health risk because they can lead to illness (severe pneumonia) in case of inhalation.
In the research project "Detection of *Legionella* in aerosols from cooling towers" (UFOPLAN FKZ 3712 61 202) a sensitive detection method for *Legionella* in the air from cooling towers (emission measurement) and in the ambient air should be developed. It should also be investigated, how *Legionella* bacteria are released from biofilms in cooling plants into the air and how long the Legionella can survive there under different conditions (temperature, humidity).
For a sampling of possibly Legionella-containing aerosols from cooling towers a process using wet cyclone (Coriolis µ) was developed. For the subsequent quantitative detection of *Legionella* in the collected air samples both a cultural method (ISO 11731 and DIN EN ISO 11731) and a molecular biological qPCR detection was established. In the combination of both methods, a detection limit of 5.5 CFU/m³ air or 60 GU/m³ air could be achieved. An adverse impact of potential air matrix components on the detection method could not be shown.
Regarding the possible release of *Legionella* from biofilms, no quantitative relationship between *Legionella* concentrations in the cooling water, in biofilms and in the emitted aerosols were detected in 10 practice facilities. In none of the aerosol samples Legionella were detected culturally. However, by qPCR Legionella were detected in the same air samples. Generally, even at high concentrations of Legionella in cooling waters it is not necessarily to expect *Legionella*-contaminated aerosols.
Laboratory studies on the survival of *Legionella* in aerosols showed that cultivability decreased considerably after a short period during which the bacteria remained in the air of a bioaerosol test chamber. Variations in temperature and humidity didn't influence the cultivability of *Legionella* significantly.

YEV-FG01
Two novel yeast species from the gut of two different termite species
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Question: The termite gut is a complex microbiome. It contains a diverse community of microorganisms (Archaea, Bacteria and yeasts). Furthermore, flagellates are present in lower termites. The microbiotic gut community plays an important role in the degradation of lignocellulolytic material. The symbionts provide enzymes for sugar-based polymer degradation next to the termite's own enzymes (cellulases, hemicellulases; [1]). The present work focuses on the isolation of novel yeast species with lignocellulolytic abilities from the gut of termites.
Methods: Microbial enrichments of yeast symbionts were performed in Vogel's minimal salt medium and glucose-yeast extract-peptone (GYP) medium in the presence of antibiotics. The enrichment cultures were obtained on agar plates and axenic cultures by micromanipulation (membrane technique, [2]). Several tests were performed to characterize the physiological properties of the novel species (i. a. API tests, sugar-based polymer degradation assays, 4-methylumbelliferyl (4-MUF)-assays). The taxonomic position was determined by sequencing of suitable genome regions (ITS, SSU, LSU, RPB1, RPB2, TEF, MtSm).
Results: The novel yeast species were able to degrade azurine-crosslinked xylan. 4-MUF-β-xylobiose and 4-MUF-β-xylotriose were degraded by both species. Weak activities of α-L-arabinofuranosidases, α-D-glucopyranosidases and α-D-mannopyranosidases were detected for at least one of the isolated species. Cellulase and laccase activity was abscent. Vitamin free growth was possible in both cases. One species was identified as a basidiomycete which is closely related to the *Cryptococcus* clade. The second, ascomycetous species was related to the *Sugiyamaella* clade.
Conclusion: Two new yeast species are in axenic cultures. They originate from the gut of two different termite species. Both yeast isolates degrade xylan. 4-MUF-assays demonstrate the ability of hemicellulose side chain-cleavage (α-L-arabinofuranosidase, α-D-glucopyranosidase or α-D-mannopyranosidase). This implicates a symbiotic role in the degradation of hemicellulose in the respective host gut. The new species could be promising sources for the isolation of novel enzymes, such as xylanases.

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YEV-FG02
Three alcohol dehydrogenase genes are responsible for ethanol degradation in *Y. lipolytica*
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The non-conventional yeast *Y. lipolytica* is able to utilize a wide range of different substrates like glucose, glycerol, ethanol, acetate, proteins and different hydrophobic molecules [1]. Although most metabolic pathways for the utilization of these substrates have been clarified by now, the responsible genes for ethanol degradation have been neither identified nor characterized. It was still unclear whether ethanol is degraded by alcohol dehydrogenases [2] or by an alternative oxidation system [3].
In order to detect the genes that are required for ethanol degradation in *Y. lipolytica*, eight alcohol dehydrogenase (*ADH*) genes and one alcohol oxidase gene (*FAO1*) have been identified and respective deletion strains were tested for their ability to metabolize ethanol. As a result of this, we found that the availability of *ADH1*, *ADH2* or *ADH3* is required for ethanol utilization in *Y. lipolytica*. A strain with deletions in all three genes is lacking the ability to utilize ethanol as sole carbon source. Adh2p is considered to be the main enzyme for ethanol degradation because it showed by far the highest enzyme activity of the three enzymes.
As *Y. lipolytica* is a non-fermenting yeast, it is neither able to grow under anaerobic conditions nor to produce ethanol [1]. To establish alcoholic fermentation, the respective key genes of *S. cerevisiae*, *ScADH1* and *ScPDC1*, were overexpressed in an *ADH* deletion strain of *Y. lipolytica*. But instead of producing ethanol, the respective strain regained the ability to use ethanol as single carbon source and was still not able to grow under anaerobic conditions.

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YEV-FG03
Triterpenoids from *Saccharomyces cerevisiae*
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S. cerevisiae is a potential source for industrial production of triterpenoids. Numerous articles were published that describe modifications of the isoprenoid biosynthesis in yeast underlining the importance of the organism as well as the particular pathway for metabolic engineering approaches. In addition to the terpenoids found in yeast a vast number of molecules from this compound class could potentially be produced by means of metabolic engineering (Immethun *et al*. 2013).
Pentacyclic triterpenoids are found in plants and some of these molecules - like betulinic acid - show antifungal, antibacterial, and antiviral activity (Moses *et al.* 2013). Therefore, these molecules are of economical relevance and scalable production processes from renewable resources are needed. Here we present the introduction of enzymes needed for betulinic acid production in yeast. A major success factor for betulinic acid production is the choice of the best CYP (cytochrome P450 monooxygenases) and CPR (cytochrome P450 oxidoreductase) combination that allows oxidation of lupeol to betulinic acid. The selection of an optimal heterologous oxidosqualene cyclase that form lupeol from 2,3-oxidosqualene is additionally of importance to maximize betulinic acid yield. Together with the choice of a suitable host strain the generation of a new production strain for pentacyclic triterpenes is well underway (Lewandowski *et al.*, in preparation).

Moses T, Pollier J, Thevelein JM, Goossens A. 2013. Bioengineering of plant (tri)terpenoids: from metabolic engineering of plants to synthetic biology *in vivo* and *in vitro*. The New phytologist 200 (1): 27-43.
Immethun CM, Hoynes-O'Connor AG, Balassy A and Moon TS 2013 Microbial production of isoprenoids enabled by synthetic biology. Frontiers in Microbiology 4 doi: 10.3389/fmicb.2013.00075.

YEV-FG04
Urm1 – a unique ubiquitin-like protein that functions in protein and tRNA modification
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Urm1 is a dual-functional protein, acting either as a ubiquitin-like modifier in a protein conjugation pathway termed urmylation or as a sulfur carrier in tRNA anticodon thiolation. Activation of the modifier depends on the E1-like enzyme Uba4 and involves the formation of a Urm1 thiocarboxylate. Since both Urm1 functions rely on thiocarboxylate formation, they are thought to be mechanistically linked. But this has not been investigated so far.
Therefore, we investigated how urmylation is affected under conditions, which are known to negatively affect tRNA thiolation. Here, we demonstrate that the conjugation of Urm1 is mediated by the same sulfur transfer pathway also responsible for tRNA thiomodification. This is also supported by a down-regulation of urmylation during sulfur starvation. In addition, we find that elevated temperatures decrease Urm1 protein levels and therefore overall urmylation in yeast, revealing a new possibility for environmental regulation of this protein modification pathway. Moreover, Uba4 mutants that were previously described to abolish thiocarboxylate formation were still able to allow impaired urmylation and tRNA thiolation, suggesting an alternative mechanism for Urm1 activation.
Taken together, our results indicate that tRNA thiolation and urmylation are tightly linked processes, which rely on the same activation mechanism and have a comparable regulation.

YEV-FG05
Hypermodification of eukaryotic 18S rRNAs
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The biogenesis and maturation of ribosomes in eukaryotes is a highly complex process requiring not only the ribosomal building blocks such as the ribosomal RNAs (rRNA) and ribosomal proteins (r-proteins). Ribosome biogenesis also depends on the concerted action of a large number of non-ribosomal proteins and small non-coding RNAs.
Ribosomal RNA precursors become heavily chemically modified already during transcription as well as during their subsequent processing. A most conspicuous and unique modification in eukaryotic rRNAs is the hypermodification of U1191 of 18S rRNA to 1-methyl-3-(3-amino-3-carboxypropyl)-pseudouridine ($m^1acp^3\Psi$). This nucleotide, located in the vicinity of the decoding center of the ribosome, is synthesized in three steps, beginning with the snoRNA35 guided conversion of U1191 to Ψ1191. Already in the nucleolus the essential SPOUT-class protein Nep1 methylates the pseudouridine at N1 resulting in $m^1\Psi$ (Meyer, *et al.*, 2011; Wurm, *et al.*, 2010). Similar to observations for an increasing number of other ribosome biogenesis factors, a Nep1 point mutation has been described as the underlying cause of the Bowen-Conradi syndrome, characterized by impaired growth, psychomotor retardation and early childhood death (Armistead, *et al.*, 2009). As the last puzzle, we have very recently identified the enzyme Bam1 (**b**ase **a**minocarboxypropyl **m**odification) responsible for acp-modification of Ψ1191. As expected Bam1 binds SAM but different to methyl transferases not SAH and the protein has a unique structure.

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YEV-FG06
Analysis of 25S rRNA Base modifications
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Ribosomal RNA undergoes various modifications to optimize ribosomal structure and expand the topological potential of RNA. The most common nucleotide modifications in ribosomal RNA (rRNA) are pseudouridylations and 2'-OH-methylations (Nm), performed by H/ACA box snoRNAs and C/D box snoRNAs, respectively. Furthermore, rRNAs of both ribosomal subunits also contain various base modifications, which are catalysed by specific enzymes. These modifications cluster in highly conserved areas of the ribosome.
Although most enzymes catalysing 18S rRNA base modifications have been identified, little was known about the 25S rRNA base modifications. In recent years, we identified all these base methyltransferases of the 25S rRNA. The m^1A modification at position 645 in Helix 25.1 is highly conserved in eukaryotes and catalysed by the Rossman-fold like methyltransferase Rrp8 (Peifer *et al.*, 2013). Using a combination of RP-HPLC, mung bean nuclease assay and rRNA mutagenesis, we discovered that instead of one, yeast contains two m^5C residues at position 2278 and 2870 and identified Rcm1 and Nop2 which are both highly conserved in eukaryotes (Sharma *et al.*, 2013a). m^1A2142 modification of helix 65 of 25S rRNA is catalysed by Bmt2 (Sharma *et al.*, 2013b), whereas m^3U2634 and m^3U2843 methylation are catalzyed by Bmt4 and Bmt5 (Sharma *et al.*, 2014).

Peifer, C., Sharma; S., Watzinger; P., Lamberth; S., Kötter; P. und Entian, K.-D. (2013) *Yeast Rrp8p, a novel methyltransferase responsible for m1A 645 base modification of 25S rRNA.* Nucl. Acids Res.41. 1151-1163 doi: 10.1093/nar/gks1102 (Epup 2012, Nov. 23.
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Sharma, S., Yang, J., Watzinger, P. Kötter, P. and Entian, K.-D. (2013b). Yeast Nop2 and Rcm1 methylate C2870 and C2278 of the 25S rRNA, respectively. Nucl. Acids Res. 41: 9062-9076 [Epub ahead of print] doi:10.1093/nar/gkt679.
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AEP01
Requirement of the energy-converting methyltransferase MTR in *Methanosarcina acetivorans*
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Methanogenesis is an important part of the global carbon cycle and exclusively carried out by methanogenic members of the domain Archaea. Most methanogenic archaea can use only one energy substrate, which makes it difficult to study the function of factors involved in methanogenesis by mutational analysis. *Methanosarcina* (M.) species are more metabolically versatile allowing disruption of individual methanogenic pathways by targeted mutagenesis. *M. acetivorans* cannot use hydrogen and carbon dioxide as growth substrates due to the lack of a functional hydrogenase system, but is able to grow on methylated compounds (e.g. methanol), acetate or carbon monoxide (CO). Common to methanogenesis from any of these substrates is involvement of the energy-converting N5-methyl-tetrahydrosarcinapterin (H4SPT): coenzyme M (CoM) methyltransferase (MTR), which couples the exergonic methyl-group transfer from H4SPT to CoM to extrusion of sodium ions, and, thus, serves as a coupling site for chemiosmotic energy conservation. However, previous genetic and biochemical analyses of *M. barkeri* and *M. acetivorans* led to the proposal of a cytoplasmatic methyl-transfer from H4SPT to CoM (and vice versa) not involving MTR. During carboxidotrophic growth *M. acetivorans* produces, beside methane, substantial amounts of acetate, which is generated through a pathway analogous to that found in acetogenic bacteria and could, thus, be coupled to energy conservation by substrate-level phosphorylation without methanogenesis. In order to address the requirement of MTR for CO-dependent growth in *M. acetivorans* and to shed more light on the potential methyl-transfer bypass, the encoding operon, mtrEDCBAFGH was deleted from the chromosome using a combination of methanol and CO as the growth substrate. A preliminary physiological analysis of the mtr mutant, i.e., its growth phenotype on single methanogenic substrates and combinations thereof, as well as the nature and amounts of metabolites produced, will be presented and discussed in light of the well-established unconditional requirement of methanogenesis in methanogenic archaea.

AEP02
The Temperature Gradient Forming Device TGFD – a simple apparatus allowing high temperature microscopy
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Question: Light microscopy of (hyper)thermophilic microorganisms should be performed at high temperatures, e.g. 100 °C. Otherwise motility - which is often temperature-dependent - would go undetected.
Methods: A rather complex constructed thermomicroscope, developed in 1998, allows analyses at only one preset high temperature [1]. Here a newly developed apparatus is presented, which can be added onto any commercial light microscope, converting it into a thermomicroscope [2].
Results: The temperature gradient forming device TGFD allows analyses in temperature gradients of up to 40 °C over a distance of just 2 cm. Only this device, which can be used with heating rates of > 5 °C/s, enabled us to show that the hyperthermophile *Methanocaldococcus villosus* [3] can react within seconds to high temperature by starting motility - even if prestored for 9 months in the cold.
Conclusions: The TGFD can convert any commercial light-microscope into a "thermomicroscope". The main advantages of the TGFD are the extreme fast heating time and the ability to analyze cells in a temperature gradient of up to 40 °C. Only use of this instrument enabled us to demonstrate temperature taxis in a hyperthermophile [3]; thermotaxis of prokaryotes had been described before only *Escherichia coli*.

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AEP03
Analysing essential proteins with CRISPRi – the tRNA splicing endonuclease and ligase
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The tRNA splicing endonuclease and splicing ligase are two enzymes involved in archaeal tRNA maturation. The splicing endonuclease, encoded by the gene *endA*, is an endonuclease that recognizes and cleaves the bulge-helix-bulge (BHB) motif, present in the introns of tRNAs and ribosomal RNAs (1,2). The splicing ligase joins the ends generated by the endonuclease yielding the mature tRNA and rRNA respectively (3). To investigate all biological functions and substrates of both enzymes in the archaeon *Haloferax volcanii*, we aim to down regulate expression of both genes using CRISPRi.
CRISPRi, short for CRISPR Interference, is a method to down regulate transcription using the CRISPR-Cas system. CRISPRi has been succesfully employed to down regulate genes in *E. coli* as well as in mammalian cells with a catalytically inactive CRISPR-Cas type II Cas9 from *S. pyogenes* (4). In bacteria the CRISPR-Cas type I-E has been developed into a CRISPRi system that successfully down regulates transcription (5).
Here, we employed the endogenous CRISPR-Cas system type I-B of *H. volcanii* to knock down the expression of both tRNA maturation enzymes. We used two different icrRNAs directed to the transcription start site of the *endA* gene. One icrRNA resulted in growth defects compared to the control strain. In addition, unspliced $tRNA^{Trp}$ precursors accumulated in the strain expressing this icrRNA and a reduction of mature $tRNA^{Trp}$ levels was observed.
Expression of the tRNA splicing ligase, encoded by the gene *rtcB*, was down regulated using icrRNAs targeting the promoter region and the region around the transcription start site.

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AEP04
Possible SAM-dependent methyltransferase involved in [Fe]-hydrogenase cofactor biosynthesis
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[Fe]-hydrogenase is found in many hydrogenotrophic methanogenic archaea and is over-produced when nickel is limiting. The iron in [Fe]-hydrogenase is part of the FeGP cofactor, which is composed of a redox-inactive low-spin Fe(II) ligated to a Cys176-thiolate, two CO, one pyridinol N, and one acyl-C of the acylmethyl substituent of pyridinol. The pyridinol ring is conjugated to guanosine monophosphate. The pyridinol is highly substituted with two methyl groups and an acylmethyl group. In many methanogenic archaea, the *hmd* genes ([Fe]-hydrogenase structural genes) are clustered with *hmd*-co-occurring (*hcg*) genes (*hcgA-G*). The *hcg* genes are found in the genome of all methanogenic archaea containing the *hmd* genes, which suggests that the *hcgA-G* genes are involved in biosynthesis of the FeGP cofactor. To identify the biosynthetic precursors, we retrosynthetically analyzed the FeGP cofactor isolated from the enzyme based on stable-isotope-labeling patterns. This analysis indicated that in the biosynthesis of the FeGP cofactor, a methyltransferase catalyzes formation of the 3-methyl group of the pyridinol moiety [1]. To analyze the function of the *hcg* genes, we employed a "structure-to-function" strategy [2,3]. Here we show that the crystal structure of HcgC resembles that of *S*-adenosylmethionine (SAM)-binding enzymes. In co-crystallization studies, SAM and its demethylated product, *S*-adenosylhomocyteine, bound HcgC, suggesting that HcgC is a SAM-dependent methyltransferase. Genetic studies confirmed HcgB and HcgC as key biosynthetic enzymes. Base on the results, we propose that HcgC is a methyltransferase catalyzing formation of the 3-methyl group of the pyridinol moiety.

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AEP05
New insights into the substrate spectrum of *Methanomassiliicoccus luminyensis*
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Question: Little is known about the energy-conserving pathway of the human gut archaeon *Methanomassiliicoccus luminyensis*. Therefore, experiments with resting cells were performed to analyze the substrate spectrum of the organism in detail. Until recently it was thought that growth of *M. luminyensis* strongly depends on hydrogen as electron donor. Hence, the question was addressed whether other compounds could also deliver electrons to the energy-conserving system of the organism.
Methods: *M. luminyensis* cultures were grown to mid-exponential phase with trimethylamine or methanol in combination with H_2 as substrates, harvested by centrifugation and washed twice with stabilization buffer to remove substrates while keeping the cells intact. All manipulations were done under strictly anaerobic conditions. Experiments were carried out in stoppered 9 mL bottles containing 2 mL of cell suspension. The bottles were flushed with oxygen-free N_2 or H_2 and substrates were added. Methane production was determined by gas chromatography. Substrate consumption was also confirmed by growth experiments.
Results and conclusion: The experiments with resting cells of *M. luminyensis* demonstrated that the organism has a selective preference for certain substrates. While cells fed with methanol + H_2 revealed the highest methane production rate, cells with methylamines + H_2 as substrates showed a significantly lower methane production activity. Among methylamines, trimethylamine + H_2 were the best substrates for *M. luminyensis* while dimethylamine + H_2 resulted in a reduction of the methane production by about 50 %. Resting cells could not use monomethylamine + H_2 when the cells had been grown on methanol or trimethylamine + H_2.
Additionally H_2 was substituted by formate as electron donor in resting cell experiments. Cells could produce methane when formate plus

methanol was present as substrates. These results indicate that *M. luminyensis* can use H_2 and formate as electron donors, respectively. In summary, the metabolism of *M. luminyensis* could be far more complex and diverse than previously thought.

AEP06

Variants of the accessory gas vesicle protein GvpM and its interaction partners during gas vesicle formation

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Fourteen *gvp* genes are involved in gas vesicle formation. The two major structural proteins are GvpA and GvpC, whereas the eight proteins GvpF through GvpM are accessory gas vesicle proteins. Not very much is known about the function of them. They occur in the exponential growth phase prior to the presence of large amounts of GvpA. Except GvpH and GvpI, all of them are required for gas vesicle formation. We propose that they are involved in early stages of gas vesicle formation, aggregates or form pre-structures that are ultimately enlarged by the addition of GvpA and GvpC. GvpJ and GvpM both exhibit sequence similarities to GvpA (50-60 %). Gas vesicle formation is inhibited by large amounts of GvpM and this inhibitory effect can be compensated by the simultaneous presence of comparable amounts of GvpH, GvpL or GvpJ but not of GvpG [1].

Objectives: Variants of GvpM were constructed to investigate their influence on gas vesicle formation. Also, we searched for potential interaction partners of GvpM to analyze its role in gas vesicle biogenesis.

Methods: Mutations in *gvpM* were introduced by mutagenesis PCR and the resulting plasmids were used to transform *Hfx. volcanii* WR340 together with a DM construct, containing all *gvp* genes required for gas vesicle formation except *gvpM*. Transformants were inspected for their ability to form gas vesicles. Ni-NTA agarose affinity chromatography was used to investigate protein-protein interactions.

Results: The exchange of non-polar to polar aa in the N-terminal region of GvpM with high similarity to GvpA (aa 13-24) resulted in Vac^- cells implying that the non-polar residues are essential for gas vesicle formation and might offer contact sides to partner proteins. Point mutations in the region with high similarity to GvpJ (aa 44-48) resulted in minor amounts of gas vesicles suggesting that this region is important for the function of GvpM. Deletions in the N-terminus indicated that the first ten aa are essential for gas vesicle formation whereas deletions of the last ten aa at the C-terminus were not required. Partner proteins of GvpM could be detected like GvpH, GvpJ and GvpL. An interaction of GvpM and GvpG was not observed [2].

Conclusion: GvpM plays an important role in gas vesicle biogenesis since single point mutations abolish gas vesicle formation. The hypothesis that accessory Gvps are involved in early stages of gas vesicle formation and potentially form pre-structures could be strengthened.

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AEP07

Strangers in the archaeal world – osmostress-responsive biosynthesis of ectoine and hydroxyectoine by the marine thaumarchaeon *Nitrosopumilus maritimus*

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Questions: Microorganisms have to cope with a multitude of environmentally imposed stresses in their natural habitats. Increases in the external salinity belong to these types of stresses. Microbial cells can accumulate compatible solute via uptake or *de novo* synthesis, to cope with high osmolarity surroundings [1,2]. Ectoine and hydroxyectoine are compatible solutes, that are widely synthesized by members of *Bacteria* [3]. Database analysis of all sequenced bacterial and archaeal genomes revealed that only a resrticted number of *Archaea* possess the genes encoding the enzymes for ectoine production.

Methods: Bacterial and archaeal genomes were analysed bioinformatically for the presence of *ect* genes and their gene neighbourhood. Growth of *Ca.* Nitrosopumilus maritimus was analysed with the addition of different salt concentrations and HPLC analysis revealed the production of ectoine/hydroxyectoine. The characteristics of the key enzymes EctC and EctD were studied biochemically. The transcriptional unit of the *ect* genes was studied in a PCR based method and qRT-PCR was used to analyse the expression levels.

Results: Database analysis of 557 archaeal genomes revealed that only 12 strains of the genera *Nitrosopumilus, Methanothrix*, or *Methanobacterium* harbor ectoine/hydroxyectoine gene clusters. Using the thaumarchaeon *Ca.* N. maritimus as a model, we demonstrated that its *hyp-ectABCD-mscS* gene cluster is functional and co-transcribed from a salt-inducible promotor leading to the production of both ectoine and hydroxyectoine in response to enhanced osmotic stress. The ectoine synthase (EctC) and the ectoine hydroxylase (EctD) were biochemically characterized and their properties resemble those of their counterparts from *Bacteria*. The *mscS* gene, cotranscribed with the *ect* genes, encodes a functional mechanosensitive channel (NmMscS), as demonstrated through complementation experiments with an *E. coli* mutant lacking all known mechanosensitive channel proteins (MscM, MscS and MscL).

Conclusions: Our data show for the first time that the effective stress protectants ectoine and hydroxyectoine are not only present in 19 bacterial phyla but are also found in at least 12 archaeal strains. We found that *Ca.* N. maritimus cells cope with high salinity not only through the synthesis of osmostress-protective ectoines but they already prepare themselves for an eventually occurring osmotic down-shock by simultaneously producing a safety-valve (NmMscS). Hence, the *ectABCD-mscS* transcriptional unit present in *Ca.* N. maritimus is a sophisticated genetic device that allows osmotically challanged cells to sequentially cope with increases and decreases in the external osmolarity of their marine and estuarine habitat.

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AEP08

454 Pyrosequencing revealed the bacterial and archaeal communities for an ancient crater as an archaeobiological niche

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Al Wahbah Crater, located at the west of Saudi Arabia as a part of The Harrat extinct volcanic chain, as big as 2.5 km wide with 300 m depth and it is registered in General Commission for Tourism & National Heritage as an ancient and archaeological site. It is considered as a remote area with extreme conditions, where the bottom of the crater is formed and frequently accumulated by rainfall and mudflow leaving behind a dried thick white sodium phosphate crystals. Total sodium concentration was with range of 28,000-46,700 ppm and calcium within range of 31,400-56,500 ppm. In addition, the samples were very sufluric whereas sulfate and sulfite exceeded 2157 mg/l and 5.54 mg/l, respectively. The favourable and source of energy like ferric ions was less than 0.2 mg/l and nitrogen soluble forms of nitrate, ammonium and nitrite were less than 2 mg/l, 1.5 mg/l and 0.05 mg/l, respectively. This work aimed to reveal the bacterial and archaeal communities from archaeobiological niches from the surface down to 100 cm at interval of 20 cm using 454 Pyrosequencing. Therefore, a pit (approx. depth of 100 cm and diameter of 40cm) was dug in the centre of the Crater allowing to collect six samples at following distance; I = 2 cm, II = 20 cm, III = 40 cm, IV = 60 cm, V = 80 cm and VI = 100 cm. Archaeal genera dominated the surface and the bottom of dug pit with 91 % and 80 %, respectively, while bacterial genera was at the optimum survival conditions (approx. 50 %) between 20 cm to 60 (II-IV). At the species level, extremely halophilic archaeon and bacteria were identified cross the samples and they are *Halorhabdus* spp. (*H. tiamatea* and *H. utahensis*), *Halorubrum* spp. *Salinibacter iranicus* and *Halorhodospira halophila*. Moreover, a noticeable existence percentage (22 %) of *Halanaerobiaceae* family distinguished as anaerobic and halophilic bacteria, which is comprised of *halanaerobium*, *halarsenatibacter*, *halocella*, was identified at the top centimetres. Having the last 40 cm of the dug pit with its extreme conditions and Al Wahbah Crater by itself as an archaeology site, *S. iranicus* and species belongs to *Halorhabdus* and *Halorubrum* are archaeobiological organisms.

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AEP09

Biochemical studies of the two rhodanese-like proteins in *Hydrogenobacter thermophilus* TK-6

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Question: *Hydrogenobacter thermophilus* TK-6 is a thermophilic and obligately chemolithoautotrophic hydrogen-oxidizing bacterium which was isolated from Izu, Japan. The optimum temperature of this bacterium is 70 °C and it can utilize hydrogen and thiosulfate as an energy source. Besides, phylogenetic analyses of 16S rRNA genes suggest that *H. thermophilus* is a member of the deepest branching order in the domain Bacteria. It is thought that ancient organisms utilized reduced inorganic sulfur compounds as the energy sources. Therefore, understanding the sulfur metabolisms in *H. thermophilus* is effective for the elucidation of the energy acquisition evolution of the life.
In sulfur metabolism, rhodanese is one of the most distinctive enzymes. Rhodanese is conserved in the three evolutionary domains and it catalyzes sulfane sulfur within thiosulfate to cyanide *in vitro*. In bacteria, the role of rhodanese as sulfur carrier and sulfur donor to another enzyme *in vivo* is known while the detailed function is still unclear.
In this study, we identified two genes which contain rhodanese-like domains in *H. thermophilus* and analyzed physiological functions to clarify the role of those two genes.
Methods: The genes which include rhodanese-like domains in *H. thermophilus* were identified by Basic Local Alignment Search Tool (BLAST). The motifs were characterized by multiple sequence alignment.
To clarify the function of the genes, the mutant strains were constructed. The growth profiles of the mutants were measured. Sulfur and thiosulfate in the culture medium were also measured using colorimetric method and ion chromatography, respectively.
Results: Two genes containing rhodanese-like domains (HTH_0621 and HTH_1596) were identified from *H. thermophilus* genome and were named rhd1 and rhd2, respectively. Multiple alignment analysis showed both genes had active site residue for rhodanese. Furthermore, rhd1 had the homology to tRNA 2-thiouridine synthesizing proteins at its N-termini. Three disruptant mutants (Δrhd1, Δrhd2 and Δrhd1Δrhd2 double mutant) were constructed and the biochemical growth profiles were measured. Growth rate in Δrhd1 strain did not change while accumulation of a large amount of sulfur in the culture medium was observed. Besides, consumption of thiosulfate became faster, suggesting Rhd1 is involved in sulfur and thiosulfate oxidation. Δrhd2 did not affect the growth profiles. It was suggested that Rhd2 was not able to complement the function of Rhd1 because Δrhd1Δrhd2 double mutant showed a similar phenotype as that of Δrhd1.
Conclusion: Two rhodanese-like proteins were identified in the genome of *H. thermophilus*. Growth profiles of disruptants suggested that Rhd1 had a role for sulfur and thiosulfate metabolism.
Enzymatic analyses by using purified protein will be carried out to get further knowledge about those genes.

AEP10

Towards a genetic system for the thermophilic acetogenic bacterium *Thermoanaerobacter kivui*

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Question: We aim to develop a genetic system for the thermophilic acetogenic bacterium *Thermoanaerobacter kivui.* The conversion of hydrogen and carbon dioxide to acetate is considered one of the most ancient energy-conserving biochemical pathways (1). In recent years, acetogenic bacteria which reduce CO_2 with electrons either derived from biomass, from hydrogen and/or carbon monoxide oxidation have attracted interest as potential whole cell catalysts for future biotechnological applications (such as syngas-to-biofuel or syngas-to-top value chemical conversions). A prerequisite for industrial application, but also for in-depth studies of physiology and gene regulation is the availability of a genetic toolbox. Many acetogenic bacteria, however, belong to the Firmicutes, a phylum with effective defense mechanisms against foreign DNA. In consequence, only recently genetic systems became available for a few acetogenic bacteria.
Methods: A method for cultivation of *T. kivui* on solid medium was developed. The minimal inhibitory concentration (MIC) for kanamycin was determined. Complementation of the inhibition was tested using an *Escherichia coli-Thermoanaerobacterium* shuttle plasmid containing a kanamycin resistance cassette and a *Thermoanaerobacterium* origin of replication.
Results: *T. kivui* optimally grows optimally at 60-65 °C with either hydrogen/carbon dioxide or C_6 sugars as substrates, with a doubling time of about 2-2.5 h with glucose (2). On solid medium, colony formation was observed after 2-3 days in anoxic jars; with a plating efficiency of about 10-15 %. Similar to other *Thermoanaerobacteraceae* (3), *T. kivui* was relatively tolerant to kanamycin; the addition of at least 200 mg mL^{-1} kanamycin was necessary to completely inhibit growth. Inhibition on solid medium was complemented by the plasmid with the kanamycin resistance cassette. The plasmid efficiently replicated in *T. kivui*, as it could be re-isolated from the latter with yields comparable to those from *E. coli* (0.5 mg DNA per mL of culture).
Conclusions: *T. kivui* exhibited natural competence and acquired the *E. coli-Thermoanaerobacterium* shuttle plasmid during growth on glucose on liquid medium; which conferred the ability to growth on solid medium containing kanamycin. Based on the shuttle plasmid, we are currently constructing an expression vector for production of thermophilic proteins as well as a suicide vector for genetic manipulations on the genome.

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AEP11

An endoxylanase from a psychrophilic bacterium serves as a reporter protein to examine the splicing performance of an intein from an acidophilic archaeon

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Question: Inteins are protein intervening sequences that splice themselves out of a non-functional protein precursor and ligate the flanking exteins with a native peptide bond. Their autocatalytic activity is highly dependent from the adjacent extein amino acid residues at the N- and C-terminus, but due to the weak evolutionary conservation of inteins, it is not possible to develop a generalized prediction method for the production of efficient intein reporters. The aim of this study is to investigate the intein flanking amino acid residues and to integrate an archaeal intein into a foreign protein for the generation of an easy traceable reporter system [1].
Methods: The *Pto* VMA-intein encoding sequence from the thermoacidophile *Picrophilus torridus* together with its directly flanking and well-conserved extein amino acid residues has been introduced into the *xyn8* open reading frame encoding a cold-active endoxylanase from the psychrophilic bacterium *Pseudoalteromonas artica* [2]. Structure predictions of Xyn8 were used to identify putative intramolecular insertion sites in non-conserved protein regions to inhibit the hydrolytic activity of the xylanase. Insertion sites were chosen preceding natural threonin residues that are essential for intein functionality as +1-extein amino acid residue.
Results: The residue Thr75 is the penultimate amino acid of a N-terminal beta-sheet structure element of Xyn8, which is located on the protein surface. Integration of the intein between Tyr74 and Thr75 resulted in its inactivity, but the additional incorporation of the intein preceding lysine residue restored the autocatalytic activity. Moreover, protein splicing revealed the generation of full-length xylanase, whose activity was easily detectable on plate assays using dye-linked xylan-substrates.
Conclusion: In this project, a tool has been developed to investigate the splicing activity of the *Pto* VMA-intein at room temperature in a fast-forward assay.

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AEP12

Novel promoters provide flexible cellulase expression for metabolic engineering and biotechnolocical application of *Sulfolobus acidocaldarius*

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The thermoacidophilic lifestyle of the crenarchaeon *Sulfolobus acidocaldarius* (78 °C, pH 2-3) matches the conditions required for the conversion of lignocellulosic biomass. Together with an advanced genetic toolbox [2], *S. acidocaldarius* thus represents a versatile chassis for the biotechnological production of value added compounds from renewable second generation substrates. To further increase the flexibility of *S. acidocaldarius* as expression host and for metabolic engineering, the two promoters precede the genes *sso1273* ($P_{SSO1273}$) and *sso2619* ($P_{sso2619}$), each encoding a binding protein of a peptide-ABC-transporter [1], were established. Vector construction based on a modified pSVA1450 [2] resulting in the constructs pBSMB1 and pBSMB2, accompanied by thermophilic β-galactosidase (*lacS*) reporter gene assays, indicated that both promotors allow for both the induction by cold shock (65 °C) and tryptone (80 °C). $P_{SSO2619}$ turned out to be advantageous in terms of efficiency whereas $P_{SSO1273}$ offered a higher temperature specificity which allows for tight regulation of induction. The applicability of the $P_{SSO1273}$ was further confirmed by cloning the eukaryotic gene encoding the thermophilic cellulase *HM003039* from the fungus *Phialophora* sp. [3] under the control of the $P_{SSO1273}$ into a newly designed pCmalLacS derived expression vector. This provided the encoded protein with an N-terminal *S. solfataricus* derived signal peptide for protein excretion into the medium, as well as a C-terminal His_6-StrepII-tag. Upon transformation and tryptone/cold shock induction, the tested thermophilic cellulase gene, confered the ability to the cellulose negative *S. acidocaldarius* MW001 to degrade the cellulose analogue carboxy-methyl-cellulose (CMC) as well as crystalline cellulose (Avicel) and beechwood xylan. This was indicated by halo formation on indicator plates and demonstrated metabolic engineering of *S. acidocaldarius*. These results further increase the applicability of *S. acidocaldarius* for metabolic engineering approaches which offer several advantages for industrial application.

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AEP13

A sulfur oxygenase from the haloalkaliphilic bacterium *Thioalkalivibrio paradoxus* with almost no reductase activity

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Question: Sulfur oxygenase reductases (SOR) catalyze the oxygen-dependent disproportionation of elemental sulfur with sulfite, thiosulfate and sulfide as products and without external cofactors or electron donors. Usually, oxygenase and reductase reactions have a molar ratio between 4:1 and 10:1 and both activities cannot be separated. SORs are not widespread in nature and before long, their occurrence was restricted to (hyper-) thermophiles. Therefore, the best-studied SORs derive from the thermoacidophilic Archaea *Acidianus ambivalens* and *A. tengchongensis* [1,2]. More *sor* genes were recently found in the genomes of several haloalkaliphilic, mesophilic *Thioalkalivibrio* strains, which were isolated from soda lakes and grow around pH 10. Together with two other variants, the *T. paradoxus* SOR (*Tp*SOR) branches deeply in a phylogenetic dendrogram with a sequence identity of merely 29 % compared to other SORs. From this, the question arose whether the protein has SOR activity or whether the deep branching point represents the development of paralogous subgroups.
Methods: The *sor* gene was heterologously expressed in *E. coli*. Specific enzyme activities were determined by colorimetric assays after purification of the protein via strep-tactin affinity chromatography. The molecular mass of the enzyme was determined by gel filtration, its diameter was measured in electron micrographs.
Results: Electron micrographs and gel filtration showed that the *Tp*SOR forms large hollow ball-shaped homo-oligomeric structures of 15 nm in diameter similar to both *Acidianus* enzymes. Thiosulfate and sulfite formation was detectable over a broad temperature (10-98 °C) and pH range (6.5-11) with optima around 80 °C and pH 9, respectively (265 U/mg protein). In contrast, hydrogen sulfide formation was detected at a maximum specific activity of only 0.03 U/mg protein, corresponding to less than 1 % of other SORs. NaCl and glycine betaine concentrations up to 1 M did not have a significant influence, while higher concentrations lead to a decrease of enzyme activity. The *Tp*SOR showed residual product formation with both solutes up to saturation conditions, suggesting that the enzyme activity depends on total osmolyte concentration rather than salt or compatible solutes.
Conclusion: Overall, the properties of the *Tp*SOR are comparable to other SORs with respect to structure and oxygenase reaction, the latter of which is nevertheless higher than usual. In contrast, the reductase activity is very low with product ratios almost down to 1:10.000. Hence, the *Tp*SOR represents a sulfur oxygenase with almost no reductase activity. The results suggest, that sulfide formation and sulfur disproportionation of the *Tp*SOR comes from a side reaction rather than from an integral step in the reaction mechanism of the enzyme.

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AEP14

Regulation of UV inducible pili system in *Sulfolobus acidocaldarius*

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Microarray studies on *Sulfolobus* species in response to UV stress revealed a clear transcriptional response, including the repression of DNA replication and chromatin proteins and the upregulation of an operon which is responsible for biogenesis of a special type IV pilus - ups pilus (UV inducible pilus of Sulfolobales). There are five proteins encoded in this operon: an ATPase (UpsE), a membrane protein (UpsF), two pilin subunits (UpsA, B) and a protein of unknown function (UpsX). The formed pili lead to species specific cellular aggregation in which the cells exchange DNA to repair the UV damaged genomic information.
Deep sequencing studies on *S. acidocaldarius* cDNA suggested a primary TSS (transcription start site) in front of *upsX* and secondary TSSs in front of *upsE* and *upsA*. However, so far, it remained unclear how the *ups* operon is regulated. In order to understand the promoter of *upsX*, which is thought to be the primary promoter of the *ups* operon, a LacS reporter assay was employed.
With the use of this assay we determined the minimal size of the *upsX* promoter to be 53bp. Interestingly, we found a 6 nucleotide-motif within this region that could also be found in other UV-inducible promoters at approximately same location. Substitution of the nucleotides within this motif resulted in a significant reduction of the promoter activity. Thereby we might have found a "UV inducible box" in the promotor regions of genes related to UV response in *Sulfolobus* species.
This study has given the first hints about regulation of the UV response in *Sulfolobus* species, which could lead to further understanding of responsive mechanism to environmental stress in Archaea.

AEP15

Sugar degradation in *Sulfolobus solfataricus* – new promiscuous pathway for L-fucose degradation

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Sulfolobus solfataricus is a thermoacidophilic crenarchaeon with optimal growth at 80 °C and pH 2-3. The organism possesses a great physiological versatility and is capable to utilize different organic compounds as carbon and energy source such as a variety of different C5 and C6 sugars (e.g. D-

glucose, L-arabinose, D-arabinose, D-xylose), sugar acids, alcohols, and peptides [1, 2]. Whereas the degradation pathway of D-glucose and D-galactose is well understood in hyperthermophilic archaea (for literature see [3]) only little is known about the degradation of other hexoses. The hexose L-fucose is a constituent of cell-envelopes in many pro- and eukaryotes as a fundamental component of exopolysaccharides [4]. However no information is available about the metabolism of L-fucose in archaea so far. Here we report - for the first time in archaea - on the L-fucose degradation pathway in *S. solfataricus*. The organism is able to utilize L-fucose as a sole carbon and energy source. Proteome and transcriptome studies on L-fucose compared to D-glucose revealed that during growth on L-fucose a small set of enzymes was strongly up-regulated, previously characterized from the D-arabinose degradation pathway. Crude extract measurements and characterization of recombinant proteins from *S. solfataricus* indicated that L-fucose is first oxidized to L-fuconate by the D-arabinose-1-dehydrogenase (SSO1300) and then converted to 2-keto-3-deoxy-L-fuconate via the D-arabinoate dehydratase (SSO3124). Subsequently, 2-keto-3-deoxy-L-fuconate is further converted to L-lactaldehyde and pyruvate by an KD(P)G aldolase (SSO3197), which is a key enzyme in sugar degradation in archaea. Thus, the pathway previously shown for D-arabinose degradation in *S. solfataricus* appears to be promiscuous for L-fucose degradation.

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AEP16

AP-MS to detect novel RNA polymerase binding proteins in the hyperthermopilic archaeon *Pyrococcus furiosus*

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Question: DNA dependent RNA polymerases (RNAPs) are multisubunit enzymes that carry out transcription in all three domains of life. The overall structure and subunit composition between archaeal RNAP and eukaryotic RNAP II are highly conserved. In general the archaeal transcription system is a simplified version of the eukaryotic system, but it lacks some of the well-described transcription factors found in Eukarya (e.g. TFIIH). We investigated affinity purification coupled to mass spectrometry to find novel RNAP interacting partners.
Methods: Modified strains of *Pyrococcus furiosus* were created with HisTags on RNAP subunits D, A`` and H. We used affinity chromotography under low salt conditions for purification to allow binding of additional proteins to RNAP. Samples of affinity purified RNAP were analyzed with MALDI-TOF mass spectrometry.
Results: After statistical analysis of the resulting MALDI data we ended up with a short list of interacting proteins. Bioinformatical tools allowed us to identify novel as well as already known proteins. Furthermore transcription rate of the affinity purified RNAP containing additional bound proteins was significantly different from a highly pure reference RNAP.
Conclusion: This investigation made it possible to detect novel proteins, that physically interact with RNAP from *Pyrococcus furiosus*. Further analysis showed that some of these proteins might play a role during archaeal transcription. In order to get an idea at which step of the transcription cycle they are involved, we currently try to express and characterize those proteins.

AEP17

The effect of point mutations on the activity of the glutathione-dependent, ETHE1-like sulfur dioxygenase from *Acidithiobacillus caldus*

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Question: The oxidation of elemental sulfur (S^0) in *Acidithiobacillus spp.* is an essential step in metal sulfide bioleaching. *Acidithiobacillus* species are dominant bacteria in heap bioleaching sites, where they perform the extraction of metal from poorly soluble metal sulfides like pyrite (FeS_2). Oxidation of sulfur and inorganic sulfur species is an integral part of bioleaching. Recently, a gene was identified in *A. caldus* encoding a sulfur dioxygenase (*sdo*), which catalyzes the oxidation of S^0 to sulfite with reduced glutathione (GSH)[1]. The protein shows a high similarity to a human mitochondrial protein, whose dysfunction leads to ethylmalonic encephalopathy (ETHE), a lethal hereditary disease based on an impaired sulfide detoxification in mitochondria. The active site contains a mononuclear iron site, however the reaction mechanism is not known. Here we present results of mutational studies on the iron ligands and the cysteine residues present in the amino acid chain in order to determine basic properties of the enzyme.
Methods: The *sdo* gene was heterologously expressed in *E. coli* and protein was purified via strep-tactin affinity chromatography. The enzyme activity was determined by colorimetric measurement of sulfite formation from sulfur. Point mutations of amino acids, presumed to be essential for enzyme activity, were created via site-directed mutagenesis of the gene.
Results: The specific SDO activity was optimal at 45˚C in 100 mM phosphate buffer pH 8 (14.5 U/mg) with 1 % S^0, 50 µM $FeCl_3$ and 200 µM GSH in the assay buffer. Mutation of the predicted iron ligands, (H113A, D130A, D130E and D130H) resulted in less than 3 % of wild type activity while H57A has 6 % residual activity. The iron content of the as-isolated mutant proteins was ≤0.05 mole per mole subunit with the exception of the D130E mutant (0.6:1). Mutants of the five cysteine residues (C87A, C117A, C137A, C180A and C224A) had residual activities between 14 and 30 % of the wildtype.
Conclusion: Altogether the SDO enzyme assay was optimized showing that not only GSH but additional iron is beneficial for activity. Activity assays and iron content determination confirmed the prediction of the iron ligands. In contrast, the cysteine residues seem to be non-essential for enzyme activity, although they might contribute to the overall performance of the protein.

1 H. Wang *et al.*, 2014 Appl Microbiol Biotechnol 98:7511-22

AEP18

The role of apparent malate synthase and β-methylmalyl-CoA lyase in acetate assimilation in *Haloarcula hispanica*

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The methylaspartate cycle is an anaplerotic acetate assimilation cycle recently proposed in haloarchaea (***1***). In this cycle, acetyl-CoA is transformed to glutamate via the reactions of the tricarboxylic acid cycle and glutamate dehydrogenase. The rearrangement of glutamate into methylaspartate and its following deamination lead to mesaconate, which is then activated to mesaconyl-CoA, hydrated to β-methylmalyl-CoA and cleaved to propionyl-CoA and glyoxylate. Propionyl-CoA carboxylation leads to succinyl-CoA, thus closing the cycle, whereas the apparent malate synthase catalyzes the condensation of the glyoxylate with acetyl-CoA to malate. *Haloarcula hispanica* is a genetically tractable haloarchaeon which is capable to grow on acetate. This archaeon possesses all the enzymes required for the methylaspartate cycle, and deletion of the genes for the key enzymes of the methylaspartate cycle (glutamate mutase, methylaspartate ammonia-lyase, succinyl-CoA:mesaconate CoA-transferase and mesaconyl-CoA hydratase) results in disruption of the growth with acetate as a sole carbon source, thus confirming their participation in acetate assimilation (***2***). Surprisingly, however, the deletion mutants of two other key enzyme of the methylaspartate cycle, malate synthase (Hah_2476) and β-methylmalyl-CoA lyase (Hah_1341) were capable to grow on acetate, although the growth was impaired compared to the wild-type. Interestingly, these two enzymes are homologous, and the double mutants are not capable to grow on acetate. The biochemical analysis reveals that both enzymes catalyze (*i*) malyl-CoA lyase reaction (malyl-CoA → acetyl-CoA + glyoxylate), (*ii*) β-methylmalyl-CoA lyase reaction (ß-methylmalyl-CoA → propionyl-CoA + glyoxylate), and (*iii*) malyl-CoA thioesterase reaction (malyl-CoA → malate + CoA). Enzyme assays and metabolite concentration determinations in the wild-type and mutant cells have shown that Hah_2476 is adapted to catalyze malyl-CoA formation from acetyl-CoA and glyoxylate, Hah_1341 to catalyze β-methylmalyl-CoA lyase reaction, and that malyl-CoA thioesterase activities of both enzymes are not physiologically relevant. Our data indicate the existence of a novel, yet-to-be-determined malyl-CoA thioesterase in *H. hispanica*, and further confirm the functioning of the methylaspartate cycle in haloarchaea.

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AEP19

Differentiation of the species of the genus *Methanothermobacter* by SAPD PCR and MALDI-TOF-MS

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Question: The family Methanobacteriaceae contains the three mesophilic genera, *Methanobacterium*, *Methanobrevibacter*, and *Methanosphaera*, and the only thermophilic genus *Methanothermobacter*, respectively. We wanted to find out whether there is a possibility to differentiate the species of the genus *Methanothermobacter* more precisely. So far, the species of the genus *Methanothermobacter* are difficult to distinguish by the described methods. Therefore, we checked two additional methods, the SAPD PCR and the MALDI-TOF-MS, for their potential to differentiate the thermophilic species of the genus *Methanothermobacter*.
Methods: The Specifically Amplified Polymorphic DNA PCR (SAPD PCR) and MALDI-TOF-MS (matrix-assisted laser desorption ionization time-of-flight mass spectrometry) were used throughout this study. SAPD PCR is a DNA fingerprinting method developed by Fröhlich and Pfannebecker to differentiate species and strains of different organisms including Bacteria, Archaea and Eucarya (Fröhlich and Pfannebecker 2007). Species-specific spectral patterns can be obtained by MALDI-TOF-MS that are not influenced by the applied medium, the age of the culture, or the growth conditions (Mellmann *et al.* 2008, Jensen and Arendrup 2011)
Results: *Methanothermobacter crinale, Methanothermobacter defluvii*, *Methanothermobacter marburgensis*, *Methanothermobacter tenebrarum*, *Methanothermobacter thermautotrophicus*, *Methanothermobacter thermoflexus*, *Methanothermobacter thermophilus* and *Methanothermobacter wolfeii* of the genus *Methanothermobacter* were investigated. Each of the investigated eight species of *Methanothermobacter* showed a species-specific banding pattern by SAPD PCR and a species-specific spectral pattern by MALDI-TOF-MS.
Conclusion: The differentiation of the species of the genus *Methanothermobacter* was possible by SAPD PCR and MALDI-TOF-MS. These two methods can therefore be used to identify thermophilic methanogens besides morphology, nutritional versatility, growth temperature, cell wall structure and G + C content of chromosomal DNA.

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Jensen R. H., Arendrup M. C. (2011) *Candida palmioleophila*: characterization of a previously overlooked pathogen and its unique susceptibility profile in comparison with five related species. J Clin Microbiol 49:549-556.

AEP20

Biogas production under high pressure

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Question: Today, the rising global energy demand is predominantly met by fossil energy resources. Thus, the utilization of renewable energy resources has gained increased political attention. The European Union approved a strategy for covering 20 % of the gross domestic energy consumption by renewable energy resources until the year 2020. In this context biogas produced in rural biogas plants plays a significant role. We proved whether high pressure conditions could stimulate biogas production. This novel approach integrates the previously separated processes of biogas production, upgrading and pressure boosting for grid injection in one step. The growth of methanogens under elevated pressures has been demonstrated [1].
Methods: Three high-pressured experimental biogas reactors with a capacity of 640 ml were designed. The test series included culture conditions under pressures of 1.1 bar, 50.5 bar and 100.1 bar. A mixture of 35.3 % corn silage hydrolysate and 64.7 % effluent from a pressure-free methane reactor of a two-stage biogas plant was used as liquid substrate (500 ml). It contained a mixture of short chain fatty acids such as acetate, propionate, n-butyrate, isovalerate, n-valerate and caproate, but no sugars or alcohols. A 16S rDNA database of methane producing archaeal clones was generated for all three reactors. In each case, more than 90 clones from the 16S rDNA amplicons per reactor were analysed by ARDRA (amplified rDNA restriction analysis) and Sanger sequencing.
Results: After an incubation time of 21 days several known and as yet undescribed methanogenic species of the families Methanosarcinaceae, Methanomicrobiaceae and Methanospirillaceae were isolated and identified. Those isolates were able to use H_2 and CO_2 as substrate. Furthermore, a new H_2 and methanol consuming species of the order Methanomassiliicoccales was discovered. In addition, members of the Methanosarcinaceae were able to use acetate, methyl-amines or methanol as energy source. The methane yield in the gas-phase increased up to 83 % after a significant increase of pressure.
Conclusion: Apparently, the applied high pressure conditions favoured the growth of hydrogenotrophic methanogens and an increased diversity of methanogenic species in comparison to atmospheric pressure [2]. In addition the proportion of methane in the gas phase was increased.

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AEP21

Ammonia-oxidizing Thaumarchaeota and potentially fermenting Woesearcheota dominate the archaeal community diversity in pristine limestone aquifers

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A considerable proportion of microbial communities in subsurface habitats is accounted for by Archaea, however knowledge of their role in biogeochemical cycles is limited. Furthermore, information on archaeal diversity in the terrestrial subsurface is even scarcer. Hence, we sought to assess the archaeal community diversity and their potential involvement in carbon fixation and utilisation, in two superimposed aquifers each characterized by different oxygen availability. Samples were obtained from eight groundwater wells ranging from 12 to 88 m depth in the Hainich region (Thuringia, Germany).
Quantitative PCR of archaeal 16S rRNA genes revealed that Archaea accounted for up to 9 % of the total groundwater prokaryotes. Analysis of the metabolically active archaeal communities, based on Illumina MiSeq RNA-derived archaeal 16S rRNA gene analysis, showed an overall dominance of two archaeal groups: the ammonia-oxidizing thaumarchaeotal Marine Group I (MG-I), and the presumably heterotrophic Rice Cluster V (RC-V) clade belonging to the recently identified Woesearchaeota phylum. More specifically, the lower oxic limestone aquifer was dominated by MG-I Thaumarchaeota, whose cultured representative - *Nitrosopumilus maritimus* - was shown to use an autotrophic pathway fixing HCO_3^- as source of carbon. The upper oxygen-deficient aquifer was dominated by the RC-V archaea which could potentially use plant-derived carbohydrates as substrate. DNA was also extracted from rock samples recovered during the drilling of the wells. Initial results show that diversity is very low and the archaea detected so far belong to the Bathyarchaeota. $^{13}CO_2$ DNA SIP carried out on passive sampler material revealed that sequences affiliated to a soil inhabiting uncultured archaeal clade, i.e. the soil crenarchaeotal group (or 1.1b group), was the only detectable group within the labelled DNA. Our results also detected soil-specific archaea in the groundwater community albeit extremely limited; suggesting an input from the surface soils. Ammonia oxidation could be an important metabolism of Archaea in deep limestone aquifers making a considerable contribution to autotrophic archaeal CO_2-fixation.

AEP22
Functional analysis of multiple general transcription factors in *Sulfolobus acidocaldarius*
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Beside unique features, archaea combine typical characteristics of bacteria and eukaryotes. Promoter structure and genetic information processing such as replication, transcription and translation, closely resemble that of their eukaryotic counterparts.
The archaeal transcription machinery consists of a multi-subunit RNA-polymerase (RNAP) and the general transcription factors (GTF) representing homologues of the eukaryotic TATA-binding protein (TBP) and the transcription factor TFIIB (TFB).
The actual understanding of the transcription initiation implies that TBP binds to the TATA-Box. Subsequently, TFB binds to the TBP-DNA-complex and forms sequence specific interactions with a purine-rich TFB-responsive element (BRE). The N-terminal region of TFB recruits the RNAP to build the ternary pre-initiation complex. Generally, the archaeal transcription machinery is considered a simplified model of the more complex processes which are known from eukaryotes.
The thermoacidophilic crenarchaeon *Sulfolobus acidocaldarius* possesses three TFBs (TFB1, TFB2 and TFB3) and one TBP. TFB1 seems to be the most commonly expressed TFIIB homologue under standard growth conditions and supports transcription initiation *in vitro* (Bell and Jackson, 1998), whereas TFB3 is upregulated following UV-exposure (Götz *et al.*, 2007) and acts as a co-activator in the presence of TFB1 (Paytubi and White, 2009). However, the role of multiple GTFs like TFB1-3 in crenarchaeota is still unclear and functions similar to bacterial sigma factors have been suggested.
The aim of this project is to study the functions of the TFB homologues, especially TFB2, and to investigate their roles in stress response. Overexpression and purification of recombinant GTFs was performed successfully for TBP, TFB1 and RNA polymerase (in genome tagging in *S. acidocaldarius*). For TFB2 and TFB3 insoluble protein was obtained and alternative expression/purification strategies are currently established and optimized. The purified proteins were used for *in vitro* transcription, *in vitro* phosphorylation assays and EMSAs. Furthermore, reporter gene constructs (*lacS*) were established to determine promoter activities of the different GTFs *in vivo* in response to different stress conditions.

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AEP23
Identification and effects of potential biofilm modulators on the intestinal archaea *Methanosphaera stadtmanae* and *Methanobrevibacter smithii*
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Question: *Methanosphaera stadtmanae* and *Methanobrevibacter smithii* are the most common archaea in the human gut identified in 32.6 % and 99.2 % of 500 individuals, respectively [1]. Despite their significant prevalence, only little is known about their appearance in the human gastrointestinal tract compared to bacterial mucosa-associated representatives [2]. As previously shown, *M. stadtmanae* and *M. smithii* are able to form biofilms and are hence potentially present as such in the human intestine. Elucidating the effect of potential modulators on biofilm structure and identification of functional factors in biofilm regulation will provide a deeper understanding of methanoarchaeal biofilms.
Methods: *M. stadtmanae* and *M. smithii* were grown on plastic µ-dishes™ with 3 mL of minimal medium (DSM medium 120). Cells were fixed to the surface of µ-dishes™ by 2 % glutaraldehyde. Prior to imaging by confocal laser scanning microscopy (CLSM), cells were stained with a PBS buffer solution containing SYTO 9 and propidium iodide (LIVE/DEAD staining). For extracellular structure investigation, propidium iodide was substituted with DDAO (7-hydroxy-9H-(1,3-dichloro-9,9-dimethylacridin-2-one)). The antimicrobial peptide FF24, a derivative of the human cathelicidin LL37, was added to planktonic and biofilm cultures in the exponential growth phase and after 2 days of growth, respectively (*M. stadtmanae* 7.5 µM, *M. smithii* 2 µM). For transcriptional profiling, total RNA was isolated from both, planktonic and biofilm cultures.
Results: DDAO-staining revealed particularly strong fluorescence signals in an area where *M. stadtmanae* cells appeared to be highly accumulated with other structural components. Exposure to the antimicrobial peptide (AMP) FF24 exhibited a deleterious effect on *M. stadtmanae* as well as *M. smithii* which was less pronounced for biofilms compared to planktonically grown cultures. High quality total RNA in high yields has been successfully obtained after establishing a modified RNA extraction protocol for planktonically grown and biofilm cultures of both strains.
Conclusion: Cells within a methanoarchaeal biofilm appear to be embedded in an extracellular matrix-like structure. Using DDAO, which is reported to be a nucleic acid dye completely impermeable to cells, we clearly demonstrated that extracellular DNA is a structural component of *M. stadtmanae* biofilms. Extracellular matrix potentially acts as a diffusion barrier which might explain why the cells grown as a biofilm are less affected than the planktonic ones by the AMP applied in this study. In future, transcriptome analysis of RNA extracts will help identify functional factors in methanoarchaeal biofilm regulation.

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AEP24
3-Phosphoglycerate, a novel allosteric activator of pyruvate kinases from Thermoproteales
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Pyruvate kinases (PKs) catalyze the last step of glycolytic pathways in all three domains of life. PKs of bacteria and eukarya are allosterically activated by sugar phosphates, e.g. fructose 1,6-bisphosphate (1), or by AMP as shown for PK of *Thermotoga maritima* (2). So far, only a few PKs from archaea, from the hyperthermophiles *Pyrobaculum aerophilum*, *Thermoproteus tenax* and *Aeropyrum pernix*, were characterized; these PKs did not respond to classical allosteric effectors (2, 3). Recently, we solved the crystal structure of PK from *P. aerophilum* which resulted in the identification of the novel type of allosteric activator, 3-phosphoglycerate (3PG) (4).
Here we report the allosteric properties of PKs from various archaea including PKs of hyperthermophiles, thermoacidophiles, methanogens and halophiles: All PKs were purified as homotetrameric enzymes and exhibited sigmoidal saturation curves with PEP and/or ADP indicating positive cooperative substrate binding. The response of PKs to classical allosteric effectors and to 3PG was analysed: (I) As shown for PK from *P. aerophilum*, PKs from other Thermoproteales, *Pyrobaculum islandicum*, *Thermoproteus uzoniensis* and *Caldivirga maquilingensis*, were also activated by 3PG rather than by sugar phosphates or AMP. An exception was the PK from *Thermofilum pendens* which was not activated by 3PG. The crystal structure of that PK was solved giving an explanation for the absence of 3PG activation. (II) The PKs from thermoacidophiles *Sulfolobus solfataricus and Picrophilus torridus* and PK from *Methanospirillum hungatei* did not respond to either classical allosteric effectors or to 3PG. (III) PKs from haloarchaea and from *Methanocaldococcus jannaschii* were positively regulated by AMP; these PKs are phylogenetically related to bacterial AMP activated PKs and are likely acquired via lateral gene transfer from bacteria. Together, the data indicate that the novel allosteric regulator 3PG represents an activator of PKs from most Thermoproteales rather than of other archaeal PKs.

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(2) Johnsen U *et al.* (2003) JBC 278, 25417-25427
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AEP25
Insights into the life of nanoorganisms
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Microbial nanoorganisms thrive using a minimum of genetic information contained in cells with a size that is in some cases just enough to hold the necessary number of enzymes and ribosomes to sustain growth.

Here we present first insights into the life of Archaeal Richmond Mine Acidophilic Nanoorganisms (ARMAN) in highly enriched cultures under laboratory conditions. These enrichment cultures arose from acidophilic, stalactite-like biofilms of an abandoned pyrite mine in the Harz Mountains (Germany) (Ziegler *et al.*, 2013). We designed an acidic, anoxic medium which contains Fe(III)SO_4 as well as an organic carbon source as necessary compounds. It has a pH of 2.5 and a H_2/CO_2 atmosphere in the head space. According to 454 data only three different species remain in this enrichment cultures: two belonging to the *Thermoplasmatales* and one to the ARMAN.
Culturing experiments controlled with CARD-FISH show an active phase of the ARMAN and the *Thermoplasmatales* after four to eight weeks. In this time a clear Fe(III) reduction is detectable as well as a decline of the pH. QPCR analysis revealed a concentration of 5*10^7 cells per mL for all *Thermoplasmatales* and 2*10^7 cells per mL for the ARMAN after seven weeks of growth.
First metagenomic analysis of the community revealed three almost complete genomes (99 % or 86 % for the *Thermoplasmatales* and 82 % for the ARMAN-species). As one would expect, we found the genome size of the *Thermoplasmatales* with 1,9 Mb or 1,6 Mb bigger than the ARMAN-genome with 1 Mb whereas the coding density is likely the same (87 % or 89 % for *Thermoplasmatales* and 91 % for ARMAN). Besides the metabolic capabilities contained in the genomes, we will also present transcriptomic data describing the way how the organisms thrive in the enrichment culture.

Ziegler, S., Dolch, K., Geiger, K., Krause, S., Asskamp, M., Eusterhues, K., Kriews, M., Wilhelms-Dick, D., Göttlicher, J., Majzlan, J., and Gescher, J. (2013) Oxygen dependent niche formation in an acidophilic consortium built by archaea and bacteria. *ISME J*. Sep; 7(9): 1725-37.

AEP26
Acetate formation and acetate activation in the halophilic archaeon *Haloferax volcanii*
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Various halophilic archaea were found to generate acetate during exponential growth on glucose and to utilize acetate as a carbon and energy source [1; 2]. Here we report the identification and characterization of enzymes involved in acetate formation and acetate activation in *Haloferax volcanii*. Transcript analyses of the encoding genes as well as growth studies with deletion mutants were performed.
Acetate formation: It was shown that the formation of acetate from acetyl-CoA is catalyzed by an ADP-forming acetyl-CoA synthetase (ACD, acetyl-CoA + ADP + P_i ⇄ acetate + ATP + CoA) encoded by *HVO_1000*. As shown by northern blot analysis this gene was specifically upregulated during exponential growth on glucose. The recombinant protein was purified and characterized as a 160 kDa homodimer. The functional involvement of the ACD in acetate formation was demonstrated by an ACD knockout strain showing an up to 70 % reduction of acetate formation.
Acetate activation: AMP-forming acetyl-CoA synthetase (ACS, acetyl-CoA + AMP + PP_i ⇄ acetate + ATP + CoA) that catalyzes the activation of acetate to acetyl-CoA was purified from acetate grown cells and the encoding gene (*HVO_0896*) was identified by MALDI-TOF. However, deletion of this gene did not affect growth of the mutant on acetate. Since eight additional paralogues ACS-genes are annotated in the genome of *H. volcanii*, a functional replacement by other ACS is suggested. Transcript analyses and enzyme characterizations of selected ACS-paralogues were performed. Three ACS-paralogues were found to catalyze the activation of acetate *in vitro*. Single, double or triple deletion mutants of the respective genes were generated and growth on acetate was analyzed. With these methods two ACS-enzymes involved in acetate activation were identified. Further, enzymes involved in phosphoenolpyruvate formation from acetate were identified.

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AEP27
Investigation of archaeal diversity of Saline Lakes by using Denaturing Gradient Gel Electrophoresis (DGGE)
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Culture-independent methods were used to evaluate haloalkaliphilic archaeal diversity in saline lakes. Archaeal diversity were assessed by denaturing gradient gel electrophoresis (DGGE), and quantifying copy numbers of archaeal 16S rRNA genes in the environmental DNA extracts using *Archaea*-specific primers. The archaeal diversity was highest in the sediment sample of and water samples; as well the number of bands derived from archaeal DNA was higher in the DGGE profiles of sediment samples than those of water samples. By molecular-based culture-independent analysis, all sequences fell into the phyla *Euryarchaeota* and *Crenarchaeota*. The library comprised new phylotypes The library comprised new phylotypes which consisted of five clones that exhibited low 16S rRNA similarity (90 - 95 %). two clones with 94 % and 95 % similarity to *Halogranum* and *Halalkalicoccus* which could represent new species, as well as two unclassified archaeal phylotypes which exhibited 97 - 99 % similarity to uncultured archaeon clone.

AEP28
Gene knock down in Archaea using CRISPRi
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Currently no methods are available to knock down genes in Archaea, in addition only a few regulatable promoters are at hand. Therefore, to study the function of essential genes *in vivo*, we developed a CRISPR-Cas based tool for the downregulation of genes in the halophilic archaeon *Haloferax volcanii*.
CRISPR-Cas systems can be repurposed as effective tools for genome editing and gene regulation in bacteria and eukarya, using the CRISPR-Cas type II specific protein Cas9 (1, 2). Archaea do not possess type II CRISPR-Cas systems, and since *H. volcanii* is a halophilic archaeon, the known bacterial Cas9 proteins might not be active in *Haloferax*. Therefore, we take advantage of the endogenous type I-B system of *H. volcanii* to repress gene expression. The endonuclease that degrades the target DNA in this system is Cas3.
In a *cas3* deletion strain, Cas proteins together with the crRNAs will bind to the target DNA, but since Cas3 is missing, the target DNA is not degraded. If the crRNAs are binding to the promoter region or coding region, transcription intitiation or elongation is blocked (3, 4), thus expression of the respective gene is down regulated.
Here, we show that CRISPRi can be employed successfully in *Haloferax*. For convenient and easy design of crRNAs for desired target regions we express the crRNAs independently of the Cas6 pathway using tRNA processing enzymes (5), resulting in so called icrRNAs (independently generated crRNAs). For efficient down regulation we use CRISPRi in a *cas3*/*cas6* deletion strain. Deletion of the *cas6* gene prevents generation of endogenous crRNAs, therefore the only crRNAs present in this deletion strain are the icrRNAs. Thus Cascade complexes in this strain contain only icrRNAs. We could furthermore show that overexpression of a catalytically inactive Cas3 variant as well as the proteins comprising the Cascade complex can enhance knockdown efficiency.

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AEP29
Analysis of the signal transduction by heme-based sensor kinases from the methanogen *Methanosarcina acetivorans*
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The multidomain protein MsmS from *Methanosarcina acetivorans* is one of the first examples for the biochemical characterization of an archaeal sensor kinase with autophosphorylation activity. It consists of two alternating PAS and GAF domains and a C-terminal H_ATPase domain. A homolog to MsmS is the putative sensor kinase MA0863, which shares

68 % identity and 84 % similarity with MsmS and contains an additional PAS domain at the N-terminus. The second GAF domain of both proteins covalently binds a heme cofactor via a cysteine residue. For MsmS, the redox state of the heme cofactor was shown to influence the autophosphorylation activity of the adjacent kinase domain [1]. For the investigation of the function of these archaeal signal transduction systems and their redox sensory function, the heme coordination structure was analyzed using UV-vis and Resonance Raman spectroscopy. Therefore, several variants of truncated MsmS were analyzed to identify the heme coordinating residues. First UV-vis spectroscopic analysis identified a histidine residue as the proximal ligand for the heme cofactor. Furthermore, the redox potential of wild type MsmS-sGAF2 and a protein variant lacking the cysteine residue for heme binding were determined to investigate the redox sensory function and the influence of the covalent linkage on the oxidation state of sGAF2. In order to increase the efficiency of heme incorporation into recombinant hemoproteins in *Escherichia coli,* a new procedure was established. Finally, the presented results will be discussed in the light of the putative cellular function of both heme-based sensor kinases.

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AEP30

Methanoarchaea as part of the human microbiota: Friends or Foes?

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Question: The archaeal strains *Methanosphaera stadtmanae*, *Methanobrevibacter smithii* and *Methanomassilliicoccus luminyensis* are known to be part of the human gut microbiota. Since the potential impact of these strains on human immune homeostasis was rarely evaluated until now, our study aims to elucidate the interaction of these archaeal gut inhabitants with the human immune system.
Methods: The response of human epithelial as well as immune cells due to the exposure of *M. stadtmanae, M. smithii* and *M. luminyensis* was elucidated by quantification of released cytokines, CLSM analysis, qRT-PCR and DNA microarray analysis. Isolation and purification of archaeal cell components was performed in order to examine the respective involved archaeal-associated molecular pattern.
Results: Whereas exposure to *M. stadtmanae* leads to substantial release of pro-inflammatory cytokines after phagocytosis by immune cells, only weak activation was detected after incubation with *M. smithii*. Although *M. luminyensis* was also found to induce high amounts of pro-inflammatory cytokines, activation by this archaeon was shown to be independent of phagocytosis by immune cells. By studying purified cell components of *M. stadtmanae* and *M. smithii*, we found immunogenic properties of cell wall compounds as well as archaeal nucleic acids that might be recognized by known human recognition receptors.
Conclusions: Overall, our findings strongly argue that archaeal gut inhabitants are specifically recognized by the human immune system. Interestingly, all examined strains lead to strikingly different immune responses. The discovered high immunogenic potential of *M. stadtmanae* might argue for its potential involvement in the development of systemic intestinal diseases.

AEP31

Markerless gene deletion in *Thermus thermophilus* HB27

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Thermus thermophilus HB27 is a gram-negative, aerobic bacterium growing at temperatures up to 85 °C. With its specific amenable characteristics like high growth rates, cell yields, constitutive natural competence, high GC content (69 %), and published genome sequence, *T. thermophilus* emerged as a laboratory model for studying the molecular basis of thermophilia. The small genome of *T. thermophilus* contains few functional paralogues and consequently studying knock-out mutants is one appropriate approach to elucidate specific gene functions in this organism. The limited number of selection markers and the demand on mutants pushed the development of alternative, counter-selectable systems for markerless genome manipulation in *T. thermophilus*. Cytosine deaminase CodA (EC 3.5.4.1) catalyzes the deamination of cytosine and its analog 5-fluorocytosine (5-FC) to uracil and 5-FU, respectively which are subsequently converted to UMP and 5-FUMP by Upp. Gene deletion system based on CodA has been widely used in other organisms. In *T. thermophilus* HB27 no *codA* gene or orthologs have been identified. An application of heterologous *codA* as counter-selectable marker for *T. thermophilus* HB27 has the great advantage that the wild type can directly be used because prior construction of a *codA* deletion strain is not required.
We developed a new markerless deletion system for *T. thermophilus* HB27 using *codA* (Tmar_1477) from *Thermaerobacter marianensis* DSM 12885, whose properties are comparable to the GC-content and optimal growth parameters of *T. thermophilus* HB27. The *codA* deletion system based on the sensibility against the antimetabolite 5-FC was used to delete the *bglT* gene (TT_P0042) encoding a b-glycosidase and three carotenoid biosynthesis genes, *CYP175A1*, *crtY*, and *crtI* (TT_P0059/60/66) encoding a b-carotene hydroxylase of the P450 superfamily, a lycopene b-cyclase, and a phytoene desaturase, on the megaplasmid pTT27 from the genome of *T. thermophilus* HB27.

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AEP32

Crystal structure and activity profiles of promiscuous carboxylesterases from the Thaumarchaeon *Nitrososphaera gargenis* linked to their metabolic function

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Candidatus *Nitrososphaera gargensis* Ga9.2 is a mesothermophilic member of the phylum Thaumarchaeota and its genome contains six putative α/β hydrolase genes (Spang *et al.* 2012). In an attempt to elucidate the function of the corresponding enzymes, the putative ORFs were cloned and successfully expressed in *E. coli* Rosetta-gami 2 (DE3). Assays for lipolytic activity resulted in two clones with reproducible esterase activity. The two carboxylesterases, namely EstN1 and EstN2, were biochemically characterized in detail. They showed a preference for *para*-nitrophenol esters with short-chained residues (C2; C4; C6), were cofactor-independent and had a temperature optimum of 40 °C with an optimal pH at 7. Interestingly, the two esterases did not show any homology based on blast-searches (blastn, blastp, blastx) to already known esterases of other genera than *Nitrososphaera* and not more than 76 % identity to putative α/β hydrolases from its closest relatives *Nitrososphaera evergladensis* SR1 and *Nitrososphaera viennensis* EN76 and their function is not known. While conserved domain searches suggest that EstN1 could be involved in the depolymerisation of polyhydroxyalkanoates, the role of EstN2 remained unclear. The crystal structure of EstN2 was solved and diffracted X-rays to 1.5 Å resolution (Kaljunen *et al.* 2014). EstN2 revealed a classical α/β hydrolase fold. A structural alignment did not result in any similar enzyme structure, but a virtual substrate screening, i.e. a docking study against 3000 molecules of the BRENDA database, indicated a rather promiscuous binding pocket for various ester substrates. Furthermore, transmission electron microscopy (TEM) after immunogold labelling of an EstN2-specific antibody revealed that the enzyme is present both within and outside the *N. gargensis* cells indicating also a possible function as an exoenzyme.

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BDP01
Factors impeding efficient polystyrene biodegradation by fungi employing extracellular Fenton chemistry
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Synthetic polymers, commonly known as plastics, are a widespread and severe factor of pollution in natural environments. Their solid nature and their chemical inertness are both known to be important contributors towards their recalcitrance against biodegradation [1]. Recently, we reported the biodegradation of the water-soluble synthetic polymer polystyrene sulfonate (PSS) by the brown-rot fungus *Gloeophyllum trabeum* via a hydroquinone-driven Fenton pathway, while oxidative attack by white-rot fungi producing lignin-modifying exoenzymes was inefficient [2].
Here, we focused on the applicability of this biodegradation mechanism to solid polystyrene (PS), the fourth-most widespread synthetic polymer. No substantial biodegradation of PS films by *G. trabeum* could be observed via gravimetric weight loss, while surface investigations with X-ray photoelectron spectroscopy and contact angle measurements indicated oxidative attack on the film surface.
In order to elucidate reasons for the apparent inability of *G. trabeum* to substantially attack PS in contrast to PSS, low molecular weight model compounds were also applied. We found that PS likely gains its recalcitrance to biodegradation by *G. trabeum* not only from its poor bioavailability, but also from the inertness of its basic building blocks towards Fenton's reaction. Nonpolar model compounds were essentially not biodegraded. On the other hand, the presence of sulfonate groups led to substantial degradation of sulfonated models, especially if two sulfonate groups in close proximity were present as in a dimeric model. Here, metabolite investigations suggested Fenton chemistry as the main degradation pathway.
All together, these results establish novel constraints for the biodegradation of synthetic polymers.

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BDP02
Beta beware – microbial degradation of aromatic β-amino acids
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The metabolism of proteinogenic α-amino acids is well investigated with respect to uptake, enzymatic conversion reactions and degradation as well as production and excretion. By contrast, little is known about the fate of β-amino acids (β-aa) which are often included in peptide-based natural compounds, presumably not least due to the high protease resistance of such "β-peptides".
So profound knowledge of the biodegradation mechanisms of β-aa is essential in terms of (A) understanding defense mechanisms of microorganisms affected with these natural compounds (B) environmental aspects referring to the persistence of β-aa in soil and water (C) pharmacokinetics of these natural compounds when used as a drug, e.g. cytostatics containing aromatic β-aa.
Using β-phenylalanine as model substrate, we were able to calculate growth parameters, substrate usage and product formation during the fermentation of Burkholderia sp., thus gaining a first quantitative insight in the biodegradation of this β-aa [1] which is initiated by a transaminase reaction [2].
We are now testing additional β-aa as substrates and started to purify the responsible transaminase in order to investigate the substrate spectrum of our model organism and its enzymes.

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BDP03
Polysaccharide decomposition – the physiological and genomic differences among *Natrialba* species
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The production of bioethanol and other bio-based industrial chemicals from aqua-/agriculture waste products is becoming increasingly more important. Extremophiles, such as halophilic *Archaea*, harbor enzymes, which function optimally at high temperatures, high salt concentrations and in the presence of organic solvents. We investigated further the phenotypic and genomic adaptation strategies of members of the genus *Natrialba* (*Halobacteriaceae*), with the focus on polysaccharide decomposition in significantly distinct habitats.
Natrialba species grow at NaCl concentrations ranging from 15 % to saturation (~ 32 %) and were isolated either from alkaline lakes (growth optimum pH 9) or marine saltern (growth optimum pH 7). Most physiological characteristics corroborate this ecological differentiation, dividing them into two evolutionary groups. The major ether-bound isoprenoid lipids are PGP-Me and PG. However, *Natrialba* species from marine saltern contained further ether lipids, such as the sulphated glycolipids DGD-S, DGD-S2. The optimal growth temperature and the DNA G+C content are higher in alkaliphilic species. Alkaliphilic species decompose peptides, such as casein and gelatin, much faster, which correlates with a greater number of peptidases (mostly metallo- and serine-peptidases). In contrast, species from the marine salterns not only utilized a greater diversity of saccharides, but utilized them faster. Furthermore, these species decomposed the polysaccharides starch, galactomannan, pachyman and cellulose, which can be explained by a considerably greater number of carbohydrate active enzymes (glycoside hydrolases, polysaccharide lyases, carbohydrate esterases) in their genomes. The finished genomes of the *Natrialba* species comprised two chromosomes and one plasmid, as shown for *Nab. magadii* DSM 3394^T. Interestingly, *Natrialba* species from marine saltern contained up to four additional plasmids. Genes potentially encoding for starch, galactomannan and pachyman hydrolysis cluster together in the genomes and are syntenic among the genomes. Finally, one of the key pathways was identified that explains the differences among the *Natrialba* species.
These results suggest that the contrasting phenotypic characteristics could be linked to genomic features and explain some adaptations to cope in their habitats. Furthermore, all enzymes identified, potentially involved in polysaccharide decomposition, are of biotechnological interest.

BDP04
Paths and processes of contaminant translocation by fungal mycelia
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Question: Mycelia act as effective transport routes for optimal resource supply of hyphal organisms in water-unsaturated soil. They also can take up and actively translocate highly immobile substances like polycyclic aromatic hydrocarbons (PAH), act as physical PAH dispersal paths and influence PAH bioavailability and biotransformation. We propose that PAH translocation along mycelia involves active transport, and passive diffusion via the gas, aqueous and biomass phases, with the translocated amounts depending on the PAHs water solubility, volatility and sorption behavior and biodegradation by the fungus during transport.
Methods: To discriminate the contributions of individual processes, we developed an agar-based model system mimicking unsaturated environments to which we added mycelial structures that stepwise offered additional PAH transport paths (gas-phase and biomass-based diffusion and active transport). The effect of biodegradation was studied by comparing able to degrade the two mycelia-forming microorganisms (*Cunninghamella elegans* and *Pythium ultimum*) differing in their ability to degrade five model PAH of differing physico-chemical properties (FLU, PHE, ANT, PYR, FLA) studied.
Results: Besides time and distance, the octanol-air partition coefficient (KOA) controlled the PAH translocation in both organisms. Gas-phase diffusion was the main path for highly volatile PAH, whereas PAH with log KOA>7.5 were most effectively mobilized by active transport in living mycelia (50-90 % of transported PAH mass). PAH-degrading *C. elegans* in addition showed a compound-specific overall translocation, decreasing in the order FLU > PHE = ANT > PYR = FLA and a clearly distance-dependent translocation in reaction of its PAH biodegradation kinetics.

Conclusion: Physiologically active and dead mycelia both enhance PAH translocation in unsaturated porous media. Mycelia-based transport processes may be of particular importance at interfaces of air-, liquid- and solid-phases as often found in the vadose zone above NAPL contaminated groundwater.

BDP05
Bioaugmentation of PAH-contaminated groundwater
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Polycyclic aromatic hydrocarbons (PAHs) are a public health concern as in high concentrations they are toxic, mutagenic and carcinogenic. They result at the incomplete combustion of organic substances and are persistent in soil due to strong adsorption to soil organic matter and low degradation rates especially of high molecular PAHs. A former gas plant site in Eisenach, Germany, is heavily PAH-contaminated due to decades of gas production and discharge of coal tar waste into pits. A leaky tar pit lead to the formation of a PAH-contamination plume in the groundwater, associated with strong depletion of oxygen, as oxygen acts as an electron acceptor and is the limiting factor during aerobic microbial PAH-degradation. To assess bioremediation potential at the site, the indigenous microflora was investigated.
Microorganisms were isolated from the contaminated groundwater and selected for their ability to degrade PAHs. The most capable strains were inoculated as a consortium into the groundwater accompanied by oxygen injection. Microbial activity was monitored by monthly groundwater sampling and the determination of colony forming units (cfu). The effect of the bioaugmentation on groundwater bacterial community was determined by 16S rRNA sequencing of community DNA with Illumina MiSeq.
The inoculated consortium comprised species of the genera *Pseudomonas*, *Acinetobacter*, *Sphingobium* and *Streptomyces*. CFU was generally very low (< 10^3 cfu/ml) compared to uncontaminated sites. The addition of oxygen increased of cfu compared to unstimulated groundwater. Bioaugmentation increased the microbial population additionally, with visible effects for three month. These effects were restricted to sampling sites close to the infiltration zone. PAH-contamination decreased downstream of the oxygen injection locations, which was mostly based on decreasing concentrations of low molecular weight PAHs. After bioaugmentation, a temporary decrease of high molecular weight PAHs could be observed. The bacterial community was predominated by *Betaproteobacteria* and *Epsilonproteobacteria.* Bioaugmentation transitionally promoted the abundance of *Gammaproteobacteria* and increased species diversity.
It could be shown that microbial PAH-degradation was temporarily improved by bioaugmentation and biostimulation, therefore substantiating bioremediation potential. We recommend repeated inoculation treatments combined with oxygen augmentation for a sustainable vitalization of the aerobic PAH-degrading microflora.

BDP06
Anaerobic two-member bacterial communities degrade sulfoquinovose concomitant with release of sulfide
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Sulfoquinovose (SQ, 6-deoxy-6-sulfoglucose) is a prominent natural organosulfonate produced by, essentially, all plants and other phototrophic organisms, and SQ is a relevant component of the biogeochemical sulfur cycle. Two bacterial degradation pathways for SQ have been demonstrated recently: First, a 'sulfoglycolytic pathway', in which SQ is catabolized to dihydroxypropanesulfonate (DHPS) in direct analogy to the Embden-Meyerhoff-Parnas pathway, and second, an Entner-Doudoroff-type of pathway, in which SQ is catabolized to sulfolactate (SL). The excreted DHPS and SL can then be utilized completely by other bacteria, concomitant with release of sulfate. Hence, SQ can be mineralized to CO2 and sulfate by two-member bacterial communities under oxic conditions. Now, we explore the degradation pathways for SQ in bacterial communities under anoxic conditions, and present our preliminary results: For a first tier, we use Escherichia coli K12 as a model organism, which apparently is able to catalyze a fermentation of SQ to DHPS, succinate, acetate, and formate, thus, a modified mixed-acid fermentation with SQ. For a second tier, we use a novel Desulfovibrio sp. isolate, which apparently is able to catalyze a fermentation of DHPS to acetate and, importantly, sulfide (H2S), in contrast to the release of sulfate as with aerobic bacteria. Since SQ is also a relevant component of the vegetable diet, we predict that anaerobic degradation of SQ is a relevant, but yet unrecognized, source of sulfide in the human gut.

BDP07
Anaerobic acetone degradation by *Desulfococcus biacutus* – identification and characterization of potentially involved enzymes
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In acetone degradation by aerobic and nitrate-reducing bacteria an ATP-dependent carboxylation reaction activates acetone to acetoacetate, which is subsequently converted to acetoacetyl-CoA. In sulfate-reducing bacteria, a similar activation is energetically not possible and also acetoacetate was ruled out as a reaction intermediate [1].
Recent studies on the sulfate-reducing, acetone-utilizing bacterium *Desulfococcus biacutus* exhibited an ATP- and TDP (thiamine diphosphate)-dependent activation, leading finally to acetoacetyl-CoA [2, 3]. Furthermore, the genome of *D. biacutus* was sequenced and comparative 2D-PAGE revealed some proteins which are specifically induced during growth with acetone, and therefore are potentially involved in acetone degradation [4].
Several candidate enzymes (two dehydrogenases, a TDP-dependent enzyme and a B_{12}-dependent mutase) were successfully cloned and overexpressed in *Escherichia coli*. Purified recombinant enzymes were used for further analysis. One of these enzymes exhibited an aldehyde/ketone oxidoreductase activity, another enzyme showed acetoacetyl-CoA reductase activity. Further characterization of each of these enzymes is supposed to elucidate the mechanism of acetone activation in *D. biacutus*, which would represent a new biochemical pathway of acetone degradation.

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BDP08
Applying immobilized laccase for removing pharmaceuticals from wastewater
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Questions: An emerging issue of the 21st century is related to micro-pollutants increasingly detected in waters. Such compounds are typically found in the ng/L to the lower µg/L range and are often not or not sufficiently removed in conventional wastewater treatment plants [1]. The development of innovative (waste) water treatment methods using enzymes offers the possibility to overcome known drawbacks of conventional wastewater treatment processes. The use of free enzymes in solution is limited due to lake of reusability, rapid denaturation and requirement of large quantities which will impact the overall cost of their use [2]. Immobilization is one of the most effective methods used to circumvent these drawbacks.
Methods: The present study addresses the immobilization of produced laccase from the aquatic ascomycete *Phoma* sp. UHH 5-1-03 on commercial membranes via electron beam irradiation [1]. The efficiency of immobilized and free laccase in the removal of environmentally highly relevant pharmaceuticals was investigated and compared using batch reaction mixtures at laboratory scale. A mixture of acetaminophen, mefenamic acid, naproxen, fenofibrate, bezofibrate, indomethacin, and ketoprofen was applied in influent, effluent from the WWTPs located in Magog (Quebec, Canada), buffer and Milli Q pure water. The (apparent) catalytic parameters k_m and V_{max} and the catalytic efficiency (in terms of V_{max}/k_m) between immobilized and free laccase for transformation of acetaminophen as a model micro-pollutant was assessed.
Results: Both free and immobilized laccase were quite efficient in the removal of pharmaceuticals under the conditions of real wastewater, without the need for buffering the respective reaction system. Acetaminophen and mefenamic acid removal by immobilized laccase in influent and effluent was around 60 % and more than 75 % for rest of batch incubation mixtures by immobilized and for all batches by free laccase. The immobilized laccase clearly showed higher V_{max} and V_{max}/k_m values despite displaying a higher k_m for acetaminophen.

Conclusion: The application of immobilized laccase for treatment of drugs in wastewater was efficient. The kinetic parameters indicated a higher catalytic efficiency for immobilized laccase than the non-immobilized.

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BDP09

Anaerobic phenanthrene degradation by a sulfate-reducing enrichment culture

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Polycyclic aromatic hydrocarbons (PAHs) are among the most recalcitrant substances occurring in nature. They have a high toxicity which can result in negative health effects for living organisms. However, the anaerobic degradation pathways for polycyclic aromatic hydrocarbons are poorly investigated. For bioremediation purposes it would be most useful to further expand the knowledge of the degradation pathways, especially in contaminated groundwater aquifers that serve as a resource for drinking water. With the world's biggest natural asphalt lake, Pitch Lake in La Brea, Trinidad and Tobago, we had a source for a range of microorganisms able to actively degrade PAHs. We enriched a culture growing on phenanthrene as sole electron and carbon source and with sulfate as terminal electron acceptor. First steps of phenanthrene degradation were assessed by metabolite analysis. We identified phenanthroic acid with LC-MS and GC-MS analysis, indicating a carboxylation as initial activation reaction. Metagenome sequencing of the enrichment culture produced an almost closed genome which is currently analyzed for phenanthrene degradation genes. Together with a biochemical characterization of the culture this will broaden our knowledge on anaerobic degradation of PAHs. Our longtime goal is the detection of novel marker genes for PAH degradation in PAH-contaminated aquifers and sediments and a more holistic understanding of the ecology of PAH-degrader populations in the pitch lake which can be taken as a proxy for oil-reservoirs.

BDP10

Elucidation of the metabolic pathway for SDS (sodium dodecyl sulfate) degradation in *Pseudomonas aeruginosa*

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Question: *Pseudomonas aeruginosa* is an ubiquitous environmental bacterium that can act as an opportunistic and nosocomial pathogen. Its metabolic versatility and pronounced resistance against toxic chemicals enables it to survive and grow in hygienic environments where it can cause outbreaks in clinical settings. In this context, the frequently utilized toxic detergent sodium dodecyl sulfate (SDS) is used by *P. aeruginosa* as a growth substrate [1]. Previous studies demonstrated cell aggregation of *P. aeruginosa* during growth with SDS as a specific survival strategy [2]. Despite of the initial hydrolysis of the sulfate ester, the metabolic pathway including responsible enzymes for the SDS degradation remains unclear. In this study we address these unknown enzymatic steps.
Methods & Results: Based on a DNA-microarray analysis comparing SDS- and succinate-grown cells several genes with a plausible function in SDS degradation were identified. These candidate genes encode alcohol dehydrogenases, aldehyde dehydrogenases and enzymes for beta-oxidation. Currently, deletion mutants of the respective candidate genes are being constructed and analyzed in physiological experiments. So far, the deletion of a special alcohol dehydrogenase system exhibits a strong and interesting phenotype during growth with SDS.
Conclusion: The analysis of this catabolic pathway will give insight in how *P. aeruginosa* copes to grow with SDS and potentially other similar toxic substrates.

[1] Klebensberger J. *et al.* (2006). Cell aggregation of *Pseudomonas aeruginosa* strain PAO1 as an energy-dependent stress response during growth with sodium dodecyl-sulfate. Arch. Microbiol. 185:417-2
[2] Klebensberger J. *et al.* (2007). Detergent-induced cell aggregation in subpopulations of *Pseudomonas aeruginosa* as a preadaptive survival strategy. Environ. Microbiol. 9(9):2247-59

BDP11

Novel polyester-degrading enzymes from plant compost metagenomes

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Question: Synthetic polyesters are present in many aspects of our life. Because of their valuable properties, synthetic polymers such as polyethylene terephthalate (PET) and polyurethane (PU) are applied in automotive, furniture, bedding, textile, packaging and other industries. The high amounts of industrially produced synthetic polyesters have resulted in a massive increase in plastic wastes. The related environmental pollution as well as the decreasing availability of raw materials require novel solutions for the recycling and degradation of these synthetic polymers. Various thermophilic actinomycetes growing in plant-containing compost materials produce enzymes capable of hydrolyzing synthetic polyesters [1].
Methods: A metagenomic approach was used to isolate novel polyester hydrolases. Metagenomic DNA was extracted from different composting sites located in Leipzig, Germany. The DNA was used as a template for the construction of fosmid libraries as well as for PCR using degenerate primers. The resulting clones were screened on turbid agar plates containing the polyester substrates PET dimer (2PET), PU or PET polymer, respectively.
Results: With both methods, fosmid libraries and degenerate primers, several novel polyester degrading enzymes could be identified which showed differences in their substrate specificities as well as in their optimum reaction temperatures. The enzymes have been purified and characterized with respect to their polyester-hydrolyzing activity.
Conclusion: Several previously unknown polyester hydrolases have been isolated from plant compost metagenomes. The use of degenerate primers was shown to be a useful approach for the identification of novel polyester-degrading enzymes.

[1] R. Wei, T. Oeser, W. Zimmermann, Synthetic polyester-hydrolyzing enzymes from thermophilic actinomycetes, Adv Appl Microbiol 89 (2014) 267-305.

BDP12

Controls of anaerobic hydrocarbon degrader community structure along a longitudinal contaminant plume transect

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Hydrocarbons are among the major pollutants endangering groundwater quality. They occur as typical point source contamination and develop into a long contaminant plume on top of the groundwater table by spreading with the groundwater flow. Microbes can oxidize these compounds in presence of electron acceptors such as oxygen, nitrate, ferric iron or sulfate, but since they are used up rapidly, they have limited availability at the contaminant plume, compared to the amount of hydrocarbons. To our understanding these biogeochemical gradients along the plume shape the structure of specialized degrader communities and also determine biodegradation rates. Evidence from a vertical plume section supports this hypothesis [1], but longitudinal plume transects are more difficult to access and therefore less investigated until now.
In this study, we collected samples from a longitudinal transect of an aromatic hydrocarbon plume, along the groundwater flow path. We measured the concentration of typical electron acceptors and hydrocarbons and linked these to the diversity of the hydrocarbon degrader and total bacterial community via T-RFLP and sequencing of the benzylsuccinate synthase (*bssA*) and 16S rRNA genes. We also performed qPCR to quantify the abundance of respective populations.
Our results show clear shifts of electron donor (BTEX) and acceptor (oxygen, sulfate, nitrate, ferric iron) concentrations along the plume transect. We observed the highest diversity of degraders at the upstream and downstream fringes of the contaminant plume, where electron acceptors were more available. In contrast, we found a very specialized low-diversity community in the plume core, where the environment causes high selection pressure on the bacteria: electron acceptors are hardly available and high hydrocarbon concentrations may also have a toxic effect as well. We also observed great shifts in overall bacterial community composition along the contaminant plume, which was also clearly linked to changing electron acceptor and donor availability. Based on these findings, we propose a conceptual model of how these

geochemical gradients affect degrader community diversity and performance at the different plume compartments, complementing current concepts of controls for biodegradation in contaminated aquifers [2].
These results suggest that monitoring bacterial or degrader abundance and diversity as a proxy of biodegradation rates can be challenging, if spatially resolved data on the whole plume system are not considered.

[1] Winderl, C., *et al.*, Appl Environ Microbiol, 2008. 74(3): p. 792-801.
[2] Meckenstock, R.U., *et al.*, Environmental Science & Technology, 2015. 49(12): p. 7073-7081.

BDP13
Global transcriptome changes in *Aromatoleum aromaticum* strain EbN1
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The facultative anaerobic denitrifying bacterium *Aromatoleum aromaticum* strain EbN1 is able to distinctively degrade hydrocarbons and phenolic compounds anaerobically (1). The anaerobic degradation of the very similar compounds toluene. ethylbenzene, phenol and *p*-ethylphenol is separately regulated for each substrate (2,3). Based on earlier experiments, sequence alignments and similarities to other hydrocarbon degrading organisms putative regulators have been assigned to the different degradation clusters (4). It was proposed that toluene, ethylbenzene and acetophenone degradation in EbN1 are under the control of the two component systems *tdiRS, ediRS* and *adiRS* respectively. On the other hand, σ^{54} dependent regulators are probably responsible for the activation of the degradation of *p*-ethylphenol (EtpR) and phenol (PdeR) (5). All of these proposed regulators can be found directly adjacent to the respective degradation operon. Recent results show a complex network of interactions between these regulators (6). To gain further insight into the matter EbN1 was cultivated with different carbon sources and the isolated mRNAs from these cultures were analyzed in a high-troughput sequencing approach. The results identify expected and unexpected changes in gene expression between the different conditions and shed new light on the complexity of the regulation network.

1. Rabus & Widdel,Arch Microbiol.1995 Feb; 163(2):96-103
2. Rabus *et al.*,Arch Microbiol. 2005 Jan; 183(1):27-36
3. Wöhlbrand *et al.*,J Bacteriol. 2008 Aug; 190(16):5699-709
4. Rabus *et al*,.Arch Microbiol. 2002 Dec; 178(6):506-16
5. Büsing *et al.*, BMC Microbiol. 2015 Nov 2; 15(1):251
6. Muhr *et al.*,Arch Microbiol. 2015 Nov 197(9):1051-62

BDP14
Regulation of *rdh* gene expression by two-component system regulators in *Dehalococcoides mccartyi*
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Reductive dehalogenases are the key enzymes in organohalide respiration, whereby halogenated compounds serve as electron acceptors for energy conservation. The genome of the strictly anaerobic bacterium *Dehalococcoides mccartyi* strain CBDB1 contains 32 different reductive dehalogenase homologous (*rdhA*) genes, reflecting its capacity to dehalogenate a broad range of halogenated compounds. Two types of regulators are encoded adjacent to *rdhA* genes: MarR-type and two-component-system (TCS) regulators, suggesting a tight control of *rdhA* transcription. For one of the MarR regulators we have shown that it acts as transcriptional repressor (Wagner at al. 2013). The aim of the current study was to elucidate the role of TCS regulators in *rdhA* gene transcription in *D. mccartyi*. We focused on the *rdhA* gene *cbdbA80*, which is part of a conserved gene cluster in *D. mccartyi* strains CBDB1 and DCMB5 and comprises two TCS genes, *cbdbA79-78* and *cbdbA82-83*, located directly upstream or more distantly downstream, respectively, of *cbdbA80*.
D. mccartyi is not accessible to genetic manipulation. Therefore, the promoters of the *rdhA* gene *cbdbA80* (P_{80}) and the TCS-encoding genes *cbdbA79-78* (P_{79}) were transcriptionally fused to *lacZ* and each was integrated in single copy into the *E. coli* host. Plasmids carrying the TCS genes *cbdbA79-78* or *cbdbA82-83* were transformed into these reporter strains. The promoter activity was assessed by beta-galactosidase assays. In addition, the number of *rdhA* transcripts was quantified in strains CBDB1 and DCMB5.
The results indicated activity of promoters P_{79} and P_{80} suggesting their recognition by the RNA polymerase of the heterologous *E. coli* host. Upon transformation with the TCS-encoding plasmids the P_{80} activity increased further compared to empty-vector controls providing first hints to an activating function of both TCS on *rdhA* gene transcription. Transcription analyses of *cbdbA80* and its orthologue in strain DCMB5 revealed two orders of magnitude higher transcript levels in strain CBDB1 compared to strain DCMB5. A natural mutation was detected in the response regulator-encoding gene *cbdbA78* not present in the orthologous gene in DCMB5, leading to the exchange of a conserved arginine to methionine within the dimerization domain. This might influence the activating function of the response regulator and explain the observed constitutive synthesis of CbdbA80 on a variety of halogenated electron acceptors (Yang *et al.* 2015) in contrast to the orthologue Dcmb81 in DCMB5 (Pöritz *et al.* 2015). The data confirm the importance of transcriptional regulation for organohalide respiration and for the expression of strain-specific dehalogenation properties.

Wagner *et al.* (2013) Phil Trans R Soc B 368,20120317
Yang *et al.* (2015) Environ Sci Technol, 49, 8497-8505
Pöritz *et al.* (2015) Appl Environ Microbiol 81, 587-596

BDP15
Analysis of the regulation of the lipopolysaccharide biosynthesis in *Escherichia coli* using quantitative mass spectrometry
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The ATP-dependent, membrane anchored and essential AAA^+ (ATPases Associated with various cellular Activities)-protease FtsH degrades the LpxC enzyme to regulate the lipopoly-saccharide (LPS) biosynthesis in *Escherichia coli* (*E. coli*). LpxC is an essential deacetylase in *E. coli* and catalyzes the first committed step in the biosynthesis of Lipid A, which forms the membrane anchor of LPS. An imbalance between LPS and phospholipids leads to an accumulation of membrane stacks in the periplasm, which subsequently results to a growth defect of the cell. The LpxC degradation is strictly regulated in a growth-rate dependent manner. We assume that additional factors (e.g. interaction partner or modulator proteins) are involved in the LpxC degradation by FtsH. However, a direct influence of already known LpxC interaction partners on the LPS regulation could not be shown so far.
In this study we established a SILAC (Stable Isotope Labeling by Amino acids in Cell culture) approach in *E. coli* to improve the identification and the quantification of the dynamic LpxC and FtsH interactome at different growth-rates. SILAC labeling drastically improves the quantification of co-purified proteins from the LpxC and FtsH complex. To maximize the labeling efficiency we used an auxotroph *E. coli* BL21 strain and tested different growth conditions. Furthermore we optimized the *lpxC* and *ftsH* expression and the respective purification protocols. After LpxC and FtsH purification and LC-MS/MS (Liquid Chromatography Tandem Mass Spectrometry) analysis 107 proteins were enriched in slow and 525 in fast growth-rates co-purified with LpxC and 327 proteins were enriched in slow and 64 in fast growth-rates co-purified with FtsH. By comparing highly enriched and overlapping proteins of the LpxC and FtsH interactome we identified 34 (slow growth-rates) and 23 (fast growth-rates) putative LpxC modulators which can provide us new insights into the regulation of the LPS biosynthesis. The influence of the identified, putative modulator proteins were validated in LpxC stability tests followed by biochemical characterization. Concluding, we were able to analyze the dynamic LpxC and FtsH interactome and enhanced the number of putative LpxC interaction partners and modulator proteins specific for different growth-rates with this quantitative mass spectrometry approach.

BDP16
2-Naphthoyl-CoA reductase – key enzyme in anaerobic naphthalene degradation
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Question: Polyaromatic compounds (PAH) are a serious threat to human health and the environment. While the degradation of PAH under aerobic conditions has been studied in great detail, little is known about the mechanisms of enzymatic anaerobic PAH metabolism.
The anaerobic degradation of the model compound naphthalene has been studied in sulfate-reducing bacteria and is initiated by carboxylation to 2-

naphthoic acid. The latter is then activated by ligation to coenzyme A and further metabolized by three consecutive reductive steps[1]. In the sulfate-reducing enrichment culture N47, two homologous enzymes are involved in the key step of ring reduction, each belonging to the old yellow enzyme family of flavoproteins and catalyzing individual two-electron reductions[2]. The 2-naphthoyl-CoA reductase (NCR) is the first member of a novel class of dearomatizing reductases and catalyzes the two-electron reduction of 2-naphthoyl-CoA (2-NCoA) at an unusually low potential of - 493 mV[2]. We studied the function of NCR, the so far only isolated and characterized enzyme involved in anaerobic PAH degradation.

Methods: Cofactors of NCR were characterized by EPR and UV/vis spectroscopy. Redoxtitration in the presence of mediators was performed to investigate the roles of the cofactors in catalysis.

Results: EPR data strongly suggests the presence of a $[4Fe\text{-}4S]^{+1/+2}$ cluster in accordance with the conserved binding motif in NCR. UV/vis spectroscopy indicated the presence of a neutral flavin semiquinone state of the semi-reduced enzyme that could be confirmed by EPR spectroscopy. Redoxtitration in the presence of mediators monitored by UV/vis spectroscopy revealed two distinct redoxtransitions of the enzyme, one of which is in good accordance to the potential determined for the substrate/product pair.

Conclusion: The presence of two flavin cofactors in NCR with similar spectral features prevents unambiguous assignment of features to individual cofactors. Future work will focus on the generation of mutants for characterization of individual cofactors. The determination of the crystal structure is in progress and should give detailed insights to possible mechanisms of the challenging dearomatization catalyzed by NCR.

Eberlein, C., Estelmann, S., Seifert, J., von Bergen, M., Müller, M., Meckenstock, R.U. and Boll, M. (2013). *Mol. Microbiol.*, 88, 1032-1039.
Estelmann S., Blank, I., Feldmann, A. and Boll, M. (2015). *Mol. Microbiol.,* 95, 162-172.

BDP17

Enzymes involved in anaerobic *ortho*-phthalate degradation

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Question: Esters of *o*-phthalate are widely used as plasticizers and significant amounts are constantly released into the environment. Primary degradation of these esters yields *o*-phthalate both under aerobic and anaerobic conditions (1). Aerobic *o*-phthalate degradation with oxygenases as key enzymes is well understood (1) while little is known about the genes and enzymes involved in the phthalate degradation in anaerobic bacteria. Different metabolic routes have been proposed (2, 3) however, *in vitro* evidence was missing.

Methods: We investigated anaerobic *o*-phthalate degradation by establishing an *in vitro* assay using cell-free extracts of three denitrifying bacterial cultures. Key enzymes were identified by differential protein induction/mass-spectrometry and genome analysis.

Results: Cell extracts of all tested denitrifyers catylzed the succinyl-CoA-dependent conversion of *o*-phthalate to benzoyl-CoA, whereas decarboxylation of *o*-phthalate to benzoate was negligible. However, detection of the free *o*-phthaloyl-CoA intermediate is not feasible due to its rapid hydrolysis via intramolecular anhydride formation.

MS-based analysis of protein induction revealed the upregulation of an UbiD-like carboxylase/decarboxylase that we assign to the *o*-phthaloyl-CoA decarboxylase. These enzymes form a separated phylogenetic cluster among UbiD-like proteins. A gene encoding an UbiX-like protein was located in the same cluster, which is expected to be involved in prenylated flavin-cofactor formation of the phthaloyl-CoA decarboxylase as reported previously for another UbiD-like enzyme (4). The phthalate-induced gene cluster comprises a type III CoA-transferase; the product was enriched and showed a succinyl-CoA: phthalate CoA transferase activity.

Conclusion: Anaerobic degradation of *o*-phthalate in denitrifying bacteria proceeds via activation to *o*-phthaloyl-CoA with succinyl-CoA as CoA-donor followed by a decarboxylation to benzoyl-CoA. Decarboxylation is catalyzed by a novel class of UbiD-like enzymes. *In vivo* the highly instable intermediate *o*-phthaloyl-CoA may be stabilized by complex formation between the CoA transferase and the decarboxylase.

(1) Liang *et al.* (2008), Appl. Microbiol. Biotechnol., 80:183-198
(2) Taylor *et al.* (1983), Appl. Environ. Microbiol., 46(6):1276-1281
(3) Nozawa *et al.* (1988), J. Bacteriol., 170(12):5778-5784
(4) White *et. al.* (2015), Nature, 522:502-506

BDP18

Characterization of 1,2-dichloroethane dehalogenation in *Dehalococcoides mccartyi* using compound-specific stable isotope analysis

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Chlorinated ethenes and ethanes belong to the most common ground water and soil contaminants as they were widely applied in agriculture, e.g. as pesticides, or produced industrial and even naturally. While certain compounds, as e.g. cis-dichloroethene (cDCE), accumulate in the environment as a result of (incomplete) reductive dehalogenation of higher chlorinated ethenes under anoxic conditions, dichloroethane (DCA) is an exclusively man-made contaminant released due to improper waste treatment at versatile production sites. Nevertheless, both are persistent, toxic or carcinogenic and influence the ground water and soil quality. In the last years, however, several *Dehalococcoides mccartyi* strains were described, known to be capable of complete dehalogenation of these compounds to non-toxic ethene, under strictly anoxic conditions.

Compound-specific stable isotope analysis (CSIA) can be applied to gain knowledge about microbial transformation reactions. The technique is based on measurements of changes in isotopic ratios of selected substrates and products, which occur during reactions. The rate of the bond-cleavage is thereby affected by the nature of the isotope - heavy or light. Therefore, with the state-of-the-art technique, carbon, hydrogen, and chlorine isotope ratios can be used to investigate transformation mechanisms on a multi-element basis.

In this study we aimed to investigate the anoxic transformation of 1,2-DCA by *Dehalococcoides mccartyi* strain 195, isolated from an anoxic digester sludge, and BTF08, which was enriched from a contaminated groundwater in Bitterfeld (Germany). Dihaloelimination of 1,2-DCA to ethene was observed with relatively higher conversion rates for strain 195 compared to strain BTF08, similar to previous observations [1]. Furthermore, the compound-specific carbon, chlorine and hydrogen stable isotope composition was analyzed for both the substrate, 1,2-DCA, and ethene to obtain information on the dehaloelimination reaction.

[1] Schmidt, M.; Lege, S.; Nijenhuis, I.: *Comparison of 1,2-dichloroethane, dichloroethene and vinyl chloride carbon stable isotope fractionation during dechlorination by two* Dehalococcoides *strains.* 2014, Water Research, 52, 146-154.

BDP19

A steroid dehydratase from *Novosphingobium* sp. strain Chol11 as a key enzyme for determining the degradation pathway of the steroid compound cholate in a homogenous and a heterologous system

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Question: Bile salts are surface-active C_{24}-steroid compounds which aid the digestion of lipophilic nutrients in the intestinal tract of vertebrates. A considerable part of them is excreted into the environment. Bacteria from different phylogenetic groups are able to degrade bile salts and use them as growth substrates. The degradation of the 3,7,12-trihydroxy bile salt cholate has been studied with *Pseudomonas* sp. strain Chol1. The metabolic pathway is initiated by oxidation reactions at the A-ring of the steroid skeleton followed by the degradation of the carboxylic side chain. The resulting C_{19}-steroid with a $\Delta^{1,4}$-3-keto structure is called 7β,12β-dihydroxyandrostadienedione (DHADD), which is further degraded via the so-called 9,10-seco pathway. The initiating reactions appear to be very wide-spread among steroid-degrading bacteria. However, recently we found that cholate degradation can be initiated by a slightly modified reaction sequence in *Novosphingobium* sp. strain Chol11. There, water is eliminated from C7 leading to the steroid degradation intermediate hydroxyandrostatrienedione (HATD) with a $\Delta^{1,4,6}$-3-keto structure of the A-ring **[1]**. In this study, we investigated this modified degradation pathway for cholate in strain Chol11.

Methods and Results: Growth experiments with different bile salts showed that 7-hydroxyl group is required for the introduction of the Δ^6 double bound. Strain Chol11 could also grow with DHADD by transforming it into HATD. Based on this reaction we established an enzymatic assay for the purification of the respective enzyme. The pure enzyme encoded by the gene *nov2c400* has a molecular mass of 19 kDa and showed similarities to steroid dehydratases from enteric bacteria. Assays with recombinant Nov2c400 indicated that 3-keto-Δ^4-structure and

an unmodified side-chain are required for the dehydration of the steroid skeleton. Deletion of *nov2c400* resulted in a prolonged lag phase during growth with cholate. Interestingly, this deletion mutant of strain Chol11 degraded cholate via DHADD. Moreover, heterologous expression of *nov2c400* in *Pseudomonas* strain Chol1 led to formation of HATD, which did not support further growth of this strain.
Conclusion: In summary, the activity of the 7α-dehydratase Nov2c400 is responsible for channeling cholate degradation to the triene intermediate HATD in *Novosphingobium* strain Chol11. The enzyme could exhibit this activity also in the heterologous host *Pseudomonas* sp. strain Chol1, in which it caused a growth arrest.

[1] Holert *et al.* (2014) Evidence of distinct pathways for bacterial degradation of the steroid compound cholate suggests the potential for metabolic interactions by interspecies cross-feeding. *Environ Microbiol* 16(5):1424-1440

BDP20
Implications for the enzymatic mechanism of the cobamide-containing tetrachloroethene reductive dehalogenase of *Sulfurospirillum multivorans* derived from an extended substrate spectrum
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Question: *Sulfurospirillum multivorans* utilizes chlorinated or brominated ethenes, such as tetrachloroethene (PCE) or trichloroethene (TCE) as terminal electron acceptors for growth via organohalide respiration. Their conversion proceeds in a hydrogenolysis reaction mediated by the PCE reductive dehalogenase (PceA). PceA is a cobamide-containing iron-sulfur protein, the structure of which was solved recently [1]. The superreduced [Co^{I}]-state of the cobamide cofactor was proposed to function as reactive species. The way of cobamide-binding to PceA was also found in the structure of the *ortho*-dibromophenol reductive dehalogenase (NpRdhA) from *Nitratireductor pacificus* [2]. Electron paramagnetic resonance (EPR) spectroscopic studies on substrate-soaked NpRdhA suggested the formation of a halogen-cobalt bond prior to halogen substraction in the catalytic cycle. Spatial restraints in the active site pockets of PceA and NpRdhA disfavored the formation of an organo-cobalt adduct. The data obtained for the binding of TCE to PceA of *S. multivorans*, did not allow for an unambiguous deduction of the enzyme's mechanism since two orientations of the substrate were found. In order to learn more about the mode of substrate binding and conversion, the substrate spectrum of PceA was revisited and aromatic halogenated compounds such as chlorinated and brominated phenols were included.
Methods: The substrate turnover by PceA was monitored via liquid or gas chromatography. Crystals of the enzyme were soaked with different halogenated phenols and subjected to X-ray structural analysis to unravel their positioning at the active site of PceA. Using EPR spectroscopy an interaction between the cobalt of the cobamide cofactor and the substrate was investigated.
Results: Next to alkenes also brominated phenols and with significant lower rates also chlorinated phenols were dehalogenated by PceA. 2,4,6-Tribromophenol (2,4,6-TBP) was completely dehalogenated to phenol. The arrangement of amino acid side chains at the PceA's active site allowed only a single mode of 2,4,6-TBP-binding. The hydroxyl group of 2,4,6-TBP and its reaction intermediates was found to be located close to the cobalt. An interaction of the halogen substituents with the cobalt was neither detected by structural analysis nor by EPR spectroscopy. This was also observed for 2,4,6-trichlorophenol (2,4,6-TCP), which was not converted by the enzyme.
Conclusion: A direct interaction of the cobalt of the cobamide cofactor of PceA with the halogen substituents of brominated phenols appears to be unlikely, which points towards a different catalytic mechanism in comparison to NpRdhA. Against the background of 2,4,6-TBP conversion but no conversion of 2,4,6-TCP, the role of the halogen substituent for the catalysis will be discussed.

[1] Bommer *et al.* (2014) Science 346: 455-458
[2] Payne *et al.* (2015) Nature 517: 513-516

BDP21
Biodegradation of pesticides in soils
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Objectives of the Study: To study the turnover mass balance (CO_2 evolution, extractable contaminant residues and non-extractable residues) of ^{13}C-labeled 2,4-D and ^{13}C, ^{15}N-labeled Glyphosate in soils under different environmental conditions.
To investigate the incorporation of ^{13}C-label into microbial fatty acids, and $^{13}C^{15}N$-labels into amino acids from ^{13}C-labeled 2,4-D and $^{13}C^{15}N$-labeled Glyphosate in soils.
To quantify the extent of "biogenic residues" formation during the degradation of two pesticides under different environmental conditions.
To define how the environmental conditions (e.g. temperature, soil organic matter and soil pH) the biodegradation of pesticides and the extent of "biogenic residues" formation.
Material and Methods: Soil samples were collected from the 0 to 5 cm soil layer in the agricultural field located in Bad Lauchstädt, Germany. The soil organic matter (OM) of the soil and the pH were modified reaching 3 %OM and 4 %OM and pH 5.5 and 6.0, accordingly. Thereafter, soil was spiked with a labeled pesticide (^{15}N-$^{13}C_3$-Glyphosate or $^{13}C_6$-2,4-D) and incubated according to the OECD 307 and at different temperatures (10 °C, 20 °C and 30 °C). The $^{13}CO_2$ evolution was quantified by Inorganic Carbon Analyzer and the isotopic composition was measured by GC-IRMS. After the incubation, the residual parental pesticide + its primary metabolites and microbial biomass components (amino acids) were extracted and quantified and identified using UHPLC (Glyphosate) or GC-MS (2,4-D, amino acids) and the isotopic composition was measured by GC-IRMS.
Results: After 39 days of incubation, mineralization of $^{13}C_3$-glyphosate in soil containing 3 %OM, 4 %OM and pH 6 at 10 °C, 20 °C and 30 °C was higher than 20 %, 45 % and 50 %, respectively. In the same experiment with soil at pH 6 and pH 5.5 at 10 °C, 20 °C and 30 °C the mineralization of glyphosate was lower and reached about 16 %, 35 % and 40 % of $^{13}C_3$-glyphosate equivalents, respectively. Mineralization of $^{13}C_6$-2,4-D in soil containing 3 %OM after 32 days of incubation constituted more than 18 %, 50 % and 70 % of $^{13}C_6$-2,4-D equivalents at 10 °C, 20 °C and 30 °C, respectively. In the soil at lower pH (5.5 and 6), mineralization of this herbicide was lower and reached accordingly 2 %, 20 % and 20 % at 10 °C, 20 °C and 30 °C.
Conclusion: The first results showed a positive influence of the environmental conditions like temperature and OM content of soil on the extent of glyphosate and 2,4-D mineralization in soil.

BDP22
Evaluation of microbial degradation of organic matter in *Kupferschiefer*
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The so called *Kupferschiefer*, originating from the late Zechstein Sea, is the most important source for copper and silver in middle Europe.
Even though the only industrial exploitation is performed pyrometallurgically, biohydrometallurgy possesses high potential for environmentally-friendly processing. Due to high carbonate loads in the host rock, flotation is strictly necessary prior to microbial leaching. Unfortunately, the organic matter leads to a decreasing flotation efficiency. Consequently a possible degradation of the organic matter has to be done beforehand to enhance the flotation efficiency and thus the leaching. Furthermore the OM degradation might lead to a release of valuable elements like vanadium bound to the organic matrix.
Due to the complex composition of the OM, degradation is hard to follow, let alone quantify. However, with the OxiTop system we found not only a relatively easy approach to follow the degradation, but also to quantify the amount of degraded organic matter in terms of n-tetradecane equivalents.
Using *Rhodococcus jostii* RHA1 and up to 75 %wt copper shale (Mansfeld area, Germany) as sole carbon source, we were able to follow the degradation in regard to consumed oxygen. A simple calculation of the degraded OM by using just the oxygen depletion is, in contrast to iron-oxidisers, not possible. Hence we have chosen to use differing amounts of

n-tetradecane as an exemplary hydrocarbon, which is also naturally present in the shale.
Checking whether degradation occurred or not is easily achievable by using the OxiTop system. This allows to investigate the influence of an increasing pulp density as well as the degradation rate. Furthermore the automated measurement provides a tool for comparison of several strains with respect to their overall degradation capacity as well as their degradation rate. Additionally to the highest possible pulp density, both parameters will play a key role in future industrial applications.

BDP23
Characterization of the cellulolytic bacterial community in mesophilic and thermophilic biogas reactors
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Anaerobic biomass digestion in biogas plants is carried out by a microbial consortium that still lacks deeper insights. Its key players and cellulolytic activities are still insufficiently characterized. In culture-based and -independent approaches we identified cellulolytic species and assessed their role and presence in the anaerobic biomass fermentation progress for biogas production. Therefore *in vivo* enrichment experiments were carried in biogas fermenters in nylon bags filled with crystalline cellulose (*in sacco*). Initially, the diversity of adherent microorganisms was characterized by 16s rRNA amplicon sequencing. To further characterize members of the enriched community we isolated cellulolytic species in selective culture media. Finally the identified bacteria were monitored by (RT)-qPCR in two mesophilic and thermophilic, constantly stirred tank reactors (CSTR) under ideal and stressed process conditions.
A 16S rRNA-gene analysis of the enriched microbial community revealed the presence of exclusively uncultured species distantly related to formerly isolated cellulolytic species. In the probed thermophilic digesters two major taxonomic groups, related to *Halocella cellulosilytica* (90-95 % 16S rRNA sequence homology) and *Clostridium thermocellum* (91-96 %), were strongly represented among other species out of the phylum *Firmicutes*. The enrichment in the mesophilic fermenter showed a more diverse taxonomic distribution of different *Firmicutes* species with 16S rRNA sequence homology to known cellulolytic species, e.g. *Acetivibrio cellulolyticus* (91 %), *Clostridium clariflavum* (90 %), *Clostridium straminisolvens* (98 %) and *Halocella cellulosilytica* (95 %).
Selective anaerobic cultivation under thermophilic conditions first showed an enrichment of the taxonomic unit related to *Clostridium thermocellum* but none of the species represented in the 16S rRNA library was finally isolated under the chosen conditions.
With our new *in sacco* approach we identified potential key players among the cellulolytic bacteria that are promising target candidates for the development of (RT)-qPCR-based marker systems to monitor the cellulolytic community in anaerobic digestion processes. Several taxonomic groups were strongly represented in the *in vivo* enrichments but were outgrown by other species when cultivated *ex vivo* for isolation.

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BDP24
Powerful interactions – Effect of electric fields on bacterial deposition and transport
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Question: There has been increasing interest in employing electrokinetic phenomena to homogenize immobilized microorganisms and diffusion-retarded chemicals and to promote the biotransformation of chemicals, respectively. Here we tested the hypothesis whether the shear force induced by electrokinetic phenomenon (electroosmotic flow (EOF), electrophoresis) over a collector allows overcoming the attractive interaction energy of initial cell adhesion and promote bacterial transport. Different to a parabolic profile of pressure driven hydraulic flows, the plug-shaped velocity profile of EOF is quasi planar and starts above the electrical double layer at a distance of a few nanometers and, hence, at a distance to the collector surface, where initial bacterial adhesion takes place.
Methods: By varying the electrokinetic conditions in laboratory percolation columns we quantified the deposition and transport of bacteria of different physico-chemical surface properties (surface charge, contacct angle, physiology). Transport and deposition data were analsed using the clean bed filtration theory and discussed in the frame of the extended Derjaguin, Landau, Verwey, and Overbeek (XDLVO) theory of colloid stability[1)].
Results: We found that the presence of DC may reduce bacterial surface coverage and initial adhesion efficiency of the cells in a range from 0 to 90 % depending on bacterial and electric field properties. Based on the data we present a model to predict electrokinetic-induced prevention of bacterial adhesion as based on XDLVO theory and the electrokinetic-induced shear forces acting on a bacterium.
Conclusion: We propose that DC fields may be used to electrokinetically regulate the interaction of bacteria with collector surfaces and, hence, also to possibly bridge the distance between bacteria and contaminants in biotechnological applications.

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BDP25
Masking of enantiomer and carbon isotope fractionation due to membrane transport in *Sphingobium indicum* strain B90A for α-hexachlorocyclohexane
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In recent years, compound specific isotope analysis (CSIA) has become a routine approach for monitoring and quantification of *in situ* biodegradation of contaminants at polluted sites. This application is based on the stable isotope fractionation upon bond cleavage during the first irreversible reaction which leads to an enrichment of heavier isotopologues in the residual substrate fraction during biodegradation. Similarly, as biological pathways can be enantioselective, for chiral compounds biodegradation can lead to enrichment of one enantiomer over the other. Therefore, enantiomer fractionation (EF) can also be used as a tool for analyzing biodegradation of enantiomeric chemicals and it can be combined with CSIA. As mass transfer steps prior to the catalytic bond cleavage may mask the real magnitude of isotope and enantiomer effects of the reaction, we aimed to compare the isotope enrichment and enantiomer enrichment of pure enzyme, crude extract and cells.
In order to model different mass transfer steps, *Sphingobium indicum* strain B90A was used for the degradation experiments, capable of the degradation of α-hexachlorocyclohexane (HCH). In this study, three sets of experiments were conducted. Intact cells (*Sphingobium indicum* strain B90A which contain both enzymes, LinA1 and LinA2), crude extract (cells of B90A disrupted via French press) and the pure enzymes LinA1/LinA2 were used for α-HCH degradation experiments, respectively.
During dehalogenation, both carbon isotope fractionation and EF were observed. Rayleigh equation was applied for describing isotope fractionation and EF. Comparing the EF factor, the absolute values were decreasing from protein over crude extract to intact cells. This was also in agreement with the enrichment factors of these three sets of experiments. Therefore we can conclude that the mass transfer steps affected the EF and isotope fractionation.

BDP26
Accumulation of N-acetylmuramic acid-6-phosphate in *murQ* mutants demonstrates peptidoglycan recycling in Gram-positive bacteria
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Bacteria degrade a substantial proportion of their peptidoglycan cell wall within one generation during vegetative growth. In Gram-negative bacteria, peptidoglycan turnover products mostly are trapped within the periplasm, wherefrom they are salvaged in a process called peptidoglycan recycling. Differently, turnover fragments in Gram-positive bacteria mostly are released into the growth medium. Whether they are recovered/recycled in these organisms is currently questioned. Here we show that *Bacillus subtilis*, *Staphylococcus aureus*, and *Streptomyces coelicolor*, differently growing and shaped Gram-positive model bacteria, recycle their own peptidoglycan wall during vegetative growth. They all possess orthologs of the *Escherichia coli N*-acetyl-muramic acid-6-

phosphate (MurNAc-6P) etherase MurQ, responsible for the reutilization of the cell wall sugar MurNAc. We showed accumulation of MurNAc-6-P in markerless chromosomal murQ deletion mutants but not the parental strains using mass spectrometry. This accumulation must be due to the recovery of the own peptidoglycan, i.e. recycling proceeds, and could be prevented by expressing MurQ in trans. MurNAc-6-P accumulation occurs during exponential growth, reaches a maximum in the late exponential/early stationary phase and remains at high level during stationary phase. Our findings indicate that in Gram-positive bacteria peptidoglycan recycling already occurs during vegetative growth, but is of major relevance during nutrient limitation, when growth is stalled and recycling may be used to overcome a shortage of peptidoglycan precursors. Accordingly, growth rates in exponential phase were not affected upon impairment of the MurNAc recycling but were reduced in stationary phase when growing in nutrient limited medium.

BDP27

Veterinary antibiotics: How to enhance their degradation in soils?

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Question: Sulfamethazine (SMZ) is a veterinary antibiotic, intensively used in Germany. By animal excretion it is transported to the fields via manure application. Once introduced into the soils, SMZ can pose several risks: The antibiotic can be taken up via the crops, the antibiotic can leach and contaminate ground and drinking water, the number of antibiotic-resistant bacteria may increase. To avoid or minimize these adverse effects on human health, it is a challenge to develop methods for the enhanced elimination of SMZ from the environment. Therefore the question is:
How to enhance the biodegradation of veterinary antibiotics in agricultural soils?
Methods: Soil incubation experiments were conducted. A microbial community that possesses the ability to mineralize SMZ was extracted from a soil, established on a carrier material, inoculated into new soil, and mineralization experiments were conducted with ^{14}C-SMZ, by measuring the evolved $^{14}CO_2$.
30 g of soil (dry weight equivalent) have been inoculated with the SMZ degrading microbial community on a carrier material in triplicates. Before inoculation to the soil, the microbial community was growing on the carrier material in liquid cultures, where ^{14}C-SMZ was the only C and N source. In one treatment the microbial community was inoculated to the soil 15 days after the start of the liquid culture. In the second treatment the microbial community on the carrier material obtained another ^{14}C-SMZ application in the liquid cultures after the first week and was thereby inoculated to the soil in a fitter stadium and probably with a higher cell number.
The soil incubators contained a cap, filled with NaOH, to trap the $^{14}CO_2$. To measure the mineralisation, the NaOH in this cap was exchanged every second day and measured in a liquid scintillation counter. The radioactivity found was then calculated to the percentage of the applied amount of radioactivity per incubator. In this way the mineralisation rate per day and the cumulative mineralisation were calculated.
To see whether this approach is still efficient after some time, a second application on the same soil incubators was conducted after 112 days. The same amount of radioactivity was added again, to see whether it can still be degraded as good as the first time.
Results: The results reveal a very efficient SMZ mineralization. 46 days after the first soil inoculation 44-57 % of the applied SMZ has been mineralized, depending on the treatment.
After the second application, the microbial community was still very active and able to degrade the ^{14}C-SMZ also very efficiently (36-38 % after 46 days).
Conclusion: These results indicate that the method of inoculation of SMZ degrading microbes on carrier material seems to be a highly promising approach for remediating agricultural soils. The microbial community on the carrier material was very stable after being inoculated to new soil and didn't lose the SMZ mineralisation ability for a long time.

BDP28

Cyclohexane biodegradation using *Acidovorax* sp. CHX100: a novel strain for biocatalytic synthesis of valuable products?

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The primary aim of biodegradation is either the complete mineralization of hazardous organic compounds or their conversion to less toxic ones. Cyclohexane is a volatile recalcitrant chemical which is widespread in air, soil and water through refinery processes and petroleum spills and thereby causing several hazardous issues in the environment [1]. In this context, we investigated the biodegradation pathway of cyclohexane in *Acidovorax* sp. CHX100 and analyzed the novel genes responsible for the respective enzymes oxidizing cyclohexane to cyclohexanol.
Question: Evaluate and analyze the most important enzymes of the cyclohexane degradation pathway and evaluate their biocatalytic potential.
Methods: A novel strain able to use cyclohexane as sole source of carbon and energy was isolated using a biotrickling filter as enrichment system. The complete pathway for cyclohexane degradation was analyzed using transposon mutagenesis, multiple alignment sequences and genome analysis. The functionality of some enzymes and biotransformation capabilities were verified in a heterogeneous host.
Results: The novel strain *Acidovorax* sp. CHX100 showed a remarkable growth rate using cyclohexane as a sole carbon and energy source (generation time of 3.5 h) [2]. The strain CHX100 degraded 99.5 % of 7.5 mM cyclohexane within a period of 24 hours. Additionally, *Acidovorax sp.* CHX100 is able to use other short cycloalkanes (C5 - C8) as a sole carbon source. Metabolites analysis and alignment sequence suggested that *Acidovorax* sp. CHX100 has the enzymes for the transformation of the main cyclohexane metabolites (cyclohexanol and cyclohexanone) in a gene cluster [2]. Genetic analysis showed that *Acidovorax* sp. CHX100 possesses a novel cyclohexane monooxygenase (CYP_{450}chx) belonging to CYP_{450} class I [3]. Gene expression and enzyme activity of CYP_{450}chx were studied in the recombinant host *Pseudomonas taiwanensis* VLB120, analyzing the oxidation of cyclohexane to cyclohexanol [4]. Biotransformation of cycloalkanes (C5 - C8) to their respective cyclic alcohols was analyzed and studied by enzymatic activity [3].
Conclusion: The results demonstrate the potential applicability of *Acidovorax* sp. CHX100 and its native genes encoding enzymes for converting cycloalkanes into value added compounds.

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BEFP01

Comparative diversity of myxomycetes in two lowland forests in Mindoro Island, Philippines

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Mindoro Island is one of the Philippines' top tourist destinations. In 1973, UNESCO declared the Puerto Galera peninsula in Mindoro as part of the Man and Biosphere programme as a result of an increase in human presence near its forest habitats. However, until today its forests remain poorly documented for its microbial flora, particularly the plasmodial slime molds or myxomycetes. Thus, this research study assesses the diversity, distribution and ecology of myxomycetes in two lowland forests in Mindoro Island - in Mt. Malasimbo (MM), Puerto Galera and in Mt. Siburan (MS), Sablayan. A total of 1,260 moist chambers (540 for MM, 720 for MS) were set-up from the collected substrata, i.e. aerial (AL) and ground (GL) leaf litter, woody vines (WV), and twigs (TW). In the study, a higher MC productivity (80 %) was observed from samples collected in Mt. Malasimbo than in Mt. Siburan. Among the collected substrata, AL had the highest MC productivity. Based on different diversity indices, higher species diversity was noted for Mt. Siburan (H_S=1.27; H_G=6.94; E=0.46) than in Mt. Malasimbo (H_S=1.05; H_G=4.56; E=0.41). However,

when the taxonomic diversity was computed between the two forest sites, a lower SG ratio, hence, a higher taxonomic diversity was observed for Mt. Malasimbo (S/G=2.00) than Mt. Siburan (S/G=2.65). In summary, a total of 52 species of myxomycetes belonging to 6 taxonomic orders and 17 genera were recorded from this study. Although both study sites have almost the same type of vegetation, a relatively high number of species (45) was recorded in Mt. Siburan than in Mt. Malasimbo (28). Perhaps the anthropogenic activities in Puerto Galera may have influence the species composition in Mt. Malasimbo. Our study is the first to extensively document myxomycetes in Mt. Siburan in Sablayan Watershed Forest Reserve and the first to compare the myxomycete assemblages in two lowland forests in Mindoro Island.

BEFP02

Gut-derived *Coriobacteriaceae* increase white adipose tissue deposition in mice

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Introduction: *Coriobacteriaceae* are dominant members of the human gut microbiome and can metabolize cholesterol-derived metabolites such as bile acids. However, consequences of these bacterial functions for the host are unknown. The aim of the present study was to characterize effects of *Coriobacteriaceae* on lipid metabolism *in vivo*.
Methods: Male germ-free (GF) C57BL/6N mice were associated at week 5 of age with a consortium of four *Coriobacteriaceae* strains (Corio). At week 10, mice were randomly divided (n=12) into 3 groups fed different diets for 16 weeks (control; high-fat; control supplemented with 0.1 % primary bile acids). GF and specific-pathogen free (SPF) mice were used as controls. Colonization and GF status were analyzed by cultivation and 16S rRNA probe-based qPCR. Quantification of bile acids was performed using a LC-ESI-QqQ system.
Results: High-fat feeding resulted in significantly increased body weight (in g: GF, 42±3; Corio, 44±4; SPF, 49±5; $p < 0.001$), and mice in all three colonization groups developed steatosis. All mice fed the bile acid diet stayed lean, but those colonized by *Coriobacteriaceae* were characterized by a significant increase in white adipose tissue (WAT) depots (in mg/g body weight: GF, 37±15; Corio, 65±15, SPF, 26±16, $p<0.001$). WAT gene expression of leptin was significantly higher and expression of the bile acids receptor TGR5 and the transcription factor FXR was lower in Corio vs. SPF mice fed the bile acid diet. Measurement of bile acids in the caecum and WAT revealed increased levels of taurine-conjugates in GF and Corio mice in contrast to higher levels of free primary, secondary and oxo-bile acids in SPF mice.
Conclusion: Interactions between *Coriobacteriaceae* and bile acids influence fat tissue deposition in mice primarily due to hyperplasia and not hypertrophy.
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BEFP03

Application of different *Pseudomonas aeruginosa* strains in bioelectrochemical systems.

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Among other virulence factors *Pseudomonas aeruginosa*, produces a range of redox active mediators called phenazines. These compounds have been shown to enable *P. aeruginosa* to interact with the anode of a bioelectrochemical system (BES), which leads to the generation of electrical current [1].
Question: Here we want to characterize the electroactive physiology of distinct *P. aeruginosa* strains with regard to the substrate and further investigate changes in the transcriptome, responsible for the observed behavior.
Methods: Using a three electrode setup, the electrochemical performance of *P. aeruginosa* PA14, PAO1 and a BES isolate (KRP1) were investigated. Thereby chronoamperometry technique was used to measure current generation and cyclic voltammetry to assess the redox activity of the cultures. Cultivation was performed under micro aerobic conditions to allow interaction with the anode as terminal electron acceptor. The phenazines quantification was done through HPLC analysis. The physiological evaluation of the planktonic and biofilm fraction of the BES active cultures was performed by transcriptome analysis via RNA sequencing.
Result: The three strains of *P. aeruginosa* exhibit a distinct behavior in the BES setup. On average, the BES isolate KRP1 generated higher current densities, which were mediated by increased amounts of phenazine-1-carboxylate (PCA).
PA14, on the other hand, generated increased current densities when provided with the fermentation product 2,3-butanediol. Hereby the PCA derivate pyocyanin (PYO) was produced in prevalent amounts.
Interestingly the widely studied strain PAO1 generated low current densities. While having the same genetic ability for phenazine production (two almost identical *phz*-operons for PCA generation) almost no redox mediators were produced. Hence, only low interactions with the electrode were possible.
Due to the lack of full aerobic conditions, a thick biofilm was formed at the headspace-media interface, leading to two metabolic subpopulations within the reactor. To elucidate their contribution to mediator production and to back up the link to current generation, RNA profiles were investigated.
Conclusion: Due to its phenazine production *P. aeruginosa* is able to interact with the anode in a BES setup and thereby produces different current densities, depending on the given substrate. Even though the different strains have the same genetic capacities for phenazine production, big differences in their levels and spectra occur. Due to the complex quorum sensing network of *P. aeruginosa* these physiological changes are not limited to phenazine production but also have a substantial effect on biofilm formation along with other alterations. This study provides insight into the applicability of the different *P. aeruginosa* strains in redox mediated current production.

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BEFP04

Ecological functions of microbial communities in the enigmatic desert Fairy Circles

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Questions: Fairy Circles (FCs) are vegetation-free circular areas with diameters up to 40 m that occur in Subsahara Africa in the arid grassland belt extending from southern Angola through western Namibia, to northern South Africa. The origin and maintenance of FCs has recently been linked to the activity of the sand termite *Psammotermes allocerus* that removes grass plants by localized herbivory [1]. Alternative hypotheses exist [2]. Whereas soil microbial communities differ between FCs and the surroundings, their role for the transformations in FCs is entirely unknown [3]. In the present study, a series of FCs were studied with respect to (i) the composition of microbial community structure, (ii) the spatial and temporal dynamic of microbial communities, and (iii) the role of particular taxa of microorganisms in the origin and development of FCs.
Methods: High throughput sequencing of bacterial 16S rRNA and rRNA genes and of fungal internal transcribed spacer sequences were conducted along transects across different FCs. Sandy soils as well as the tapetum (the organic layer covering the tunnels) of termite nests were analyzed. Bacteria and fungi from the tapetum and surrounding grasses were also recovered by high throughput cultivation techniques.
Results: The microbial communities present in the sandy soils of the circle center were highly similar for different FCs whereas the community composition changed markedly across single FCs. Even more pronounced was the difference between the microbial communities of the tapetum that contained mainly lignocellulose and hydrocarbons-degrading bacteria (*Planomicrobium-Planococcus*, *Flavobacterium*) and those found in the unconsolidated sandy soils (*Bacillus*, *Geodermatophilus*).
Conclusions: Particular bacterial groups were associated with the different environments, indicating their participation in the origin and development of FCs. One of the prominent microorganisms that might be involved is the fungal plant pathogen *Culvularia* sp. which could be isolated from dead grasses.

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BEFP05

Phosphate solubilization and multiple plant growth promoting properties of rhizobacteria isolated from chickpea *(Ciceraeritinum L.)* producing areas of Ethiopia

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Chickpea is one of the major legume crops widely grown in Ethiopia. The low availability of phosphorus in soil is among the stresses that constrain the production of this crop in the country. However, there are bacteria which capable of solubilizing insoluble form of phosphorus in soil and make it available to the plant. Thus, this study was aimed at isolation and characterization of phosphate solubilizing bacteria from the chickpea rhizosphere. Fifty phosphate solubilizing bacterial strains were isolated from the soil samples, characterized biochemically and identified by 16S rDNA sequences analysis. The results indicated the presence of genera *Acinetobacter, Bacillus*, *Brevibacillus*, *Burkholderia*, *Empedobacter, Enterobacter*, *Pseudomonas*, *Ralstonia,Sphingomonas and Stenotrophomonas*. Phosphate solubilizing efficiencies of the strains were analyzed using different insoluble phosphorus sources and the results showed that most isolates released a substantial amount of soluble phosphate from tricalcium phosphate, rock phosphate and bone meal. Screening for multiple plant growth promoting attributes results showed that 44 % and 18 % of them were capable of producing indole acetic acid and inhibiting the growth of *Fusarium oxysporum*under *in vitro* conditions. A direct impact of several strains (*Bacillus flexus* (PSBC17), *Pseudomonas fluorescence* (PSBC33), *Enterobacter* sp. (PSBC35), *Enterobacter sakazaki* (PSBC79) and *Enterobacter* sp. (PSBC81)) on the growth of chickpea in pot culture has been demonstrated by the increase in the number of nodules, shoot dry matter, nitrogen and phosphorus concentration of shoot. Based on the results, we conclude that chickpea rhizosphere harbor phosphate solubilizing bacteria which are diverse in taxonomy and phosphate solubilizing efficiencies. Thus, consecutive studies should focus on field studies on those strains due to their potentially high importance for the nutrition in this area and in this context for the improvement of the sustainability of land use in the country.

BEFP06

Deep sequencing of V3 16S rRNA amplicons reveals the diversity of gut-associated Planctomycetes

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Planctomycetes are a phylum of bacteria inhabiting a variety of different environments. First isolated from fresh water lakes, next generation sequencing revealed the ubiquitous presence of Planctomycetes in various habitats including marine and terrestrial environments. In addition, various microbiome projects have shown that even in the gut of different animals planctomycetal sequences can be found. In aquatic habitats, Planctomycetes are believed to play an important role in global carbon- and nitrogen-cycles e.g. the degradation of organic debris from higher organisms such as marine snow. An analogous function could be assumed for gut-associated Planctomycetes, as different phyla have been found to encode chitinases in their genomes. As wild boars are omnivores, their diet contains various sources of chitin e.g. fungi and insects. Since Planctomycetes are able to degrade a variety of other complex polysaccharides, an involvement in the degradation of complex biopolymers in animal guts was proposed. Playing crucial roles in the gut flora of animals, it is also likely that Planctomycetes are associated with disease, should a shift in the gut microbiome e.g. triggered by antibiotic treatment occur. Although all currently described Planctomycetes are classified as non-pathogenic, recent case reports point towards pathogenic members of this phylum as planctomycetal DNA profiles were found in immunocompromised patients, suffering from pneumonia previous to antibiotic treatment. Here we present deep sequencing data of the gut microbiome of wild boar (*Sus scrofa*) and roe deer (*Capreolus capreolus*), giving an insight into gut-associated planctomycetal genera. The phylogenetic reconstruction based on near full-length 16S rRNA sequences reveals a distinct group of gut-associated Planctomycetes. With this approach we go one step further to reveal the relevance of Planctomycetes in the animal gut and their potential as crucial degraders in mammal-associated bacterial communities.

BEFP07

Taxonomic characterization of *Corynebacterium* isolates from bulk tank raw cow milk of different German dairy farms

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Pathogenic and non-pathogenic species of the genus *Corynebacterium* are found frequently in raw milk and raw milk products. Among the raw milk associated *Corynebacterium* species are the human and animal pathogenic *Corynebacterium amycolatum*, *Corynebacterium bovis*, *Corynebacterium ulcerans* and *Corynebacterium minutissimum,* and the non-pathogenic species *Corynebacterium casei* that is well known as a starter culture for the production of smear ripened cheese. Routine identification to species level is difficult for *Corynebacterium* species because of high 16S rRNA similarity values, limited phenotypic markers and the presence of yet undescribed species in this habitat. The incompleteness of data about the genus *Corynebacterium* in raw milk is partly due to the low abundance and the lack of selective media for this genus. In this study, we used a tellurite containing selective medium to isolate *Corynebacterium* species from raw milk samples from seven dairy farms in Germany during a seven-month period, in order to complete the information about the diversity of *Corynebacterium* species in raw milk. We detected *Corynebacterium* species in raw milk samples from three farms. In samples from four dairy farms, no Corynebacteria were detected. Some of the isolated strains could not be reliably identified based on their 16S rRNA gene sequences and needed further identification by sequencing of their partial *rpoB* gene sequences. *Corynebacterium xerosis* and *Corynebacterium variabile* were detected in the samples of three resp. two different dairy farms. *Corynebacterium callunae* and *Corynebacterium confusum* were detected in several samples of the same dairy farm, whereas *Corynebacterium lactis* and *Corynebacterium glutamicum* were isolated from just one sample of one farm. The isolated strains showed the typical chemotaxonomic features for the genus. The 16S rRNA gene sequence of one of 25 isolates indicated the presence of a yet undescribed species, which demonstrates the underestimated diversity of this genus in raw milk. With these data, we were able to identify species associated with raw milk as well as farm-specific species and we could complement yet missing chemotaxonomic data of some *Corynebacterium* species. Although growth below 20 °C is rarely detected within this genus, we were able to demonstrate growth at 10 °C for single *Corynebacterium* strains from raw milk.

BEFP08

Growth at low temperature increased biomass production of food-associated Bacteria

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For several psychrophilic species, an increased biomass yield was described at temperatures lower than the optimum growth temperature, which is defined by the highest growth rate (μ_{max}). This effect is deviant to the prevailing conception for mesophilic bacteria that will have the largest cell yield at the temperature with the maximum growth rate. In contrast, various studies of our group with isolates from chilled food showed clearly increased biomass formation at low temperatures as well. All of these isolates were characterized as mesophiles by their growth rates, which are significantly higher at 30°C than at 10°C. Five isolates from chilled food and refrigerators and closely related reference strains of the species *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus xylosus*, *Bacillus cereus*, *Pedobacter nutrimenti* and *Pedobacter panaciterrae* were tested for the effect of growth temperature (30 °C and 10 °C) on the following parameters in complex and defined media: optical density, viable cell count, total cell count, cell dry weight, whole cell protein content and cell morphology, in order to determine at which temperature maximum biomass formation is obtained. Growth was monitored via OD_{625nm} and culture parameters were measured at the early stationary phase and then used for statistical calculation and analysis of the data. In addition defined media were used to determine glucose and ammonium consumption related to maximum cell yield. Except for the protein content, temperature had a significant (LSD, $P < 0.05$) effect on all growth parameters for each strain. The results showed a significant difference between the isolates and their related reference strains. Isolates achieved

an increase in biomass production up to 110 % at low temperatures. In contrast, some reference strains showed only a maximum increase of about 25 % whereas some reference strains showed no increase or a decrease of approximately 25 %. As expected, growth rates for all strains were higher at 30 °C compared to growth at 10°C, while biomass production for isolates was higher at 10°C than at 30°C. In contrast, the reference strains showed similar growth yield at both temperatures. The quantification of the residual content of carbohydrates and ammonium in defined media at the stationary phase, demonstrate an enhanced conversion rate of nutrients into biomass at low temperature and therefore a more efficient use of nutritional resources also for mesophilic organisms.

BEFP09

Acidobacterial key players of subdivision 4 and 6 liberate limited nutrients in subtropical savannah soils of Namibia, Angola and Botswana

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Questions: *Acidobacteria* are abundant in different habitats like hot springs, wastewater, and polar deserts. Soils represent a typical habitat where *Acidobacteria* constitute on average 20 % and in some instances even 70 % of the bacterial community. So far, the environmental determinants of *Acidobacteria* community composition and the role of *Acidobacteria* in the biogeochemical cycles are little understood. Especially in semiarid areas, which cover 40 % of the Earth´s surface the implications of the high abundance of *Acidobacteria* is unclear.
Methods: V3-high-throughput-sequencing of 138 African different savannah soils was performed. The soils differed in physicochemical parameters and land use type. Diversity analyses were combined with the analysis of environmental parameters and the physiological characterization of novel isolates from the major *Acidobacteria* subdivisions.
Results: Up to 14 % of the active microbial community of the examined savannah soils were *Acidobacteria*. Most were affiliated with subdivisions (sd)1, 3, 4 and 6. The independent parameters included in the multivariate statistical analysis explained 80.1 % of acidobacterial abundance patterns and identified pH and C/N ratio as the main drivers of the acidobacterial community composition. While sd1 and sd3 *Acidobacteria* dominated soils with (slightly) acidic pH, sd4 and sd6 *Acidobacteria* prevailed in soils with a neutral or (slightly) basic pH. Additionally, the abundance patterns of sd1 and sd3 *Acidobacteria* positively correlated with the soil carbon content and the patterns of sd4 and sd6 *Acidobacteria* with the soil nitrogen and phosphorus content.
The physiological characterization of novel isolates from the subdivisions provided more detailed insights in their potential role in the nutrient cycles. Sd1 *Acidobacteria* are capable of the degradation of complex carbon compounds (cellulose, starch), while sd4 and sd6 *Acidobacteria* mostly secrete degradation enzymes of complex nitrogenous and phosphorus compounds.
Conclusions: Due to their high abundance in subtropical savannah soils and their broad degradation potential of complex organic compounds, *Acidobacteria* play an important role in the carbon, nitrogen and phosphorus cycling of nutrient limited subtropical savannah soils. The results presented within this study suggest that the ecological functions fulfilled by the *Acidobacteria* can be separated along the different acidobacterial subdivisions.

BEFP10

Abundance and community structure of groundwater microorganisms that pass through 0.2 µm pore size filters

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Analyses of microbial community structure in samples of aquatic environments are traditionally based on microbial biomass collected on 0.2 µm pore size filters. However, the abundance, diversity and taxonomic affiliation of microorganisms smaller than 0.2 µm that may escape standard molecular surveys, especially in oligotrophic environments such as the terrestrial subsurface, have rarely been addressed. We evaluated the abundance and community composition of bacteria and archaea <0.2 µm in groundwater samples from two superimposed limestone aquifers in the Hainich region (Thuringia, Germany), obtained from seven wells along a transect with sampling depth ranging from 12 to 88 m. Groundwater samples were filtered through 0.2 µm pore size filters and the flow-through was subsequently filtered through 0.1 µm pore size filters. Quantitative PCR targeting bacterial and archaeal 16S rRNA genes revealed that about 0.6 to 8.8 % of the total bacterial population and 0.5 - 7.9 % of the total archaeal population was found in the 0.1 µm filter fraction. Across sites, this percentage fraction did not appear to be linked to sampling depth nor oxygen availability. qPCR-based quantification was complemented with flow cytometry experiments, showing corresponding population sizes in non-filtered and 0.2 µm-filtered water, respectively, after staining with SYBR Green. Quantification of genes involved in ammonia oxidation (*amoA*) and CO_2-fixation via the Calvin-Benson-Bassham cycle (*cbbL, cbbM*) indicated that the genetic potential for nitrification and RubisCO-based CO_2-fixation was also represented in the microbial communities of the 0.1 µm filter fraction. DGGE-fingerprinting of 16S rRNA genes revealed distinct differences in the community composition between the 0.2 µm and 0.1 µm filter fractions at each site and pointed to more pronounced differences for archaeal than for bacterial communities. Our results have clearly shown that a substantial fraction of microorganisms in groundwater may be overlooked in standard analyses and that this fraction may harbour an unknown diversity of organisms involved in processes such as carbon and nitrogen cycling. Ongoing investigations using Illumina MiSeq amplicon sequencing of bacterial and archaeal 16S rRNA genes will provide more insight into the taxonomic affiliation of the community members of this overlooked fraction.

BEFP11

The bacterial diversity of the phycosphere

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Question: Many marine bacteria live in symbiotic association with eukaryotic microalgae. We investigated the composition of the bacterial communities accompanying non-axenic algal cultures in order to quantify the amount of epibionts and to reveal specific associations.
Methods: Partial bacterial 16S rRNA gene sequences from 23 non-axenic marine algal cultures, including *Apicomplexa* (n=3), *Dinophyta* (n=7), *Haptophyta* (n=7), *Cryptophyta* (n=3) and *Bacillariophyta* (Diatoms; n=3), were amplified with barcoded V3/V5-specific primers; amplicons were pyrosequenced on a Roche 454 Life Sciences Titanium platform and taxonomically classified [1]. Bacterial isolates were retrieved from the cultured algae, grown on Marine Agar 2216 (Difco) and classified on basis of their nearly complete 16S rRNA gene sequences.
Results: 48,093 partial bacterial 16S rRNA gene sequences (~ 500 bp) were obtained from the non-axenic algae cultures by barcoded pyrosequencing and analyzed. With a relative abundance of 61 %, *Alphaproteobacteria* clearly dominated the phycosphere of the algae, followed by *Cytophagia* (11 %), *Flavobacteriia* (10 %) and *Gammaproteobacteria* (8 %). Among *Alphaproteobacteria* members of the *Rhodobacteraceae* (52 %) and *Sphingomonadaceae* (18 %) revealed highest abundances. Approximately 450 bacterial strains were isolated from the cultured algae. Phylogenetic 16S rRNA gene sequence analyses assigned various isolates to putative novel taxa, i.e. a putative novel order related to *Rhizobiales* as well as putative novel genera and species within the *Rhodobacteraceae*, *Rhodospirillaceae*, *Flavobacteriaceae* and *Cytophagiaceae*. In case of the apicomplexan alga *Chromera velia* CCAP1602/2 - a free-living relative of the malaria parasite [2] - the nearly complete accompanying bacterial flora consisting of seven strains could be isolated. We determined the physiological characteristics of the epibionts and use their antibiograms to establish an axenic culture of this evolutionary key species [3].
Conclusions: The marine phycosphere of representative microalgae is dominated by *Alphaproteobacteria.* The isolation of the epibionts of the apicomplexan model organism *Chromera velia* offers the promising perspective to develop a test system and to study algal-bacterial interactions on the 'omics level.

[1] Wemheuer *et al.* (2015), Front Microbiol, doi: 10.3389/fmicb.2015.00805
[2] Moore *et al.* (2008), Nature, 451: 959-963.
[3] Petersen *et al.* (2014), Genome Biol Evol, 6:666-684.

BEFP12

Diversity of anaerobic fungi in horse digestive tract

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In the class Neocallimastigomycetes (Neocallimastigomycota) seven genera of anaerobic fungi are known, that could be cultivated. These genera are the monocentric *Neocallimastix*, *Piromyces, Buwchfawromyces*, the polycentric *Orpinomyces*, *Anaeromyces*, and the bulbous *Caecomyces*, *Cyllamyces.* Yet, those genera represent only a minor part of gut fungi as indicated by a large number of uncultured species in public databases. A recent study on available ITS1 sequences revealed 37 clusters with 19 clusters not related to the known genera, presumably representing new groups of gut fungi [1]. Some of these new groups seem to be host specific like Black rhino cluster, or clusters AL1 and AL3 found in fecal samples of horses, zebras and donkeys. Isolates from Equidae cluster apart from other hindgut animals share a very low percentage of OTUs with non-Equidae samples. Moreover, horses, zebras, and donkeys share a similar fungal community composed mostly of the new groups AL1 and AL3. The numbers of sequences belonging to these new groups make up to 99 % [2]. These results indicate that the digestive tract of horses is occupied by completely unknown anaerobic fungi, which differ substantially from known rumen fungi. Cultivable anaerobic fungi from the equine were commonly isolated from feces and belonged to the genera Piromyces and Caecomyces. The present study is focused on samples obtained directly from different anatomical segments (caecum, right ventral colon, left ventral colon, left dorsal colon, and right dorsal colon). Here we report the results of a molecular assessment of gut fungi in different parts of a healthy horse digestive tract to elucidate the effect of anatomical region on *Neocallimastigaceae* intestinal diversity. The luminal content of five segments of the equine gastrointestinal tract were compared with a rectal sample. Changes in the anaerobic fungal community composition along the equine hindgut were monitored using ITS1 rDNA amplicon clone libraries, revealing differences in the diversification of anaerobic fungi along the horse hindgut.

[1] Kittelmann S *et al.* 2012. Plos ONE 7 e36866
[2] Liggenstoffer AS *et al.* 2010. ISME Journal 4, 1225-1235.

BEFP13

Potential impact of salinity changes on bacterial isolates and their phages from the deep biosphere of the Baltic Sea

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Subsurface sediments from the Baltic Sea have undergone alterations between limnic, brackish and marine conditions due to repeated glaciations. While the immense prokaryotic diversity within deep sediments has been assessed by molecular methods, adaptations to specific environmental conditions often only emerge from cultivation-based approaches. We have isolated new bacterial strains from deep-subsurface sediments of the Baltic Sea sampled during Expedition 347 of the International Ocean Discovery Program (IODP) with the aim to investigate the potential impact of salinity changes on bacterial and viral communities. Four new isolates are affiliated to the genus *Marinifilum* within the *Bacteriodetes* (93-94 % 16S rRNA gene sequence similarities), potentially representing two novel species. All strains grow by fermentation of sugars, glycerol, lactate and pyruvate, with one strain exhibiting a wider substrate spectrum. The strains also differ in their growth range and optima for NaCl, one being adapted to marine conditions, while the other strains favor lower salinities. Genome sequencing showed that one of the strains might have integrated a temperate phage (siphovirus) in its genome. Three of the four strains produce virus-like particles upon induction by mitomycin C. We hypothesize that salinity changes during the paleoenvironmental history of the Baltic Sea had major impacts on the structure of the microbial communities by i) influencing their energy metabolism, ii) resulting in induction of temperate phages and finally lysis of infected cells and iii) the release of labile organic compounds by the lysis of cells and thus stimulation of the remaining cells via the viral shunt.

BEFP14

Metagenome-based metabolic reconstruction reveals the ecophysiological function o*f Epsilonproteobacteria* in a hydrocarbon-contaminated sulfidic aquifer

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The population genome of an uncultured bacterium distantly related to the genus *Sulfurovum* (*Epsilonproteobacteria*) was reconstructed from a metagenome dataset obtained by whole-genome shotgun pyrosequencing. Genomic DNA was extracted from a sulfate-reducing, *m*-xylene-mineralizing enrichment culture isolated from groundwater of a benzene-contaminated sulfidic aquifer. The identical epsilonproteobacterial phylotype has previously been detected in toluene- or benzene-mineralizing, sulfate-reducing consortia enriched from the same site. Previous stable isotope probing experiments with $^{13}C_6$-labeled benzene suggested that this phylotype assimilates benzene-derived carbon in a syntrophic benzene-mineralizing consortium that uses sulfate as terminal electron acceptor [1]. However, the type of energy metabolism and the ecophysiological function of this epsilonproteobacterium within aromatic hydrocarbon-degrading consortia and in the sulfidic aquifer are poorly understood.
Annotation of the epsilonproteobacterial population genome suggests that the bacterium plays a key role in sulfur cycling as indicated by the presence of a *sqr* gene encoding a sulfide quinone oxidoreductase and *psr* genes encoding a polysulfide reductase. It may gain energy by using sulfide or hydrogen/formate as electron donors. Polysulfide, fumarate, as well as oxygen are potential electron acceptors. Auto- or mixotrophic carbon metabolism seems plausible since a complete reductive citric acid cycle as well as an acetate import system were detected. Thus the bacterium can thrive in pristine groundwater as well as in hydrocarbon-contaminated aquifers. In hydrocarbon-contaminated sulfidic habitats, the epsilonproteobacterium may generate energy by coupling the oxidation of hydrogen or formate and highly abundant sulfide with the reduction of fumarate and/or polysulfide, accompanied by efficient assimilation of acetate produced during fermentation or incomplete oxidation of hydrocarbons. The highly efficient assimilation of acetate was recently demonstrated by a pulsed $^{13}C_2$-acetate protein stable isotope probing experiment [2]. The capability of nitrogen fixation as indicated by the presence of *nif* genes may provide a selective advantage in nitrogen-depleted habitats. Based on this metabolic reconstruction, we propose acetate capture and sulfur cycling as key functions of *Epsilonproteobacteria* within the intermediary ecosystem metabolism of hydrocarbon-rich sulfidic sediments.

[1] Herrmann, S., *et al.* 2010. Functional characterization of an anaerobic benzene-degrading enrichment culture by DNA stable isotope probing. *Environ. Microbiol*. 12: 401 - 411
[2] Starke, R., *et al.* Submitted. Pulsed 13C2-acetate protein-SIP unveils Epsilonproteobacteria as dominant acetate utilizers in a sulfate-reducing microbial community mineralizing benzene.

BEFP15

Impact of increased surface temperature on the abundance and diversity of culturable bacteria living in the phyllosphere of grasslands

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Current changes of the global climate lead to an increase in surface temperature. This will effect plants and microbes colonizing the aerial part of plants (phyllosphere), separately, and in consequence the interaction among both. The aim of this study was to gain a first insight into the effects of increased surface temperature on the composition and diversity of phyllosphere bacteria in a six year long IR-lamp field-experiment. The experiment was performed at the permanent grassland of the „Environmental Monitoring and Climate Impact Research Station Linden“ near Gießen, Germany. Two abundant plant species, *Arrhenatherum elatius* and *Galium album,* were selected as a representative grass and herb, which showed clear differences with respect to surface colonization as shown by scanning electron microscopy. Leaves were collected from four control and four warmed plots, exposed to +2 °C surface temperature. A cultivation-dependent approach was applied to monitor changes in the abundance and diversity of “general” heterotrophic bacteria using ½ R2A agar and a more specialized group, abundant in the phyllosphere, methylotrophic bacteria, using a mineral medium with methanol as sole carbon source. SybrGreen I staining and subsequent epifluorescence microscopical counting of bacteria detached from leaf material showed as expected clear differences in the abundance of phyllosphere bacteria between plant species but not within a plant species if plants were grown under different surface temperatures. A similar result was obtained for the concentration of culturable heterotrophs and methylotrophs, except for *G. album,* which showed a decreased concentration of culturable methylotrophs, if plants grew under elevated temperature. Differences among most abundant culturable heterotrophs and methylotrophs were investigated by Denaturing Gradient Gelelectrophoresis (DGGE) of PCR amplified 16S rRNA gene fragments from cultured bacteria. For this, bacterial biomass was collected from agar plates of the highest positive dilutions that showed bacterial growth. Principal component analysis (PCA) of DGGE profiles of cultured heterotrophs showed clear differences among the plant species and for *G. album* also between plants grown at normal and increased surface temperature. In contrast, no clear differences were obtained for the DGGE profiles of cultured methylotrophs of the two plant species. In comparison to the control plants, the DGGE patterns of cultured methylotrophs were much more diverse among plants derived from different plots exposed to increased surface temperature. These results are a first indication, that increased temperature affects the structure and abundance of different metabolically active bacteria of the phyllosphere.

BEFP16

Impact of elevated atmospheric CO_2 on the diversity and abundance of culturable bacteria living in the phyllosphere of a permanent grassland

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Free air carbon dioxide enrichment (FACE) experiments in a permanent grassland showed, that elevated atmospheric carbon dioxide (eCO_2) increased plant biomass production (Kammann *et al.* 2005). The effect of eCO_2 on the abundance and the community structures of bacteria inhabiting the phyllosphere was not investigated so far. Phyllosphere colonizing bacteria can strongly affect plant growth, health and yield, among those pink pigmented facultative methylotrophic bacteria (PPFM) of the genus *Methylobacterium* are well known plant growth promoting bacteria and heterotrophic bacteria of the species *Sphingomonas* are known for their antagonistic activity. The aim of this study was analyzing the effects of eCO_2 on bacterial communities inhabiting the phyllosphere of two different plant species, *Arrhenatherum elatius* and *Galium album.* For this purpose leaves were collected at two time points (spring/summer) in 2014 and 2015 from the permanent grassland of the Gießen FACE system, which is continuously exposed to eCO_2 (+20 %) since 1998. Leaves were collected from plants grown in FACE rings exposed to eCO_2 compared to ambient (aCO_2) conditions.
A cultivation dependent approach was applied to compare the concentration and diversity of culturable bacteria grown on a lower concentrated complex media (two-fold diluted R2A-agar, here called “heterotrophs”) and bacteria grown on mineral medium with methanol as sole carbon source (methlyotrophs). The concentrations of both culturable heterotrophs and methylotrophs always showed significant differences between plant species. Depending on the time of sampling and the plant species, the exposure to eCO_2 significantly increased the concentration of culturable methylotrophs and/or an cultured heterotrophs in selected eCO_2 - aCO_2 FACE ring pairs (with specific soil moisture content). A total of 1763 of the most abundant cultured bacteria were isolated and identified by partial 16S rRNA gene sequencing. Bacteria of the genus *Sphingomonas* were most often identified among cultured bacteria grown on low concentrated complex media and *Methylobacterium* spp. highly abundant among methylotrophic bacteria. A high diversity of genetically different *Sphingomonas* spp. and *Methylobacterium* spp. were obtained among those phylotypes that occurred only on plants which were exposed to eCO_2. This indicates the adaptation of bacterial ecotypes inhabiting the phyllosphere exposed to atmospheric eCO_2 concentrations as predicted for the upcoming global climate change. The role of those adapted ecotypes on plant fitness needs to be investigated in future studies in more detail.

Kammann *et al.* 2005 Response of aboveground grassland biomass and soil moisture to moderate long-term CO2 enrichment. Basic and Applied Ecology 6: 351-365.

BEFP17

Description of *Backusella constricta* sp. nov. isolated from the Brazilian Atlantic Forest

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The Atlantic Forest can be found all along the Brazilian coastline and is known for its biodiversity and endemic species of several taxonomic groups. One group of fungi frequently found in the Atlantic Forest, known as Mucorales, is ecologically important as it covers saprobes and facultative parasites of both vertebrates and invertebrates. The Backusellaceae family was first described as referring to mucoralean saprobes that inhabit soil and form transitorily curved (when young, erect at maturity) sporangiophores arising from aerial hyphae or directly from the substratum. The family is monogeneric and currently contains 10 species. During a study on Mucorales in soil from an Atlantic Rainforest in Pernambuco, Brazil, *B. constricta* sp. nov. was isolated and is being described as new to science. Morphological description were performed in triplicate in malt exctract agar and in potato dextrose agar at 15, 20, 25, 30 and 35ºC for 15 days. Phylogenetic analyses of ITS (ITS1-5.8S-ITS4) and LSU, (D1 and D2 domains) regions of rDNA showed that *B. constricta* sp. nov. formed a distinct clade with other species of *Backusella*. This novel species exhibits morphological characteristics that differers from other species of the genus, such as the presence of conical (mostly) and cylindrical columellae possessing a central constriction, and the production of subglobose sporangiospores, 7.5-15 × 5.5-10 µm in size, serving as synapomorphic characters for the new species, which of the description in conjunction with a taxonomic key for species of this genus are provided.

BEFP18
Visiting the Black Queen – the metabolic dependencies in soil bacterial communities
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Microbial interactions are essential for maintaining genotypic biodiversity and shaping the bacterial community in soil habitats. However, little is known what drives the emergence of such interactions. The Black Queen hypothesis (BQH) provides a mechanistic explanation for the evolution of metabolic interactions: community members tend to lose biochemical functions that are costly, when the corresponding metabolites are sufficiently provided by other members in the community. As a consequence of this process, bacteria are expected to loose seemingly essential functions and become metabolically dependent on other organisms producing these metabolites for them.
To test this prediction, we quantified the number and identity of metabolic auxotrophies within soil bacterial communities. For this we applied a hierarchical sampling design in which we sampled nine soil columns from different habitats (i.e. coniferous forests, deciduous forests, and meadows) in Jena, Germany. Three soil particles (1 mg) from each column were screened for the presence of bacteria that were auxotrophic for one or more of twenty amino acids, five vitamins, and four nucleosides. Six thousands bacterial strains were isolated, purified, and subjected to the auxotrophy test, in which their ability to grow on minimal media without any metabolite supplementations was verified. Interestingly, 5-15 % of all culturable bacteria were auxotroph for one or several amino acids, vitamins, or nucleosides. Moreover, the analysis of the soil samples indicated the presence of free amino acids which would be available for the auxotrophs.
The obtained data is consistent with the predictions of the Black Queen hypothesis. Amino acids production is likely a leaky function and other community members benefit from loosing the ability to produce these amino acids autonomously and instead rely on a supply by others. Ultimately, the results of this work suggest that metabolic interactions within natural microbial communities might be more common than previously thought and imply that the environmentally-compensated gene loss is an important evolutionary force that significantly shapes microbial communities.

BEFP19
Drainage and its effects on paddy soil microbial communities – a metatranscriptomic approach
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Methane is the most abundant hydrocarbon on earth and a major greenhouse gas. Rice cultivation is one of the main sources of atmospheric methane. Therefore, mitigation strategies are needed to reduce its emission from rice paddies. One of these is drainage, which is a common practice in rice cultivation. It results in a loss of water and proliferation of oxygen into the rice field soil. The aim of our study is to understand how drainage affects the structure and functioning of paddy soil microbial communities.
Using paddy soil from the Italian Rice Research Institute in Vercelli, flooded microcosms amended with grinded rice straw were set up and incubated for 7 or 28 days, followed by 9 days of drainage. The addition of rice straw is a common practice to improve soil fertility. Metatranscriptome libraries were generated using total RNA extracted from flooded and drained soil at 2 cm depth. Libraries were sequenced using Illumina HiSeq 2500.
Our results showed that after 9 days of drainage, oxygen concentration reaches ~ 240 µmol/L across all soil depths (atmospheric concentration = 283 µmol/L). Comparative analysis of bacterial 16S rRNA reads (ribo-tags) revealed that drainage induced major changes in the community rRNA pool. *Firmicutes* decreased, while *Proteobacteria* increased in relative abundance. On family level, *Comamonadaceae, Nocardioidaceae,* and *Streptomycetaceae* increased in ribo-tag and mRNA abundance, while *Clostridiaceae*, *Bacillaceae, Lachnospiraceae,* and *Ruminococcaceae* decreased. Among methanogens, ribo-tag abundance of the *Methanosarcinaceae* remained fairly constant during drainage, yet their relative mRNA abundance strongly declined. Functional annotation of the mRNA showed that community-wide gene expression affiliated with certain SEED level 2 categories was highly affected by drainage. Transcripts involved in 'oxidative stress', 'sulfur metabolism', and 'phosphorus metabolism' were overrepresented in response to drainage, while those related to 'sporulation', 'flagellar motility', and 'fermentation' were underrepresented. In particular, transcripts encoding reactive oxygen species (ROS)-scavenging enzymes were highly enriched in the mRNA datasets from drained soil, while those of methanogen-specific *mcrA* were strongly underrepresented.
We conclude that oxygen availability is the main driving force for the observed community dynamics. Apparently, drainage selects for microbial populations that are able to combat oxidative stress through the expression of ROS-scavenging enzymes, thereby resulting in a complex community composed of aerobes and anaerobes.

BEFP20
Biodiversity and strategic perspectives for Glomeromycota conservation in four egyptian protected area
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Protected areas represent an appropriate place for the *in situ* conservation of AMF where they are under the care of national and international authorities, providing them with adapted situations together with established complex networks of interactions with different components within each specific ecosystem. The diversity of AMF communities in Egyptian Protectorates can decline due to natural and/or anthropogenic disturbance and many valuable ecotypes could become extinct before they are even discovered. In order to investigate the effect of edaphic factors and anthropogenic disturbance on diversity and conservation of AMF, spore density and mycorrhizal colonization of rhizsopheric soils and plant roots of the dominant plant species in El-Omayed, Saint Katherine, Wadi El-Alaqi and Wadi El-Assuiti Protectorates were analyzed. The results showed that the majority of wild plants could form strong symbiotic relationships with AM fungi. There existed obvious differences in AM fungal colonization status among the four protectorates (PP*in situ* conservation of wild plants reserves and ecological niches though awareness campaigns of local Bedouin tribes. Here, we shed the light on the current status of AMF diversity and the main present-day threats to AMF conservation and the current state of knowledge about their occurrence in four protected areas in Egypt. Consequently, long-term strategies are urgently needed as Egyptian conservation legislation is strongly focused on protecting animals and plants and their habitats. Fungi are still neglected and to solve such problems there should be collaboration between mycologists, amateur fungal groups, fungal conservation societies, regional natural parks and environmental agencies.

BEFP21
Comparative genomics on Acidithiobacillus ferrooxidans
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Introduction: Recently, isolation of bacteria from Theisen sludge, a fine grained by-product of former copper smelting, was successful. Theisen sludge contains remarkable amounts of heavy metals and metaloids. Furthermore, Theisen contains 10 % total organic carbon among which are toxic compounds (biphenyls). The 16S rRNA gene of the isolate showed > 99 % similarity to *Acidithiobacillus ferroo*xidans DSM 14882^T. The new strain was designated as *A. ferr*ooxidans Hel18. These indigenous microorganisms are supposed to be especially adapted in terms of resistances agains zinc, lead, cadmium, mercury, arsenic, and toxic organics. Therefore, the genome of *A. ferrooxidans* Hel 18 was sequenced and compared with the genome of *Acidithiobacillus ferroo*xidans DSM 14882^T.
Methods: DNA was extracted using the UltraClean Microbial DNA Isolation Kit (MO BIO Laboratories, Inc.). PCR amplification of the bacterial 16S rRNA genes was with 0.25 µM (final concentrations, respectively) of primers 27F and 1387R using a PCR master mix from Thermo Scientific Fisher. The mix was supplemented with 5 % (v/v) DMSO and 0.2 µg µL^{-1} bovine serum albumin. PCR program was the following: 2 min initial denaturation, 30 cycles of 30 sec at 94 °C, 30 sec at 55 °C, 90 sec at 72 °C and a final incubation at 72 °C for 5 min. PCR products were purified with UltraClean PCR Purification Kit (Mo Bio Laboratories Inc). The sequence of the 16S rRNA gene was deposited at NCBI (accession number KU057357). Next generation sequencing of the genome was with Illunina Miseq using the Nextera DNA library preperation kit, resulting in paired-end reads of 300 bp. Quality check, trimming of adapters, and assembly was with the web-based platform Galaxy (http://galaxy.uni-freiburg.de). Quality assesment of the genome

assembly was with QUAST. RAST (http://rast.nmpdr.org) was used for genome annotation. Genome alignment for comparative genomics was with the program Mauve.
Results: Comparative genomics lead to the following findings: The genome of *A. ferrooxidans* Hel18 is greater (3,103,965 bp) than the genome of *A. ferrooxidans* DSM 14882^T (2,982,397 bp). Large parts of both genomes consist of homologous genes. However, regions with genes could be identified which occur only in Hel18. Among them are genes belonging to the mercury resistance operon, and genes involved in zinc and cadmium resistance. Furthermore, genes for the degratation of biphenyls could be found.
Conclusion: The findings indicate a special adaption of *A. ferrooxidans* Hel18 to conditions that prevail in Theisen sludge, which are hight heavy metal contents and presence of toxic organic compounds. Further cultivation dependent studies can underline these findings.

BEFP22
The brackish bacterial sediment community has a distinct bacterial community compared to marine and freshwater sediment communities
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While there are various studies about marine and freshwater sediment bacterial communities, only few studies investigated the transition between these environments. Here we focus on the bacterial community in the Baltic Sea that covers a stable salinity gradient from marine to almost freshwater conditions over a large geographic distance. These conditions allow autochthonous brackish sediment bacterial communities to establish. We studied six silty sediment samples from marine (salinity 32) to oligohaline conditions (salinity 3), which were compartmentalized in five layers (0.5 cm steps) and analyzed for abundances of 16S rRNA amplicon sequencing reads, total organic carbon, total sulfur and nitrogen, grain size and pore water nutrients. A non-metric multidimensional scaling plot indicated that the primary environmental factor structuring the bacterial communities was salinity and the second most important factor total organic carbon. However, a parallel increase in total sulfate concentration with salinity and a change in organic matter quality (indicated by decreasing C/N ratio) may also have influenced shifts in the bacterial community composition. Especially in the marine - brackish sediments (salinity >7) Gammaproteobacteria and Deltaproteobacteria were the dominant classes that were often represented by families involved in sulfate reduction. In contrast, the bacterial communities in brackish - oligohaline sediments (salinity <7) were a rather divers combination of abundant Bacteroidetes, Chloroflexi, Alphaproteobacteria, Nitrospirae, Gemmatimonadetes, and the Candidate phyla BRC1, WS3. Moreover, opposed to what is known for macrozoobenthos, highest numbers of bacterial taxa were detected at brackish conditions. This study provides a first framework of the structure of the bacterial community in brackish-oligohaline sediments, revealing clear differences to freshwater and marine sediment bacterial communities.

BEFP23
Effects of high ammonia concentration on microbial community composition and transcription in a biogas reactor
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Question: As fossil fuels are a finite resource the production of biogas from renewable sources or biological waste in terms of energy production comes more and more into public focus. The process of biogas production is carried out by a diverse microbial community under anaerobic conditions. This complex food web can be disturbed by increased ammonia concentration introduced into the system by insufficient nitrogen removal or imbalanced "feeding" of the system. In this experiment we simulated an increased ammonia concentration of a biogas plant in a small scale biogas reactor addressing the questions how does the microbial community react to such changes in the environment and how is the transcriptional profile, reflecting metabolic activity, altered under such conditions.
Methods: This approach combines two up-to-date methods for monitoring microbial communities and ecosystem function. 16S rRNA gene amplicon sequencing shows high potential for monitoring large numbers of environmental samples while metatranscriptomic analysis allows the investigation of potential active pathways under different conditions. By the combination of these two methods we aim to link taxonomy to function and vice-versa. We incubated small scale experimental reactors (1L) as previously described by Refai and Wassmann *et al.* 2014 over 10 days with two experimental conditions, high ammonia and control conditions, each with 4 replicates. 16S rRNA gene region was analysed by MiSeq amplicon sequencing and using mothur. RNA was isolated from an experimental reactor running under similar condition converted to cDNA and sequenced by NextSeq. The obtained reads were analyzed using an in-house pipeline.
Results: The community composition as well as the transcriptional profile differed strongly between the two experimental conditions. Several OTUs in the amplicon sequence set showed potential behavior as marker species for the two conditions (ammonia concentration high/low). The transcriptional activities of the selected marker species were investigated under the different experimental conditions by their corresponding contigs and reads in the metatranscriptome. We observed quite different specific transcriptional activity for the taxa themselves and in response to the increased ammonia concentrations.
Conclusion: Combining the applied methods might be an improvement from the sheer observation of taxa and their abundance towards a linking of taxonomy to function within an ecosystem. The transcriptional activity of selected marker species will be compared and discussed.

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BEFP24
Mineralogical control on microbial N cycling in soils
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Question: Present ecological concepts in N-cycling largely ignore the role of mineral-organic associations, although, minerals are relevant for ecosystem productivity by directly and indirectly influencing microbial carbon (C) and nutrient cycling. Therefore our main research questions were i) if mineral-bound organic N (ON) is an important bioavailable N source for soil microorganisms, and, ii) which soil parameters control the bioavailability of mineral-bound ON?
Methods: To clarify these questions we set up a microcosm experiment with bulk soil and the mineral-associated organic matter fraction (MOM) of four soil ages (different mineralogical assemblage) under oxic and anoxic conditions. Thereby, we tested the effect of different nutrient additions (without, phosphate, cellulose, and phosphate + cellulose). During a 125 d incubation CO_2 and N_2O production as parameters for microbial activity were measured regularly, and at the end the net N mineralization and gene copy numbers of phylogenetic and N-cycle related marker genes were determined, as well as the microbial community composition pattern by T-RFLP.
Results: Most of the measured microbial activity and molecular parameters showed distinct pattern between oxic and anoxic conditions. The CO_2 production was highest with cellulose addition under oxic conditions, whereas under anoxic conditions higher values were determined for bulk than for MOM samples. A detectable N_2O production was only determined for bulk samples under anoxic conditions and here P addition resulted in higher values. The net N mineralization tended to be higher in MOM than in bulk samples. The 16S and 18S rRNA gene copy numbers differed significantly between oxic and anoxic conditions for *Bacteria* and *Fungi*, but not for *Archaea*. Interestingly, bacterial 16S rRNA gene copy numbers were higher for MOM samples under oxic conditions, whereas bulk sample copy numbers were higher under anoxic conditions. Archaeal *amoA* gene copy numbers were higher in bulk samples of older soils under oxic conditions. Surprisingly, the range of *narG* gene copy numbers was similar under oxic and anoxic conditions, and cellulose had a clear negative effect on numbers under anoxic

conditions. Archaeal and bacterial community composition patterns were mainly related to O_2 status but also to the soil age.
Conclusion: Our results demonstrate that the soil mineralogy has a pronounced effect on microbial ON cycling. Most ON cycling processes and corresponding microorganisms were inhibited in the mineral fraction suggesting that soil microbes could less efficiently use mineral-bound ON in agreement with our findings to enzyme activities (Turner *et al.* 2014, SBB). In addition, the redox regime is the most important factor in determining microbial C and N cycling. Nevertheless, the mineralogical effect varied between processes and was related to the nutrient situation.

BEFP25
Accurate detection and quantification of anammox bacteria using specific primers
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Anammox bacteria are ubiquitous in the environment and play an important role in the global nitrogen cycle. However, the identification and quantification of Anammox bacteria are sometimes problematic due to the fact that the 16S RNA gene sequences of Anammox strains share low identity (ranging from 89 % to 97 %). In this study, we aim to validate the accuracy of a pair of Anammox-specific primers with a set of 29 DNA samples that come from various geographical origins and possess different levels of diversity in their microbial communities. Base on alignment with 16S rRNA gene sequences of all currently known Anammox species, this pair of primers is able to match all Anammox species except *Scalindua marina*. With this primer pair, quantitative real-time PCR (qPCR), 16S-DGGE, 16S-clone library, and Illumina sequencing of PCR amplicons all showed satisfactory results in detection and quantification of Anammox populations and agree well with each other. Based on sequences similarity, new Anammox species were found through Illumina sequencing. Detection and quantification using this primer pair may prove useful in monitoring Anammox community in reactors and in the natural environment.

BEFP26
Activated sludge harbours a novel moderately thermophilic *Nitrospira*
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Nitrification is a crucial step in the treatment of wastewaters with nitrite oxidation being more sensitive than ammonia oxidation. Today, there is a growing interest in the development of thermophilic nitrogen removal processes for the treatment of warm wastewaters up to 50 °C, but only mesophilic nitrifiers have been detected so far in activated sludge of municipal full-scale plants. Members of the genus *Nitrospira* are the most dominant NOB in activated sludge as well as in thermophilic environments, such as hot springs. At least six sublineages of this highly diverse genus are known; nevertheless the diversity seems to be much higher. Apart from temperature, oxygen was identified as one selective factor for the dominance of single sublineages of *Nitrospira*. The objective of this study was to search for novel NOB by enriching the latter from activated sludge under modified cultivation conditions. Identification of dominant members was done by specific PCR as well as electron microscopy.
A new *Nitrospira* grew up in mineral salts media containing nitrite, after the initial enrichment was performed under denitrifying conditions. It forms a separate 16S rRNA- as well as nxrB- cluster with *N. bockiana* and *N. calida* as next taxonomically described organisms, whereat both strains are characterized as (moderately) thermophilic. Fastidious nitrite oxidizing activity of the new culture at 28 °C could be enhanced, when the incubation temperature was shifted to 35-42 °C. The same organism was selectively enriched, when activated sludge was directly incubated at elevated temperature with nitrite as sole energy source. Growth of the highly enriched culture was accelerated by the addition of an additional nitrogen source or by heterotrophic "helper" bacteria, derived from other nitrite oxidizing enrichments. The new *Nitrospira* could be further purified by use of an optical tweezers system coupled with a micromanipulator.
This study reveals that activated sludge in Köhlbrandthöft / Dradenau, Hamburg has the potential for nitrite oxidation in a broad temperature spectrum between 4 and 46 °C, caused by highly specialized microorganisms. The new *Nitrospira* is highly resistant to elevated temperature and probably to low dissolved oxygen concentrations. Further characterization of the phenotypic properties is in progress.

BEFP27
Anaerobic oxidation of methane (AOM) at the thaw front of subsea permafrost
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Introduction: Thawing arctic subsea permafrost is a source of organic carbon in deep sediment layers. The permafrost that is at its thermal equilibrium releases biologically produced methane and a deep sulfate-methane transition zone (SMTZ) is formed due to sulfate-rich overlaying marine sediment layers. The process of methane oxidation in this anaerobic environment has been suggested[1] but AOM associated microbial communities remain to be identified.
Objectives: We aimed at providing evidence for anaerobic methanotrophic (ANME) archaeal communities at the deep SMTZ of the north-east Siberian Laptev Sea shelf.
Material and methods: Two sediment cores were retrieved (77 m and 47.4 m deep) from the coastal shelf north of Cape Mamontov Klyk 'C2' (11.5 km offshore) and west to the Buor Khaya Peninsula 'BK2' (800 m offshore), respectively. Methane and sulfate concentrations as well as ^{13}C isotope values of CH_4 were measured and correlated with molecular analysis of microbial communities along the thaw front.
Results: At the thaw front of BK2, at 23.7 meters below sea floor (mbsf) biologically produced methane (^{13}C= -70‰ VPDB) gets oxidized (^{13}C= -29.8 ‰ VPDB)[1]. At the same depth, we found an increase in functional genes of methanogenic archaea (*mcrA*) and sulfate reducing bacteria (*dsrB*) analysed by quantitative PCR. Massive parallel tag-sequencing of the 16S rRNA gene showed an increase of ANME-2a/2b and ANME-2d sequences towards the thaw front in both cores. At the thaw front of the BK2 core, typical ANME-2 partners of the *Desulfobacterales*[2] were found to dominate the sulfate reducing bacterial community, whereas *Desulfobacca* sequences dominate in all samples of the C2 core. Theoretical methane oxidation rates (0.4-6 nmol cm^{-3} d^{-1})[1] based on estimated methane fluxes showed higher values than typically found in subsurface sediments and are more similar to rates of margin SMTZs[3].
Conclusion: Our data indicate that active anaerobic methane oxidizer communities at the thaw front of subsea permafrost prevent methane from being released into the water column and subsequently to the atmosphere. Further analyses on lipid biomarkers and ^{14}C-CH_4 isotopic rate measurements will determine how active these communities are *in situ*.

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BEFP28
Differential utilisation of methylamine as a nitrogen source by marine microorganisms
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Methylamine is a breakdown product of osmolytes produced by marine animal and plant species like glycine betaine and trimethylamine N-oxide, and is typically present in concentrations up to a few 100 nM in marine habitats. As a volatile organic compound, methylamine can enter the atmosphere, where it is involved in aerosol formation (1) and consequently influences climate patterns. Certain microorganisms have the ability to

break down methylamine to CO_2 and ammonium, recycling the contained carbon and nitrogen. This biodegradation plays a major role in modulation of methylamine emission from aquatic habitats. Microorganisms using methylamine as sole carbon source belong to the methylotrophs, and have been identified in various aquatic environments. However, also some non-methylotrophic organisms have the ability to degrade methylamine, using it as nitrogen source. Little is known about the diversity and activity of the latter.

Stable isotope probing (SIP), the metabolic labelling of microbial communities using substrates enriched with heavy, nonradioactive isotopes, is a powerful tool in environmental microbiology (2). Using SIP, the microorganisms that actively contribute to the metabolic processes of interest can be identified against the background of a complex microbial community, directly within environmental samples. A combination of the SIP approach with metaproteomic analysis, named protein-SIP, has been introduced recently, and uses mass spectrometry to identify peptides and simultaneously assess their enrichment in heavy isotopes (3). This approach also allows the detection of ^{15}N labeled biomolecules, and consequently is an ideal tool to study assimilation of nitrogen in a microbial community.

Here we report the first application of a ^{15}N-SIP approach combined with metagenomic and metaproteomic analysis of a marine microbial community. Multiple microorganisms assimilating methylamine derived nitrogen in environmental samples were identified. These organisms showed a correlation between the mode of nitrogen assimilation and the employed pathway for methylamine degradation. Only one organism detected was using the methylamine dehydrogenase pathway typically found in methylotrophs. In contrast, multiple microbial species from the Alpha- and Gammaproteobacteria were found using the Glutamylmethylamide pathway, which is also present in non-methylotrophs. Draft genomes of two of these organisms were recovered by ^{15}N-DNA SIP, showing a diverse metabolic potential. We conclude that various microbial species employing the GMA pathway are present in the investigated environment, playing a dominant role in methylamine degradation and are potentially involved in a variety of other biogeochemical processes.

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BEP01

Active site amino acid composition determines the catalytic bias of [NiFe] hydrogenase

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Question: Hydrogenases catalyse the reversible splitting of H_2 into $2H^+$ and $2e^-$. There are two main types of hydrogenases which are discriminated according to their active site metal composition [1]. [FeFe] hydrogenases operate mainly in the direction of H^+ reduction and are extremely sensitive towards O_2. [NiFe] hydrogenases are generally less sensitive towards O_2 and are either biased towards H_2 oxidation or H^+ reduction. Tolerance towards O_2 is a prerequisite for the biotechnological applicability of hydrogenases. Fortunately, nature has evolved O_2-tolerant variants of [NiFe] hydrogenases. A well characterised example of such an oxygen-tolerant enzyme is the membrane-bound hydrogenase (MBH) from *Ralstonia eutropha* H16 [2,3]. MBH is strongly biased towards H_2 oxidation and shows strong product inhibition upon H^+ reduction [4]. [NiFe] hydrogenases possess an invariant arginine residue at their active site forming salt bridges with two aspartate side chains. One of these aspartate residues is replaced by serine in those [NiFe] hydrogenases biased towards H^+ reduction. The question was whether replacement of Asp117 for serine alters the catalytic bias of MBH.

Methods: We generated a mutant strain synthesising the protein variant MBH^{D117S}. The recombinant strain was grown with H_2 and CO_2 under varying O_2 levels to analyse its oxygen tolerance. Isolated MBH^{D117S} protein was compared to native MBH using activity assays for H_2 oxidation and H^+ reduction.

Results: The MBH^{D117S} variant showed <1 % of the native enzyme's H_2 oxidation activity. Despite this low catalytic activity the mutant strain grew well lithoautotrophically in the presence of 1 % O_2. However, growth was strongly diminished at 5 % O_2 and not detectable at 10 % O_2, indicating higher sensitivity towards O_2 than the wild-type strain. Comparing the relative ratios of H_2 oxidation and H^+ reduction of purified native MBH vs MBH^{D117S}, the D117S variant showed strongly elevated H_2 production capabilities. This is also manifested in the apparent inhibition constant (K_i^{app}) for H_2 which increased fourfold in case of MBH^{D117S} compared to native MBH.

Conclusion: The bias towards H_2 oxidation of [NiFe] hydrogenases is obviously related to the presence of an aspartate residue forming a salt bridge to an invariant arginine residue at the [NiFe] active site. Exchanging this aspartate for serine enables significantly higher H_2 production rates. This knowledge has implications on biotechnological H_2 production and the design of efficient artificial H_2 producing catalysts.

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BEP02

Ethanol and ethylene glycol metabolism in *Acetobacterium woodii*

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Question: Acetogenic bacteria can grow lithoautotrophically on H_2+CO_2 but also heterotrophically on a variety of different substrates like sugars, alcohols and carboxylic acids. Here, we have studied the enzymology and bioenergetics of ethanol and ethylene glycol metabolism in the model acetogen *Acetobacterium woodii*.

Methods: Pathways were constructed by activity measurements in cell free extracts, gene expression was monitored by RT-PCR. Protein levels were quantified by Western blotting and the key enzyme was purified by chromatographic steps.

Results: The endergonic oxidation of ethanol to acetaldehyde is catalyzed by a bifunctional alcohol/aldehyde dehydrogenase (AdhE) that also catalyzes the next step, the oxidation of acetaldehyde to acetyl-CoA. The purified enzyme is subject to regulation by CoA [1]. AdhE is also involved in ethylene glycol metabolism which yields acetate and ethanol first and the latter is then oxidized to acetate.

Conclusion: Ethylene glycol is first dehydrated to acetaldehyde that is further disproportionated to ethanol and acetate. In the stationary phase, ethanol is further converted to acetate in a reaction sequence that involves AdhE as initial enzyme and the Wood-Ljungdahl pathway as sink for electrons. Metabolism of ethanol requires reverse chemiosmosis to reduce ferredoxin.

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BEP03

Domain structure-function of the rhodopin 3,4-desaturase (CrtD) of *Rhodospirillum rubrum*

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The carotenoid present in the light-harvesting (LH) 1 complex of *Rhodospirillum rubrum* is in the *all-trans* configuration and transverses the photosynthetic membrane. However, it has been demonstrated that *in situ* modification of the carotenoid "ends" e.g. the 3,4,3´,4´-dehydrogenation can occur during LH1 maturation, despite the fact that only a single enzyme, rhodopin desaturase (CrtD) is present exclusively in the cytoplasm. In this study, we focus on the functional domain structure of CrtD to cast light upon this question. The *crtD* gene, separated by gene synthesis into different functional domains, was cloned into a pRK404-derived broad-host range vector and then used to complement a *crtD* deletion mutant (*R. rubrum* strain ST4 [1]). Here, we focus on the C-terminal domain, which we recently showed to be important for catalytic function [2]. Additionally, as a comparison, the *crtD* gene from *Rhodobacter sphaeroides*, which only catalyzes a chain-asymmetric dehydrogenation, was also used for ST4 complementation. The results of these studies will be presented.

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BEP04

Insights into flavin-based electron bifurcation: structural and functional studies of the NADH-dependent reduced ferredoxin-NADP oxidoreductase

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NADH-dependent reduced ferredoxin: NADP oxidoreductase (NfnAB) reversibly catalyzes the endergonic reduction of ferredoxin with NADPH driven by the exergonic transhydrogenation from NADPH to NAD^+. Coupling is accomplished via the mechanism of flavin-based electron bifurcation[1]. NnfAB is a heterodimeric complex. NfnA (31 kDa) binds one FAD (a-FAD) and one [2Fe-2S] cluster and NfnB (51 kDa) one FAD (b-FAD) and two [4Fe-4S] clusters. To understand its enzymatic mechanism on an atomic basis we determined the structure of the NfnAB complex of *Thermotoga maritima* at 2.1 Å resolution. The structure revealed b-FAD as bifurcating FAD in the center of the NfnAB complex. B-FAD is the starting point for two electron transfer routes; the first via the [2Fe-2S] cluster to a-FAD and the second via the proximal to the distal [4Fe-4S] clusters. Most likely the ferredoxin binds adjacent to the distal [4Fe-4S] cluster. Moreover, the NfnAB-NADH structure at 2.4 Å resolution revealed a-FAD as binding site for NADH. We then analyzed FBEB using Hydrogen-deuterium exchange mass spectrometry. Using this technique, we identified the NADPH binding site and tracked conformational changes in the complex upon NADPH binding. A mechanism of FAD-based electron bifurcation was postulated. Therein, we propose that the second electron transfer from b-FAD to a-FAD, the site of the exergonic reaction, is prevented by a rearrangement of NfnA and NfnB relative to each other.

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BEP05

Electron accepting units of the diheme cytochrome *c* TsdA, a bifunctional thiosulfate dehydrogenase/tetrathionate reductase

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The enzymes of the thiosulfate dehydrogenase (TsdA) family are widespread diheme *c*-type cytochromes [1]. TsdA proteins catalyze the reversible formation of a sulfur-sulfur bond between the sulfane atoms of two thiosulfate molecules, yielding tetrathionate and releasing two electrons. All TsdA enzymes characterized to date catalyze both the oxidation of thiosulfate to tetrathionate and the reduction of tetrathionate to thiosulfate at measurable rates, albeit with very different catalytic bias depending on the source organism [1, 2, 3]. Here, we posed the question which redox carriers mediate the flow of electrons arising from thiosulfate oxidation into respiratory or photosynthetic electron chains. In a number of organisms, including *Thiomonas intermedia*, *Sideroxydans lithotrophicus* and *Pseudomonas stutzeri*, the *tsdA* gene is immediately preceded by *tsdB* encoding for another diheme cytochrome. Spectrophotometric experiments [1] in combination with enzyme assays in solution showed that TsdB is not itself reactive with thiosulfate but that it acts as an effective electron acceptor of TsdA *in vitro* even when TsdA and TsdB do not originate from the same source organism. While TsdA covers a range from -300 mV to +150 mV, TsdB was found to be redox active between -100 to +300 mV, thus enabling electron transfer between these hemoproteins. The three-dimensional structure of a TsdB-TsdA fusion protein from the purple sulfur bacterium *Marichromatium purpuratum* (Mp) was solved by X-ray crystallography providing insights into internal electron transfer. In the oxidized state, this tetraheme cytochrome *c* contains three hemes with axial His/Met ligation, while heme 1 exhibits the His/Cys coordination typical for the active site of TsdA proteins. In several purple sulfur bacteria including *Allochromatium vinosum* (Av), TsdB is not present, precluding a general and essential role for electron flow. Both, AvTsdA as well as the MpTsdBA fusion protein react efficiently with high potential iron sulfur protein from *A. vinosum* (AvHiPIP: E_m +350 mV) *in vitro*. HiPIP not only acts as direct electron donor to the reaction center in anoxygenic phototrophs but can also be involved in aerobic respiratory chains.

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BEP06

ΔΨ-supported jump-start ATP regeneration after short-term anoxia by *Dinoroseobacter shibae*

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Dinoroseobacter shibae, as a member of the world-wide abundant Roseobacter clade, performs anoxygenic photosynthesis under oxic conditions while using light as additional energy source. It is known that the ATP level of the cells is drastically reduced after only 2 hours of oxygen depletion, but is quickly regenerated after 5 minutes of aeration and light exposure **(1)**. We suspected variations within the proton-motive force to contribute to the observed phenomenon. Recent experiments, including the permeabilisation of the cell membranes with butanol **(2)**, revealed that the intracellular pH of *D. shibae* lies around 7.3 and is therefore slightly more acidic than its growth medium. As a result, ΔpH is not able to support the proton-motive force in an ATP-regenerative way, leaving the membrane potential (ΔΨ) as the main force for the observed fast ATP regeneration after aeration. We were able to display changes within the membrane potential between fresh-, energetically depleted- and reenergized cells via the ΔΨ-indicating dye $DiOC_2(3)$ and epifluorescence microscopy. The advantage of this method is the *in vivo* visualization of ΔΨ-driven dye accumulates within the stained cells and therefore track changes in the membrane potential. Experiments showed that *D. shibae* in fact increases its membrane potential during phases of anoxia-induced energy depletion, compares to fresh- and reenergized cells. We hypothesize that an electrogenic succinate accumulation within the cells is responsible for this effect. It is already known that the cells undergo a metabolic crisis during short-term anoxia, and TCA-cycle intermediates tend to accumulate due to the lack of an alternative electron acceptor **(3)**. Control experiments with succinate-free cell suspensions did not show this effect. With an increased ΔΨ, the cells could be able to give their ATP regeneration a flying start after anoxia, once the oxygen is available again and explain the observed quick ATP regeneration. An intact membrane potential is essential for ATP regeneration. Our results shed new light on the cells adaptation on short-term anoxia and help explaining the fast ATP regeneration ability of *D. shibae*.

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BEP07

Molecular characterization of Na^+-translocating Glutaconyl-CoA decarboxylase (Gcd)

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Fermenting bacteria and archaea developed specific enzyme complexes for energy conservation that completely differ from those of the respiratory chain. Glutaconyl-CoA decarboxylase (Gcd), a family member of Na^+-translocating biotin-containing decarboxylases couples the exergonic decarboxylation of glutaconyl-CoA to crotonyl-CoA with the formation of an electrochemical Na^+-gradient across the membrane [1]. According to the current mechanistic proposal the carboxylate of glutaconyl-CoA is first transferred onto biotin implicating a large conformational change. Subsequently, the decarboxylation of carboxybiotin drives Na^+ translocation, which was demonstrated for all Na^+-translocating biotin-containing decarboxylases using inverted membrane vesicles or purified enzymes after incorporation into artificial liposomes [2]. Gcd is composed of four subunits: the biotin-dependent carboxylase GcdA (65 kDa), the membrane subunit GcdB (35 kDa), the biotin-binding subunit GcdC (14 kDa), and a small membrane anchor

subunit GcdD (12 kDa). The oligomeric composition of the Gcd complex and therefore also the molecular mass are unknown. Gcd was purified from 10 g *Acidaminococcus fermentans* wet cells after solubilization of the membrane fraction with Triton X 100 - 1.5 mg of homogenous enzyme was obtained. For molecular and low-resolution structural studies we initiated native gel, multi-angle light scattering, Laser-induced liquid bead ion desorption mass spectrometric and preliminary electron microscopic experiments.

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BEP08

Dimethylsulfide induces expression of heterodisulfide reductase-like proteins in different *Hyphomicrobium* strains

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A wide array of chemo- and phototrophic sulfur-oxidizing prokaryotes lacks the well-established Dsr pathway. These organisms are proposed to pursue an alternative sulfur oxidation pathway involving a heterodisulfide reductase (Hdr)-like protein complex [1, 2]. However, direct biochemical evidence for this suggestion has not been published.
We established *Hyphomicrobium denitrificans* as a new model organism for elucidating dissimilatory sulfur metabolism via the Hdr-like complex. *Hyphomicrobium* species are well known for their capacity to grow on C1 compounds like methanol or methylamine. Here, we verified previous reports that *Hyphomicrobium* strain EG can grow on dimehtylsulfoxide as the only carbon source. In addition, this strain as well as the type strain of the species *H. denitrificans* (DSM 1869) grows on dimethylsulfide (DMS). While DMS is consumed, sulfate is formed as the end product. DMS concentrations above 1.5 mM proved toxic for strain DSM 1869. Appreciable cell yields necessitate repeated feeding of cultures. The *H. denitrificans* DSM 1869 genome sequence contains several sets of genes for proteins potentially involved in dissimilatory sulfur oxidation. Besides a complete Sox system for thiosulfate oxidation, a *tsdA* gene for a thiosulfate dehydrogenase was detected, indicating the capacity for tetrathionate formation from thiosulfate. A cluster of *hdr*-like genes, *hdrC1B1AhyphdrC2B2*, is not only linked with genes encoding sulfur-trafficking and liponamide-binding proteins but also with a gene for a cytochrome P450. The latter may be involved in the first step of DMS oxidation. Previously reported methods for plasmid transfer into *H. denitrificans* DSM 1869 via conjugation and electroporation were established in our laboratory. A replicative plasmid equipped with a suitable *H. denitrificans* promoter was used to drive production of the Strep-tagged hypothetical protein encoded in the *hdr*-like gene cluster. Methods for targeted gene knock-out were also applied. A highly specific antiserum was generated against recombinant *H. denitrificans* HdrA-like protein (Hden_0691). In both *Hyphomicrobium* strains studied, crude extracts of cells grown on methylamine hardly contained any protein reactive with the antiserum. In contrast, bands exactly corresponding in size with the HdrA-like protein (37.7 kDa), were clearly present in cells grown on DMS. Thus, formation of the HdrA-like protein and probably the complete Hdr-like complex is specifically induced upon growth on the organic sulfur compound, further strengthening the notion that this complex is involved in a process functionally replacing the Dsr system.

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BEP09

Flavodoxin reduction by electron bifurcation and Na^+-dependent oxidation by NAD^+ in *Acidaminococcous fermentans*

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Electron bifurcation with electron transferring flavoprotein (Etf) and butyryl-CoA dehydrogenase (Bcd) in *Acidaminococcous fermentans* enables the endergonic reduction of ferredoxin by NADH driven by the exergonic reduction of crotonyl-CoA to butyryl-CoA also by NADH [1,2]. Here we show that recombinant flavodoxin from *A. fermentans* produced in *Escherichia coli* can replace ferredoxin. Under limiting concentrations of crotonyl-CoA, flavodoxin is reduced to the semiquinone, whereas with an excess of this oxidant the fully reduced quinol state of flavodoxin is obtained. Kinetic data show that the reduction of the quinone to the semiquinone ($E^{\circ\prime}_1$ = -60 mV) is about 3-times slower than that of the semiquinone to the quinol ($E^{\circ\prime}_2$ = -430 mV), though the opposite is expected when considering the redox potentials. Re-oxidation of the quinole is achieved by hydrogenase resulting in the equation: Crotonyl-CoA + 2 NADH + 2 H^+ → Butyryl-CoA + 2 NAD^+ + H_2. Membrane preparations of *A. fermentans*, containing a very active ferredoxin-NAD reductase (Rnf), catalyze the re-oxidation of the quinol to the semiquinone by NAD^+. Due to the high redox potential of the semiquinone, further oxidation to the quinone is not possible. The combination of Etf, Bcd and Rnf recycles not only the semiquinone but also half of the NAD^+, resulting in the simple equation: Crotonyl-CoA + NADH + H^+ → Butyryl-CoA + NAD^+. In this system the apparent K_m of flavodoxin was determined as 9 µM, 45-times higher than that of ferredoxin by recycling with hydrogenase, K_m = 0.2 µM [1]. Furthermore, this assay allowed to demonstrate the postulated dependence of Rnf from *A. fermentans* on Na^+ or Li^+ [3], which has been already verified for Rnf from *Acetobacterium woodii* [4,5]. Following values were measured: K_m = 120 µM Na^+ or 275 µM Li^+. Etf contains two FAD, one of which exhibits normal redox potentials, $E^{\circ\prime}_1 < E^{\circ\prime}_2$, whereas those of the other are similar to the potentials of flavodoxin, $E^{\circ\prime}_1 > E^{\circ\prime}_2$ (see above). This inverse behavior leads to a model of flavin-based electron bifurcation.

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BEP10

Cell biology of a purple sulfur bacterium – *in vivo* localization of a new sulfur globule protein in *Allochromatium vinosum* by fluorescence microscopy

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Many sulfur-oxidizing bacteria form conspicuous sulfur globules as intermediates during the oxidation of reduced sulfur compounds. The sulfur globules are deposited either intracellularly in the periplasm or outside of the cells. The anoxygenic phototrophic purple sulfur bacterium *Allochromatium vinosum* is a well-studied example for intracellular sulfur deposition. So far, three extremely hydrophobic structural proteins have been established as constituents of the proteinaceous sulfur globules envelope: SgpA and SgpB are very similar and can functionally replace each other. SgpC participates in sulfur globule expansion [1]. Presence of a fourth major sulfur globule protein, SgpD (Alvin_2515), was indicated by comparative transcriptomic profiling and a proteomic study of enriched sulfur globules [2]. Here, we obtained direct experimental proof for a function of SgpD as a sulfur globule protein *in vivo* by coupling its carboxy-terminus to a fluorescent protein. This enabled targeting of the fusion to the bacterial periplasm via the original Sec-dependent signal peptide of SgpD. mCherry was chosen as the reporter protein for subcellular localization of SgpD, because red fluorescent protein derivatives are known to be effectively transported to the bacterial periplasm through the Sec system. Usually, the use of fluorescent reporter proteins as non-invasive molecular tools for *in vivo* imaging is restricted to aerobic systems, as the formation of their chromophores strictly requires oxygen. However, *A. vinosum* is grown under anoxic conditions. This problem was overcome by incubation of embedded *A. vinosum* cells on microscope slides for approximately one hour at ambient oxygen concentrations before fluorescence microscopy. While a mCherry-SgpD fusion lacking a signal peptide led to an evenly distributed low fluorescence of the whole cell, the fusion protein targeted to the periplasm co-localized exactly with the sulfur deposits apparent in sulfide-fed *A. vinosum* cells as highly refractile globules by light microscopy. Insertional inactivation of the *sgpD* gene in *A. vinosum* showed that the protein is not essential for the formation and degradation of sulfur globules when cells are exposed to sulfide (range 2-6 mM) or thiosulfate (range 4-10 mM). At very high sulfide concentrations (8 mM) sulfur globule formation appeared slightly delayed in the *A. vinosum* Δ*sgpD*::ΩKm strain.

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BEP11

Membrane topology of AtpI in *Escherichia coli*

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AtpI, encoded by the first gene of the *atp* operon of *E. coli*, is a hydrophobic, chloroform/methanol extractable protein of 14 kDa, which is present in substoichiometric amounts in purified F_O and F_OF_1-ATP synthase preparations, although not necessarily part of the functional enzyme complex. Whereas AtpI is essential for the oligomerization of the *c* ring in Na^+-translocating F_OF_1-ATP synthases during assembly of F_OF_1, it is not absolutely required for the assembly of H^+-translocating F_OF_1-ATP synthases, although in its presence, stability and activity of the membrane-bound ATPase activity is modestly increased (Deckers-Hebestreit, 2013; and references therein). Nevertheless, the *atpI* gene is highly conserved in most bacterial *atp* operons, although the protein exhibits a high variability in sequence and length.
Hydropathy plots as well as the positive-inside rule suggest a membrane topology of AtpI with four transmembrane segments and a N_{in}-C_{in} orientation. To determine its topology, intact cells as well as inverted membrane vesicles enriched in AtpI carrying single cysteine substitutions were incubated with membrane-impermeable 4-acetamido-4‘-maleimidylstilbene-2,2‘-disulfonic acid to block thiol groups accessible from the water phase. Subsequently, blocked and untreated samples were labelled with membrane-permeable 3-(N-maleimido-propionyl) biocytin and detected with fluorescently labelled streptavidin after electrotransfer (Jung et al, 1998).
Our analysis verifies the presence of four transmembrane helices as predicted. At the periplasmic side of the membrane only a few amino acid residues were accessible to the non-permeable thiol reagent indicating the presence of small loops only. While at the cytoplasmic side of the membrane, the accessibility of both termini as well as of the cytoplasmic loop is more extended as initially expected. Furthermore, hydrophobic, membrane-spanning segments shorter than 20 amino acids in length indicate the presence of a hydrophilic cavity probably comparable to the one observed for F_O subunit *a* in the Na^+-F_OF_1-ATP synthase of *Propionigenium modestum* (von Ballmoos et al, 2002) or the H^+-pumping F_OF_1-ATP synthase of *Polytomella sp*. mitochondria (Allegretti et al, 2015). Further investigations are necessary to describe the interaction between those membrane-spanning segments of AtpI as well as the interface between AtpI and the subunit *c* ring.

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BEP12

A novel nitrite-reducing multienzyme complex from anammox bacteria

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Anaerobic ammonium-oxidizing (anammox) bacteria are major players in the earth's nitrogen cycle and are now frequently applied in waste water treatment. Their energy-metabolism is based on the combination of nitrite and ammonium to form dinitrogen gas which involves the highly reactive intermediates nitric oxide (NO) and hydrazine (N_2H_4). The genome of the anammox bacterium *Kuenenia stuttgartiensis* encodes ten paralogues of octaheme c-type cytochromes related to hydroxylamine oxidoreductase (HAO). Here we describe a complex of the HAO-like octaheme cytochrome kustc0458 (α-subunit) with the diheme cytochrome c kustc0457 (β-subunit) which was isolated from *K. stuttgartiensis*. Biophysical analyses using analytical ultracentrifugation (AUC) revealed a 546 kDa complex consistent with an $\alpha_6\beta_6$ stoichiometry. The molecular structure of this heterododecamer was determined by X-ray crystallography at 2.6 Å resolution. The structure shows an assembly of two kustc0458 trimers connected by a ring of three kustc0457 dimers, and in total contains sixty heme cofactors. Unlike other HAO-like proteins that catalyze the oxidation of their substrates, the kustc0457/58 complex reduces nitrite to ammonium using methylviologen as electron donor in biochemical assays. Despite the high degree of overall structural similarity to other trimeric octaheme cytochromes, there are striking differences at the kustc0458 active site. In particular, the tyrosine residue commonly forming a covalent crosslink to the active site heme of an adjacent subunit in the HAO-trimer (the P460 cofactor) is replaced by a three-tryptophan motif. Thus, the subunits of kustC0458 are not covalently bound together, and the active site heme cofactor displays less distortion from planar symmetry, which may aid in explaining the differences in reaction specificity between octaheme c-type cytochromes acting in the substrate-oxidizing or -reducing direction.

BEP13

Towards the structure of an anammox nitrite oxidase complex

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The discovery of anammox bacteria in the 1990's has dramatically changed our understanding of the global nitrogen cycle. These bacteria perform ANaerobic AMMonium OXidation, combining ammonium with nitrite into molecular dinitrogen (N_2) and water, yielding energy for the cell (see [1] for a review). In marine ecosystems, the anammox process is estimated to be responsible for up to 50 % to the total N_2 production [2].
We are studying the structure of the nitrite:nitrate oxidoreductase (Nxr) multienzyme complex from the anammox organism *Kuenenia stuttgartiensis* using protein crystallography. We have obtained large crystals containing all three subunits that diffract to 3.5 Å resolution. The ongoing analysis of the diffraction data is greatly complicated by the extremely large unit cell. However, it has already revealed large supercomplexes of Nxr. In addition we have localized five iron-sulfur clusters as well as a heme and a molybdopterin site in each protomer. The positions of these redox-active sites reveal a path for electrons through the multiprotein complex. Further analysis is being performed.

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BEP14

CO and syngas metabolism in the thermophilic acetogen *Thermoanaerobacter kivui*

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Question: *Thermoanaerobacter kivui* is an acetogenic bacterium that is able to grow autotrophically on H_2 + CO_2 [1]. A genetic system is available for some *Thermoanaerobacter* strains making *T. kivui* a good candidate for a strain to be used for the production of biocommodities from CO_2 at high temperatures [2,3]. Another interesting feedstock for 3rd generation biofuels is synthesis gas or syngas that contains carbon monoxide, carbon dioxide and molecular hydrogen in variable proportions**.** Unfortunately, it was reported that *T. kivui* is unable to grow on carbon monoxide which would exclude syngas as a feedstock for this bacterium [4]. The capability of *Thermoanaerobacter kivui* to adapt to growth on CO was re-investigated.
Methods: Growth experiments were conducted to elucidate adaptation to CO. Product formation from CO and/or in combination with H_2 was investigated by gas-chromatography. Cellular activity and the effect of the ionophore TCS was studied in cell suspension experiments. Photometric assays were conducted to shed light on key enzyme activities.
Results: *T. kivui* can be adapted to grow on CO when adapted from a culture grown on H_2 + CO_2 but not from glucose. The final optical density increases with increasing CO concentrations and reaches a maximum at 50 % CO. Growth on CO in minimal medium is slower but not affected by omission of vitamin solution. The organism forms acetate as the main end product along with molecular H_2. When subjected to synthesis gas, CO and H_2 can be co-utilized by a growing culture. The main end-product is acetate. As shown in cell suspension experiments, the oxidation of CO is coupled to ATP synthesis and acetate formation. The presence of a protonophore abolishes ATP and acetate synthesis. Hydrogenase activity is highest in CO-grown cells and carbon monoxide dehydrogenase activity is highest in H_2 + CO_2-grown cells.

Conclusion: *T. kivui* can be adapted to grow on CO. CO oxidation leads to acetate and H_2 formation and is coupled to energy conservation.

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BEP15

Hydrogen production by *Escherichia coli* wild type and hydrogenase mutants upon formate and glycerol fermentation under different growth conditions

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One of the fermentation end products during glycerol and glucose fermentation in *Escherichia coli* is formate, which is decomposed by the formate hydrogen lyase (FHL) complex leading to H_2 production. FHL is formed by formate dehydrogenase H (FDH-H) and hydrogenase (Hyd). *Escherichia coli* possesses four [Ni-Fe]-hydrogenase (Hyd) enzymes, encoded by the *hya*, *hyb*, *hyc* and *hyf* operons. In this study H_2 production was investigated with the help of two redox Pt (sensitive to H_2) and Ti-Si electrodes. From the end of the log growth phase the drop of two redox Pt and Ti-Si electrodes form positive to low negative ((Pt) - 450± 0.05) values was detected in *E. coli* BW25113 wild type upon glycerol fermentation in peptone rich medium pointing out H_2 formation. *E. coli* BW25113 wild type growth and H_2 production were also observed in minmal salt growth medium at pHs 5.5 to 7.5. H_2 production was delyed and observed after 24 h growth, moreover, the bacterial growth and H_2 production reached their maximal values at 72 h at pHs 6.5 and 7.5. Our previous findings identified the conditions when formate alone or with glycerol had stimulatory effect on bacterial growth and H_2 production. The role of deleting the large subunits of each Hyd (1-4) enzymes for bacterial growth and H_2 production was evaluated. In this study role of formate alone or with glycerol on hydrogen production and growth were investigated in double *hyaBhybC* (lacking Hyd 1 and 2; triple *hyaBhybChycE* (lucking Hyds 1-3), and *selC* (lacking FDH-H) mutants were investigated upon 24 h bacterial growth. H_2 production was absent in *selC* and triple *hyaBhybChycE* mutants during log growth phase upon glycerol or formate alone or with glycerol fermentation at pH 6.5 and 7.5 and formate supplementation had no effect. H_2 evolution was delayed at pH 7.5, but observed and stimulated at pH 6.5 upon glycerol and formate utilization in double *hyaBhybC* mutant.
The results point out the ability of *E. coli* to grow and utilize glycerol in minimal salt medium, as well as confirmed the key role of Hyd-3 at both pH 6.5 and 7.5 as well as the role of Hyd-2 and Hyd-4 at pH 7.5 for H_2 production by *E. coli* during glycerol fermentation with formate supplementation.

BTP01

Alkaline protease as detergents and solvents compatible nanobiocatalyst via immobilization onto functionalized rattle-type magnetic core@mesoporous shell silica nanoparticles

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Alkaline protease from alkaliphilic *Bacillus* sp. NPST-AK15 was immobilized onto functionalized and non-functionalized rattle-type magnetic core@mesoporous shell silica (RT-MCMSS) nanoparticles by physical adsorption and covalent attachment. However, the covalent attachment approach was superior for NPST-AK15 protease immobilization onto the activated RT-MCMSS-NH_2 nanoparticles and was used for further studies. In comparison to free protease, the immobilized enzyme exhibited a shift in the optimal temperature and pH from 60 °C to 65 °C and pH 10.5 to 11.0, respectively. While free protease was completely inactivated after treatment for 1 h at 60 °C, the immobilized enzyme maintained 66.5 % of its initial activity at similar conditions. The immobilized protease showed higher V_{max}, k_{cat} and k_{cat}/K_m, than the soluble enzyme by about 1.3-, 1.3-, and 1.6-fold, respectively. In addition, the results revealed significant improvement of NPST-AK15 protease stability in variety of organic solvents, surfactants, and commercial laundry detergents, upon immobilization onto activated RT-MCMSS-NH_2 nanoparticles. Furthermore, the immobilized protease maintained significant catalytic efficiency for ten consecutive reaction cycles, and was separated easily from the reaction mixture using an external magnetic field. To the best of our knowledge this is the first report about protease immobilization onto rattle-type magnetic core@mesoporous shell silica nanoparticles. The results clearly suggest that the developed immobilized enzyme system is a promising nanobiocatalyst for various bioprocess and protease applications.

BTP02

Construction of a recombinant biocatalyst for the production of phenylacetic acids and phenylethanols from styrenes

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Question: Numerous soil bacteria have been reported to be able to metabolize styrene via the pathway of side-chain oxygenation. This pathway comprises a styrene monooxygenase (SMO), which oxidizes styrene to styrene oxide, a styrene oxide isomerase (SOI), which converts styrene oxide into phenylacetaldehyde, and a phenylacetaldehyde dehydrogenase (PAD). The latter enzyme enables the oxidation of the aldehyde to the central metabolite phenylacetic acid. Further enzymatic steps allow subsequently the degradation of the acid to intermediates of the tricarboxylic acid cycle [1,2]. In this study the construction of a recombinant biocatalyst under consideration of suitable SMOs, SOIs and PADs was intended because this pathway is of potential relevance for the biotechnological production of phenylacetic acids and similar compounds.
Methods: During our present study various enzymes of this pathway from different microorganisms (*Rhodococcus opacus* 1CP, *Sphingopyxis fribergensis* Kp5.2, *Variovorax paradoxus* EPS, *Pseudomonas fluorescens* ST) were investigated with respect to their applicability for the transformation of styrenes to phenylacetic acids. The most active and stable ones were combined to an enzyme cascade which was expressed in suitable *Escherichia coli* strains. Enzyme assays and whole cell biotransformation were used to evaluate these putative biocatalysts.
Results: Remarkably, the integration of a styrene monooxygenase and a styrene oxide isomerase in *E. coli* BL21(DE3) and T7Express resulted in the accumulation of phenylethanols instead of - as expected - phenylacetaldehydes. It can be assumed that most probably the phenylacetaldehydes are transformed immediately into the alcohols by unspecific alcohol dehydrogenases. But, additional transformation of a gene encoding a phenylacetaldehyde dehydrogenase resulted in the formation of significant amounts of phenylacetic acids. Both biotransformations are mainly affected by the activity of the SMO. During this study some SMOs were identified which convert styrene to styrene oxide with high relative activities and allow a stable transformation rate over a period of several days.
Conclusion: Both, phenylacetic acids and phenylethanols, are important chemicals for various industries and these biocatalysts reported herein offer environment-friendly alternatives to common chemical strategies in order to synthesize such compounds.

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2 Teufel, R., V. Mascaraque, W. Ismail, M. Voss, J. Perera, W. Eisenreich, W. Haehnel, and G. Fuchs. 2010. Proc Natl Acad Sci USA 107:14390-14395.

BTP03

Optimisation of the bioleaching of REE from FP with chemoorgano-heterotrophic microorganisms

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Rare earth elements (REE) are used in mostly all new technologies and until now, there is nearly no recycling of REE containing end-of-life products [1]. Furthermore, only poor information is available regarding interactions of microorganisms with REE and there are almost no studies describing the bioleaching of REE. However, it can be assumed that microorganisms play an important role in the biogeochemistry of REE. This study investigates the potential of organic acid and metal binding molecules producing microbes to extract REE from technical waste.
During recycling of energy-saving bulbs fluorescent phosphor (FP) is collected as a distinct fraction. It contains about 10 % REE-oxides bound in the hardly water-soluble triband dyes as oxides, phosphates, aluminates and silicates [2]. Previous experiments showed, that the chemoorgano-heterotrophic, organic acid producing micrrooganisms *Yarrowia lipolytica*, *Komatogateiibacter xylinus* and *Lactobacillus casei* as well as the mixed culture Kombucha are in principle suitable for the bioleaching of REE from FP. In this presentationthe solubilisation process is investigated regarding the leaching metabolites and optimised with respect to maximal REE release.

Thereto, the results are transferred from shake flasks to bioreactor and the media were adjusted. Furthermore, the influence of metal binding molecules like siderophores was tested.
It could be shown, that bioleaching is a potential alternative to technical leaching approaches, even though, the leaching efficiency is still low. This provides the basis for the development of an eco-friendly alternative to the currently applied methods.

[1] European Commission (2014) *On the review of the list of critical raw materials for the EU and the implementation of the Raw Materials Initiative*, Brüssel. [2] Haucke *et al.* (2011) *Verfahren zur Rückgewinnung seltener Erden aus Leuchtstofflampen*, Osram AG

BTP04

Rooting of jujube (*Ziziphus Jujuba* Mill) li variety cuttings, using some root promoting micro-organisms and plant growth regulators

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This study was conducted at the experimental nursery of the Horticulture Research Institute at Giza, Egyptto study the effect of plant growth promoting rhizobacteria (PGPR) and plant growth regulators on rooting of jujube cuttings during 2008 and 2009 seasons. Sub-terminal cuttings were taken on mid April from mature 15- years old trees of jujube (*Ziziphus Jujuba* Mill) Li variety (difficult to root). Rooting treatments included inoculation with *B*acillus *polymyx, Bacillus circulance, Bacillus megaterium, Bacillus pasteruii, Pseudomans florescence* or mixed inoculants from previous PGPR strains or yeast *(Saccharomyces cerevisae)*. In addition, Indole-3- Butyric Acid (IBA) and Naphthalene Acetic Acid (NAA) were also tested at concentration of 1000 and 2000 ppm as compared with untreated (control). Data showed that the effect of *Bacillus megaterium* as PGPR resulted in the highest significant rooting percentage (60 % and 50 %). After eight months of transplanting, bacterial strain (*Bacillus megaterium)* followed by *Pseudomans* strain surpassed the other treatments in survival percentage, average number of roots/transplant, stem and root length, number of leaves, number of branches/transplant as well as leaves, stem and root fresh and dry weight (g). On the contrary, the lowest significant effect of treatments was found as a result of NAA at 1000 ppm and control during the two seasons of study. Histological studies revealed that, the callus originated from the cambial and phloem parenchyma cells below the cork cells, from these protrusions the adventitious roots were developed. The new roots established their connections with the vascular tissue of the cutting treated with *Bacillus megaterium*. Generally, it can be concluded that inoculation jujube (Li variety) sub-terminal cuttings with *Bacillus megaterium* or *Pseudomans florescence* can promote root formation as well as increase survival percentage and enhance vegetative growth of the produced transplants.

BTP05

Optimizing of γ-Polyglutamic acid production by glutamate independent *B. licheniformis* strain DSMZ 8785

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Introduction: γ-PGA is a homo amino acid polymer with various applications. Especially the use of the cell-free supernatant containing the γ-PGA itself, was of interest in this work. In fact that γ-PGA is edible, non-toxic and biodegradable the polymer should be produced with view for an industrial use in the biomining processes for the future.
Methods: The modified Medium E was used for the cultivation process. Optimization was done with view on the yield of γ-PGA with different carbon and nitrogen sources. The carbon sources were citric acid and glycerol and ammonium chloride was the nitrogen source. Cultivation was executed with a 6-fold 1L bioreactor system. During the cultivation time samples were taken, directly followed estimation the optical density and determination the concentration of γ-PGA by photometric CTAB-assay. The cells were separated by centrifugation with 20,000 g for 20 minutes. Furthermore the cultivation process was observed by estimation the carbon and nitrogen sources with enzymatic determination kits.
Results: In all triplicate trials the start concentration of the carbon sources and the nitrogen source was varied. Citric acid was depleted first, Glycerol use followed. It was possible to produce γ-PGA in the range from 1 g/L to about 26 g/L. The viscosity increased during the cultivation and the oxygen transfer was influenced.
Conclusion: In fact of the co-produced γ-PGA degrading enzyme, it´s recommended to heat the supernatant to provide a degradation of γ-PGA. By use of the CTAB assay was no further purification of the supernatant necessary. With view on the results it could be shown that DSMZ 8785 is a glutamate independent γ-PGA producer with the ability to form high concentrations of γ-PGA. Further studies will aim on an intensive use of the produced γ-PGA for biomining applications.

BTP06

***Synechocystis* biofilms as solar driven biocatalysts**

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Question: How to design a biocatalyst, which continuously produces value added compounds from sunlight, CO_2 and water?
In the course of the debate regarding fossil carbon and energy, a lot of research is invested into the development of biological catalysts fuelled by sunlight, CO_2 and H_2O. Besides focussing on the biorefinery approach and maximizing biomass, photoautotrophic microbes are harnessed for directly producing a whole bunch of interesting compounds. However, low activities, low stabilities, and slow growth are problems these approaches are facing. Here we report on utilizing a biofilm based concept to realize a truly continuous bioprocess as these microbial communities feature extraordinary robustness and permanent regeneration.[1]
Method: In this study we investigate the ability of the photo-autotroph microbe *Synechocystis* sp. PCC6803 for biofilm formation with the long-term goal to develop a biofilm catalyst able to produce value added compounds fuelled by sunlight, CO_2 and H_2O. As a first step it was necessary to find a system, allowing the long-term cultivation of a tightly attached biofilm under continuous flow conditions. In parallel a heterologous pathway for the synthesis of 1,2-propanediol directly from CO_2 was introduced into *Synechocystis* sp. PCC6803 applying a plasmid based as well as a genomic based approach.
Results*:* We successfully grew *Synechocystis* sp. PCC6803 as biofilm in a capillary microreactor.[2] However, the biofilm was characterised by the formation of long streamers (filaments) and the whole system was prone to clogging. Upon introducing air segments into the system in a segmented flow fashion, the architecture changed significantly towards a flat and homogenous structure. It was possible to operate this system for five weeks, before the experiment was actively terminated. The biofilm developed up to a thickness of 70 - 120 µm. The biofilm stopped growing at this thickness and stayed constant without any detachment events occurring afterwards. The substrates CO_2 and light were supplied in a counter-current fashion. Confocal microscopy revealed a throughout photosynthetically active biofilm, indicated by the red fluorescence of the photopigments.[3] In addition, first fermentations have been conducted yielding final product titers of 4 mM 1,2 propanediol. Strikingly, the production of the 1,2 propanediol seemed to be clearly coupled to the stationary phase of the organism, as production started when cell growth ceased.
Conclusion*:* Our findings indicate an uncoupling from cell growth and 1,2-propanediol synthesis. In addition, biofilms of *Synechocystis* sp. PCC6803 seemed to stall growth at an optimal biofilm thickness of about 100 µm. Thereby the reaction format of applying a phototroph as a catalytic biofilm for the generation of value added compounds fuelled by sunlight and CO_2 seems to be a perfect match for continuous solar driven catalysis.

[1] B. Halan et al, *Trends Biotechnol* (2012) 30:453
[2] R. Karande et al, *Biotechnol Bioeng* (2014) 111:1831
[3] C. David et al, *J Ind Microb Biotech* (2015) 42:1083

BTP07

Production of substituted phenylacetic acids by styrene-degrading bacteria

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Question: A number of soil bacteria is able to metabolize styrene via initial side-chain oxygenation into the central metabolite phenylacetic acid and harbors corresponding genes [1]. This pathway of styrene degradation is of potential biotechnological relevance for the production of especially phenylacetic acids. These aromatic acids are important for various industries. In this study [2] we report on the establishment of a process using native microorganisms for the co-metabolic production of substituted phenylacetic acids.
Methods: The styrene-degrading representatives *Rhodococcus opacus* 1CP, *Pseudomonas fluorescens* ST, *Gordonia* sp. CWB2 and the novel

isolate *Sphingopyxis fribergensis* Kp5.2 were initially investigated with respect to their applicability to produce substituted phenylacetic acids. Therefore, cultivation and inducing conditions were optimized and biotransformation strategies were developed to accumulate the products to high concentrations and yields. Products formed were quantified by HPLC. Further optimization of the process was performed in a 5L-fermenter.

Results: This study has shown that all strains differ in substrate specificity and product yields. Remarkably, the synthesis of smaller amounts of the pharmaceutical 4-isobutyl-α-methylphenylacetic acid (ibuprofen) could also be shown for *Gordonia* sp. CWB2 while all other strains were not able to form this product. Furthermore, a stereoselective potential of this multi-step biotransformation was demonstrated for some strains using 4-chloro-α-methylstyrene as substrate.

Pseudomonas fluorescens ST has been identified as a promising biocatalyst for the synthesis of 4-chloro-, 3-chloro-, 4-fluoro-, α-methyl-, and 4-chloro-α-methylphenylacetic acid. Additionally, whole-cell biotransformation of 4-chlorostyrene with cells of strain ST yielded about 27.5 mmol l^{-1} product after nearly 350 days. In the next step of this study, this first result was optimized to >7 mmol l^{-1} product per day applying concentrated styrene-adapted biomass under improved culture conditions. It was shown that a decreasing pH limits the transformation efficiency in some extent. This limitation was subsequently overcome by using a pH-regulated fermenter and the product amounts could be improved again.

Conclusion: A promising strategy to produce various substituted phenylacetic acids was established using native styrene-degrading strains, especially *Pseudomonas fluorescens* ST. In contrast to non-substituted phenylacetic acid, the substituted products were enriched during the process in the medium because the substitution reduces or prohibits the further degradation.

[1] O'Leary, N. D., K. E. O´Connor, A. D. W. Dobson. 2002. FEMS Microbiol Rev 26:403-417.
[2] Oelschlägel, M., S. R. Kaschabek, J. Zimmerling, M. Schlömann, D. Tischler. 2015. Biotechnology Reports 6:20-26.

BTP08

Biochemical characterization and stability improvement of 'thermophilic-like' ene-reductases by site-directed mutagenesis

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Ene-reductases are widely applied flavoproteins for the asymmetric reduction of activated C=C-bonds through a trans-hydrogenation reaction of relevant industrial chemicals. The characterization of novel biocatalysts is from great interest regarding to their stability against temperature and organic solvents.

Two novel `thermophilic-like` ene-reductases OYE*Ro*2 and FOYE from the actinobacterium *Rhodococcus opacus* 1CP and from the iron-oxidizing betaproteobacterium *Ferrovum myxofaciens* JA12 were discovered by genome mining and subsequent multiple sequence alignment (Riedel *et al.*, 2015). Both enzymes were overexpressed in *Escherichia coli* BL21 and biochemically characterized and classified by bioinformatically methods.

Both, OYE*Ro*2 and FOYE use non-covalently bound $FMNH_2$ as reduction equivalent and are strongly NADPH dependent. They were also active with the mimic cofactor BNAH (Paul *et al.*, 2013) and present highest specific activities (OYERo2: 45−50 U mg^{-1}; FOYE: 60−70 U mg^{-1}) on maleimides, which are efficiently converted to the corresponding succinimides. The OYE*Ro*2-mediated reduction of prochiral alkenes afforded the (*R*)-products with excellent optical purity (*ee* > 99 %) (Riedel *et al.*, 2015). A sequence alignment and dendrogram of 15 characterized `thermophilic-like` ene-reductases explains the similarity in biochemical properties of OYE*Ro*2 and FOYE.

OYE*Ro*2 was found to be not as thermo-resistant as related OYEs. Introduction of a characteristic intermolecular salt bridge by site-specific mutagenesis raised the half-life of enzyme inactivation at 32 °C from 28 min to 87 min and improved the tolerance towards organic co-solvents. The replacement and influence of a critical cysteine (Cys) by the amino acids Ala, Gly, and Ser on substrate inhibition is discussed.

Riedel, A., Mehnert, M., Paul, C. E., Westphal, A. H., Van Berkel, W. J., and Tischler, D. (2015). Functional characterization and stability improvement of a 'thermophilic-like' ene-reductase from *Rhodococcus opacus* 1CP. *Front. Microbiol.* 6:1073doi: 10.3389/fmicb.2015.01073
Paul, C. E., Gargiulo, S., Opperman, D. J., Lavandera, I., Gotor-Fernández, V., Gotor, V., Taglieber, A., Arends, I. W. C. E., and Hollmann, F. (2013). Mimicking nature: synthetic nicotinamide cofactors for C=C bioreduction using enoate reductases. *Org. Lett.* 15, 180-183.

BTP09

Release of water-soluble dibutyrin explains clearing of tributyrin emulsion by lipase *in vitro* or during screening for microbial lipase producers

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Lipase activity is known to generate clearance halos on turbid agar plates when tributyrin (1, 2, 3-Propanetriyl tributanoate) droplets are used as substrate. Such indicator plates allow screening and qualitative analysis of environmental samples for lipase producing microorganisms [1]. Recently, reliable quantification of lipase activity with enzymes from fungi (*Candida rugosa*, *Rhizomucor miehei*, *Thermomyces lanuginosus*) and bacteria (*Burkholderia cepacia, Chromobacterium viscosum, Pseudomonas fluorescens*) was shown in cavities of microtiter plates [2].

Question: How can clearing of turbid lipid emulsions in agar plates or microtiter cavities be explained?

Methods and Results: Total hydrolysis of tributyrin was excluded as explanation for the clearing phenomenon because release of glycerol was limited to 1 % of the theoretical maximum [2]. Glycerol was detected by a specific enzymatic test, by HPLC, or by titration. On the other hand up to 80 % of the expected glycerol was found when tributyrin was replaced with rapeseed oil in shake flasks where recombinant *Thermomyces lanuginosus* lipase was incubated with the triglyceride for more than 20 hours. In the case of tributyrin an accumulation of 1,2 dibutyrin and 1,3 dibutyrin was detected by thinlayer chromatography. Both products of partial hydrolysis were purified in gram scale from 50 ml shake flask lipase hydrolysis experiments by chromatography and identified by NMR.

At the moment solubility experiments and kinetic studies with water-dissolved dibutyrin forming a second phase at the critical micelle concentration (cmc) are performed. It is expected that interphase activation causing a conformational change of the lipase is also true for dibutyrin with substrate concentrations above cmc. Furthermore, it is assumed that in the turbid agar plates or microtiter cavities the concentration of dibutyrin is below its cmc. Soluble dibutyrin is a poor substrate for the lipase and therefore a low end concentration of glycerol is reasonable.

Conclusion: Clearing of agar plates turbid by immobilized droplets of tributyrin is three times faster than expected. A single hydrolysis reaction into butyric acid and dibutyrin is sufficient because both products, butyric acid and dibutyrin are diffusing into the water phase.

[1] Lawrence *et al.* (1967) Nature 213:1264-1265.
[2] Barig *et al.* (2013) Analytical and Bioanalytical Chemistry 405: 8539-47.

BTP10

25-Hydroxyvitamin D_3 synthesis using a bacterial Mo-enzyme for steroid side chain hydroxylation with water

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Question: Vitamin D_3 (VD_3, cholecalciferol) is generally known for its regulatory function in calcium and phosphorous homeostasis, but is also recognized as an anti-proliferative factor for dividing cells and tissues.[1] VD_3 is formed from proVD_3 (7-dehydrocholesterol) by UV-B irradiation and thermal [1,7] sigmatropic hydrogen shift in the skin. VD_3 is converted into its biologically active form by consecutively acting cytochrome P450 monooxygenases (CYPs) in the liver (25OHVD_3, calcidiol) and kidney ($25(OH)_2VD_3$, calcitriol). The hydroxylation into 25OHVD_3 is a crucial reaction, as 25OHVD_3 represents the circulating, and clinically most relevant form of VD_3. Insufficient levels are linked to numerous forms of cancer but also to cardiovascular diseases, immunodeficiency and diabetes. Chemical synthesis of 25OHVD_3 requires multiple steps with low yield and selectivity, whereas the use of CYPs depends on complex electron donor system. In this work we tested the use the denitrifying *Sterolibacterium denitrificans* grown on cholesterol as only carbon source for the selective hydroxylation of VD_3 to 25OHVD_3. This organisms contains eight Mo-containing hydroxylases that are potential candidates

for the specific hydroxylation of VD_3 at C25 by water, requiring only a regenerative electron acceptor such as ferricyanide.[2]
Methods: Crude extracts of *S. denitrificans* grown with different steroids were tested for the *in vitro* conversion of VD_3 and $proVD_3$ to the corresponding C25-hydroxylated forms in the presence of various solubilizing agents. The VD_3 hydroxylating enzyme was purified and the encoding gene identified. Products of hydroxylation reactions were analyzed by MS- and MNR-techniques.
Results: Extracts of *S. denitrificans* catalyzed the ferricyanide-, and cyclodextrin-dependent specific hydroxylation of VD_3 (1 mM) to $25OHVD_3$ with >99 % yield and selectivity. The VD_3 hydroxylating enzyme was purified by four chromatographic steps and identified as the previously described cholesterol C25 dehydrogenase. We demonstrate that the specific role of cyclodextrin was to alter the equilibrium between VD_3 and $preVD_3$ to right side, and that $preVD_3$ is the actual substrate for the water-dependent hydroxylation reaction. Once formed the product $25OHpreVD_3$ is released from cyclodextrins and isomerizes back to the desired product $25OHVD_3$.
Conclusion: The facile and robust method developed is a novel example for the concept of substrate-engineered catalysis and offers an attractive alternative to chemical or O_2/electron donor-dependent enzymatic procedures and may be useful for treatments of $25OHVD_3$ deficiencies and to satisfy the high demand for monitoring its plasma levels as a result of an increasing number of VD_3-linked disorders.

[1] Holick (2007), New Eng J Med, 357:266-81.
[2] Dermer and Fuchs (2012), J Biol Chem, 287(44):36905-16.

BTP11
Fate of sulfonamide and trimethoprim resistant bacteria and resistance genes in constructed wetlands
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Question: The current extensive release of antibiotic resistant bacteria (ARB), respectively antibiotic resistance genes (ARG), into the environment is of significant concern. Here, we asked whether constructed wetlands (CWs) are suitable to treat domestic wastewater containing ARB/ARG; i.e. which mechanisms are governing whether ARG are eliminated, maintained, multiplied, or distributed in near-natural habitats like CWs?
Methods: Two pilot-scale horizontal subsurface flow CWs, planted with common reed (*Phragmites australis*) and receiving the same inflow (secondary clarifier effluent from a nearby wastewater treatment plant), were investigated over a sampling period of almost 2 years. One CW was aerated, the other was not. Abundances of sulfamethoxazole and trimethoprim resistant bacteria (SMX^R and TMP^R) along the CWs' flow paths were quantified by plating coupled with phylogenetic identification of resistant isolates. Concomitantly, respective resistance genes (*sul-I*, *sul-II*, *dfrA1*) were enumerated by qPCR analysis. Those genes were selected as ARG indicators since they can be abundant in anthropogenic sources but are rare in native aquatic and terrestrial ecosystems as well have frequently different linkages to mobile genetic elements. In addition, standard wastewater parameters incl. numbers of *Escherichia coli* (quantified by MPN counting and qPCR) were recorded and bacterial community profiling was carried out via 454 pyrosequencing.
Results: The inflow displayed microbiological features typical of secondary treated wastewater, including abundances of the selected ARG ranging from ca. 10^4 to 10^5 copies/100 ml. In the non-aerated wetland there was a roughly-steady decline of the ARB/ARG by about 1 to 1.5 log units along the flow path. In contrast, in the aerated CW the numbers of SMX^R, *sul*-I, and *sul*-II increased first till a maximum at about 2 m downstream from the influent before they decreased by about 2 log units. Furthermore, the SMX^R species profile changed thru the passage of the aerated wetland. No strong shift in *sul* hosts was observed for the non-aerated bed, or for *dfrA1* in either CW. Total bacterial community profiles, enumerations of *E. coli*, and standard wastewater parameter indicate that the aerated CW became an unsuitable habitat for many bacteria present in the inflow at about the 2 m mark, while the non-aerated bed did not appear to constitute a particularly stressful habitat.
Conclusion: The CW biotechnology appears to be a promising approach to attenuate or even eliminate ARG/ARB from wastewater. However, this study also provides evidence that common habitat stress may be a significant factor governing the dispersal of ARG when those are present in a mobile genetic context.

BTP12
Keratin waste biodegradation and peptide production by keratinolytic proteinase from *Bacillus methylotrophicus* AD-AA3
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Introduction: Keratinolytic proteinases or keratinases (EC 3.4.21/24/99.11) are a particular class of proteolytic enzymes that displays the capability of degrading insoluble keratin substrates such as fibrin, keratin, elastin, collagen and soluble substrates such as sodium caseinate, albumin and gelatin or other keratin-like material [1]. Keratinolytic proteinases are produced by various microorganisms including bacteria, actinomycetes and fungi and belongs to metallo and serine or metallo-serine proteases based on their catalytic type [2]. Keratin-rich waste do not accumulate in environment due to natural biodegradation mechanism by keratinolytic proteinases [3]. Conventional not enough effective keratinous waste degradation methods can be replaced by eco-friendly enzymatic biodegradation methods. Therefore, identification, characterization and development of new powerful and efficient biocatalyst for keratin waste biodegradation are required.
Methods: The newly isolated *B. methylotrophicus* AD-AA3 strain was grown in minimal medium containing 0.1 % (w/v) keratin from wool as sole carbon and energy source. The medium was precipitated by solid ammonium-sulphate up to 80 % saturation. Partially purified keratinolytic proteinase (BMKer) was used for further analysis of substrate specificity and capability to produce value peptides. Obtained degradome was analysed by thin-layer chromatography (TLC) and compared with commercial peptides generated by non-biological treatment. Moreover, degradome was analysed with low molecular weight tricine PAGE with incorporated ethylene glycol (LMW-T-EG-PAGE) [4].
Results & Conclusions: In this study BMKer enzyme from *B. methylotrophicus* AD-AA3 strain was successfully produced and partially purified. Physical and chemical characterisation of native BMKer suggested that new keratinolytic proteinase is powerful biocatalyst for efficient keratin waste biodegradation and can replace conventional insufficient non-biological hydrolysis processes without energy, important amino acids and nutritional element loss. High value bio-active hydrolysis products - peptide obtained from keratin waste biodegradation by BMKer are suitable for industrial applications in white and green biotechnology.

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BTP13
Explorating the biosynthetic capability of ganefromycin by direct cloning and heterologous expression
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Ganefromycins produced by *Streptomyces*(*S.*)*lydicus ssp. Tanzanius NRRL* 18036, belong to the elfamycin family and display a narrow spectrum of antibacterial activity against human pathogens[1].The screening of the target cosmid library of *S. lydicus ssp. Tanzanius NRRL* 18036 has been finished by colony PCR screening. We used homologous probes to detect the PKSI genes and the 2,3- or 4,6-dehydratase genes, and then successfully identified four cosmids (cosmid 26, cosmid201, cosmid21 and cosmid19). Those cosmids were sequenced and analysed subsequently revealing the presence of 26 open reading frames (ORFs). In order to verify those cosmids containing the gene cluster for the biosynthesis of ganefromycin, we carried out the gene disrupted experiment *in vivo*. In addition, we started experiments to construct an heterologous expression system for this gene cluster by adopting the transformation-associated recombination(TAR) technology[2]. The establishment of this heterologous expression system will help us to lay a foundation for subsequent research on the biosynthetic pathway of ganefromycin.

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BTP14

Establishing the CRISPR/Cas-System in *Dictyostelium discoideum*

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The social amoeba *Dictyostelium discoideum* is a ubiquitous inhabitant of soil and is typically found on acidic leaf litter where it preys on bacteria. Upon food starvation it enters a development cycle in which up to 10^5 amobae stream together eventually to form a multicellular pseudoorganism. *D. discoideum* has served as a model organism for the last decades and major insights in the areas of social evolution, cellular function and chemotaxis have been made by use of this organism. In particular inter- and intracellular signaling processes are important in this social amoeba. Genome sequencing provides an insight in the wealth of genes devoted to these processes.[1] Some amoebal secondary metabolites, which are the product of polyketide synthases (PKS), are important communication signal molecules during the development phases. The genome of *D. discoideum* contains over 40 putative *pks* genes and the biosynthesis products of these PKS as well as their function are largely unknown. To identify and investigate these products, we want to use a combination of molecular biology and analytical chemistry tools. Therefore knockout mutants of selected *pks* genes will be generated and the resulting secondary metabolome will be compared with the wildtype strain. The *pks* genes of *D. discoideum* represent a class of genes with a high nucleotide sequence similarity. For this reason well-established methods for the editing of the social amoeba's genome do not necessarily meet with success. The aim of this project is to enable targeted genome editing in *D. discoideum* using the clustered, regularly interspaced repeated short palindromic repeat (CRISPR) RNA-guided Cas9 nuclease molecular tool. This tool is very specific for highly homologous genes, because it only needs nucleotide sequences of 20 base pairs to induce a double strand break and, consequently, generating a knockout *via* non-homologous end-joining.[2] We want to establish this technology in *D. discoideum* thus generating a mutant library of *pks* genes in *D. discoideum* to elucidate the structure and function of previously unknown secondary metabolites. Furthermore, this efficient methodology provides complementarity for existing methods for genome editing and would benefit the entire *Dictyostelium* community.

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BTP15

SoxR as a single-cell biosensor for NADPH-consuming enzymes in *Escherichia coli*

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Question: NADPH-dependent enzymes, such as stereospecific alcohol dehydrogenases, are important tools e.g. for industrial biotransformations. An ultra-high-throughput screening system for these enzymes was established that is based on the [2Fe−2S] cluster-containing transcriptional regulator SoxR of *Escherichia coli* that activates expression of *soxS* in the oxidized but not in the reduced state of the cluster. As SoxR is kept in its reduced state by NADPH-dependent reductases, an increased NADPH demand of the cell counteracts SoxR reduction and increases *soxS* expression. We have taken advantage of these properties to construct an NADPH biosensor by placing the *eyfp* gene under the control of the *soxS* promoter. The aim of this study was to test the NADPH biosensor in *E. coli* cells expressing an NADPH-dependent alcohol dehydrogenase from *Lactobacillus brevis* (*Lb*Adh), which reduces methyl acetoacetate (MAA) to (R)-methyl 3-hydroxybutyrate (MHB). Under suitable conditions, the specific fluorescence of the cells should correlate with the substrate concentration added and with *Lb*Adh enzyme activity, supporting the NADPH responsiveness of the sensor. Furthermore we tested whether the NADPH biosensor can be used for high-throughput screening of NADPH dependent alcohol dehydrogenases with optimized properties.
Methods: For the analysis of the NADPH responsiveness of the biosensor a BioLector system was used. This system allowed forrecording of eYFPfluorescence and growth. Another method that was applied due tothefluorescent properties of the NADPH biosensor was fluorescence activated cell sorting (FACS).
Results: During the NADPH-consuming biotransformation of MAA to MHB, we showed that the maximal specific fluorescence of cells expressing the NADPH biosensor correlates with the concentration of MAA reduced to MHB between 0 mM MAA and 70 mM MAA. Moreover, the properties of the NADPH biosensor enabled sorting of single cells harboring wild-type *Lb*Adh from those with lowered *Lb*Adh activity or without *Lb*Adh activity by FACS. Based on these results the NADPH biosensor was successfully used to screen a mutant *Lb*Adh library for variants showing improved activity with the substrate 4-methyl-2-pentanone.
Conclusion: The NADPH biosensor responds to alterations in the NADPH concentration of *E.coli* cells. Therefore this biosensor shall be exploited in future studies for the directed evolution of NADPH-dependent alcohol dehydrogenases, which have wide applications in industrial biotransformations.

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BTP16

Enzymatic bioreactor for simultaneous synthesis of fine chemicals and energy production

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We introduce an enzymatic bioreactor for simultaneous electrosynthesis and electricity production, which is based on a laboratory prototype of flow bioreactor with a porous activated carbon felt (CF) bioanode and a commercially available oxygen gas-flow cathode. CF was first covered with multi-walled carbon nanotubes (MWCNT) and electropolymerized methylene green (PMG), which was then covered with a silica gel layer encapsulating the D-sorbitol-dehydrogenase (DSDH). The co-immobilization of DSDH and the mediator allowed performing a stereoselective conversion of D-sorbitol into D-fructose with appreciable rate. The enzyme immobilized in the silica film showed excellent operational stability for at least two weeks. The recovery of the electric power generated during the bioconversion was achieved by means of electrochemical regeneration of the cofactor at the bioanode and use of an oxygen gas-flow cathode, without the need for a membrane. Having zero-current voltage of 0.25 V, the developed bioreactor was able to deliver a peak power of 14.6 $\mu W\ cm^{-3}$ at 0.1 V. Although DSDH does not produce compounds of high value, it was chosen as a model dehydrogenase because there are many electroenzymatic data available for comparison[1,2,3]. The results of the proof of concept are a prerequisite for later replacement of DSDH by other dehydrogenases with substrate spectra of higher interest[4,5,6,7].

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BTP17

Immobilization of cysteine-tagged dehydrogenases on macroporous carbon felt by click chemistry for electroenzymatic synthesis

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Compared to conventional organic synthesis, enzymatic electrosynthesis is a green and sustainable processes suitable for the multi-step reactions under mild conditions with higher yields. During the process, the key step to successful electrosynthesis is enzyme immobilization. Up to now, different strategies for immobilization of enzymes on porous materials has been developed in order to enhance the stability as well as catalytic activity and the recyclability of the enzymes [1]. In the present work, a facile metal-free 'click' type thiol-ene reaction [2] was utilized to immobilize the cysteine-tagged D-sorbitol-dehydrogenases (cys-DSDH) on carbon felt (CF). This bio-system is proved to be stable and efficient for enzymatic oxidation of D-glucitol, and the direct electrochemical regeneration of NADH has been successfully applied. Besides, the multi-wall carbon nanotube was deposited on the carbon felt in order to increase the surface area and efficiency of this cys-DSDH-CNT/CF system.

Meanwhile, cys-DSDH catalyzed enzymatic reduction of D-fructose with electrochemical NAD^+ regeneration was realized by using $[Cp^*Rh(bpy)Cl]^+$ mediator in the solution, which is promising in electroenzymatic synthesis.

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BTP18

From serum bottles to fermentors: Scaling up the production and upgrading of organic acids with reactor microbiomes

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Question: An increasing demand for bio-based liquid fuels and chemicals is the driving force for the investigation of alternative microbiome-based fermentation technologies to upgrade low grade biomass to value added products. One process of interest is the production of long chained volatile fatty acids (VFAs), *e.g.*, based on ethanol and acetate. Numerous studies have been devoted to the identification of suitable microbiomes that can drive this process, as well as their characterization. However, these studies were mostly only performed at one scaler, i.e. lab scale or technical scale. Consequently, we assessed the transfer of an exemplary microbiome to larger reactor volumes in order to derive universal guidelines for microbiome based reactor engineering.
Methods: Enriched reactor microbiomes were applied in serum bottles (55 mL working volume), in 500 mL tailor-made glass reactors, and in technical scale bioreactors (more than 2 L working volume) for the conversion of acetate and ethanol into a mixture of VFAs. Production of liquid and gaseous metabolites as well as cell growth was monitored and the microbial community composition was characterized.
Results: This study shows a high and stable production of butyrate and caproate by the characterized microbiome across all investigated reactor scales. 300 mM acetate (C_2) and 400 mM ethanol are converted into 93 ± 3 mM butyrate (C_4) and 106 ± 1 mM caproate (C_6) within 8 days with a carbon recovery of 62.9 ± 2.9 % in the liquid phase. During the conversion process the gas phase is composed of 79.2 ± 0.3 % hydrogen (H_2) and 19.3 ±0.3 % carbon dioxide (CO_2) but no methane (CH_4) is produced. The microbiome consists of 35 % microorganisms assigned to *Clostridium* sp., based on 16s rDNA sequencing data, with half of them being classified as *Clostridium kluyveri*. This is in accordance with current publications, which assume that this microorganism is predominant in microbiomes capable of producing butyrate and caproate from acetate and ethanol. Besides this, *Sporanaerobacter* sp. and *Terrisporobacter* sp. were found in significant proportions.
Conclusion: The successful production of butyrate and caproate from ethanol and acetate was shown at different scales from 55 mL to 500 mL. This not only indicates a high potential for further scaling up and development of the studied process, but also allows deriving more general conclusions of the engineering of microbiome based processes. Next step concerning the VFA production will target in-line acid separation and process optimization for low grade biomasses and undefined substrates.

BTP19

Upgrading conventional bioreactors for bioelectrotechnology

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Question: Conventional bioreactors (i.e. fermentors) are a well-established in-depth characterized technology platform and several parameters can be highly defined or monitored online allowing cultivations and productions to take place at optimal conditions. In contrast bioelectrochemical reactor systems, as these are used to combine microbial and electrochemical conversions of different kind, are diverse and poorly characterized. For further development of the underlying concept of microbial electrochemical technologies (METs), cross-comparisons of different studies as well as benchmarking lab processes to technical scale are urgently needed. Therefore, we assessed, if the (often already existing) infrastructure of conventional bioreactors can be easily be upgraded for bioelectrotechnology.
Methods: The experiments were performed in standard bioreactors upgraded for bioelectrotechnology with volumes of 0.5, 1.0 and 2.0 L with a constant area-to-volume ratio of the electrodes throughout the scales. For the upgrade kit different materials, including polytetrafluoroethylene (PTFE) and polyether ether ketone (PEEK) were tested regarding material strength and autoclavability. As working, counter and reference electrodes graphite rods, titanium mesh and silver/silver chloride were used. Microbial model species, like *Shewanella spec.* being a typical electroactive microorganism, were investigated in one- and two-chamber set-ups, respectively. Cultivations were typically done at 30 °C under different aeration conditions with standard online-monitoring (pO_2, T, pH, substrate). Moreover, chronoamperograms at +0.2 V were recorded as well as cyclic voltammograms at certain points of time.
Results: Most important evidence is provided that the electrochemical steering as well as measurements can be performed without disturbances on the conventional online parameter monitoring and control of the bioreactor. Further, it is shown that the set-up allows not only an easy handling of microbiomes, but also of pure cultures since all reactor components are autoclavable. Additionally, depending on the process objective, working in one- or two-chamber set-ups, i.e. with or without separation of anode and cathode, is easily possible. Here, the used membrane fixation process prevents membrane leakage, allows autoclaving and membrane exchanges. Experiments with the model organism *Shewanella* demonstrated highly reproducible results across the scales being also in line with literature values.
Conclusion: There is no need to buy new reactor systems, a reversible upgrade kit for standard bioreactors was developed to perform bioelectrochemical syntheses allowing a systematic process characterization and development [1,2].

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BTP20

The liaison between microbiomes and electroorganic syntheses: Upgrading microbial intermediates to alkanes and esters

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Question: The production of renewable electric energy, including for instance photovoltaics and wind, is highly fluctuating. Thus, more flexible technologies are required to either instantaneously exploit surplus electricity or store it effectively. At the same time, microbiomes can convert different kinds of biomass into potential platform chemicals. Among others, the conversion of biomass to carboxylic acids (CA), being considered as platform molecules, is a well-established process. Here we suggest combining the stable but relatively slow microbial production of CA with fast electroorganic synthesis in order to exploit surplus electricity for the synthesis of liquid energy carriers. Therefore, we assessed if microbially produced CAs with chain lengths of C_4 to C_8 (being continuously extracted from the fermentation broth) can be electrochemically converted to energy dense alkanes (chain length: C_6 to C_{14}) and esters (C_7 to C_{15}).
Methods: Starting from renewable substrates using a specialized microbiome for chain elongation of CAs, the microbially produced CAs are extracted and enriched continuously. Batchwise, this enriched CA mixture is electrochemically oxidized at platinum electrodes to energy dense hydrocarbons. Using HPLC and GC analysis, the production rate of CAs during fermentation, the extraction efficiency as well as the performance of the electroorganic synthesis of alkanes and esters are evaluated.
Results: For yeast fermentation beer, a sum parameter of CAs of 7.5 $g_{COD}\ L^{-1}d^{-1}$ was achieved[1]. An electrochemical conversion efficiency of $\approx 5\text{-}9\times10^{-6}$ $mol_{CA}C^{-1}$ was realized, resulting in product mixtures containing roughly 50 % alkanes. The reaction rate of the electrochemical step is dependent on the characteristics of the CA and the electrolytic conductivity of the enriched CA mixture.
Conclusion: We conclude that the suggested approach of combining microbial biomass conversion with electrochemical upgrading can become efficient and thus well suited to store surplus electric energy in liquid fuels. However, the single steps of the process line need to be optimized.

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BTP21

Transcriptional activity of the rDNA promoters in *Corynebacterium glutamicum*

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Question: *Corynebacterium glutamicum* is a Gram-positive bacterium mainly used for the biotechnological production of amino acids. It is a robust producer strain, easy to handle, and suitable for genetic engineering. However, its growth rate is significantly lower than that of other production organisms [1] and thus, there is a need for growth improvement. Possible factors influencing the growth rate are the number and the expression of the ribosomal DNA *(rrn)* operons encoding the 5S, 16S, and 23S rRNAs and in consequence, possibly the number of ribosomes.
In *C. glutamicum,* there are six *rrn* operons, which are distributed on the genome in the order *rrnA*, *rrnB*, *rrnF*, *rrnD*, *rrnE,* and *rrnC* [2]. In this study, we focus on the analysis of transcriptional activity of each of the *rrn* promoters in *C. glutamicum* under different conditions.
Methods: For investigation of the *rrn* transcriptional activity we used plasmid pET2, containing the promoterless chloramphenicol acetyltransferase (CAT) gene as a reporter gene [3]. The six *rrn* promoter regions were amplified from genomic DNA of *C. glutamicum* and cloned into plasmid pET2. The resulting vectors were transformed into *C. glutamicum* wild type and into single and multiple *rrn* deletion mutants and growth experiments were performed with different media. In the exponential growth phase, the cultures were harvested and after cell lysis, CAT assays were performed, and specific activities determined. To eliminate copy number effects, we determined the respective plasmid copy numbers by quantitative RT-PCR and corrected the observed activities accordingly.
Results: The promoter activities of the six different *rrn* operons differed significantly from each other, the *rrnF* promoter showing the highest specific activity (up to 10 U mg protein^{-1}) on all media tested. The total specific activity of all six *rrn* promoters was shown to vary on different media. Surprisingly, the *rrnF* and the *rrnE* promoter activities increased and decreased, respectively, with the number of deleted *rrn* operons, indicating a cross-regulation.
Conclusion: The results indicate that (i) the six *rrn* operons in *C. glutamicum* are differentially expressed, (ii) expression is different on different media, and (iii) the presence or absence of *rrnA, B, C,* and *D* influences the *rrnF* and *rrnE* promoters. Further experiments have to clarify the mechanism of this cross-regulation.
Acknowledgement: This work was supported by the BMBF grant 031A302E.

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BTP22

Impact of *rrn* operon deletions on growth of *Corynebacterium glutamicum*

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Question: In general, the bacterial 5S, 16S, and 23S ribosomal RNAs are encoded by the so-called *rrn* operons. Bacterial cells usually possess multiple copies of the *rrn* operon scattered across the genome. There is a distinct number of *rrn* copies in each organism, e.g. *Escherichia coli* possesses seven copies whereas slow-growing *Mycobacterium* species contain only one or two *rrn* operons. In *Corynebacterium glutamicum*, the annotation of the genome sequence provided evidence for the presence of six rDNA operons (copies *rrn*A-*rrn*F) [1].
Several studies indicate a correlation between abundance of ribosomes and growth rate and the number of ribosomes may be dependent on the availability of ribosomal proteins and rRNAs. *C. glutamicum* renders growth rates of up to 0.6 h^{-1}, which is relatively low compared to *E. coli,* which exhibits growth rates of up to 2.61 h^{-1} [2]. Our project aims to identify and characterize factors influencing the growth rate in *C. glutamicum* and finally, to improve the growth rate of this industrially important organism. One such factor presently under investigation is the *rrn* operon copy number and its impact on the growth rate of *C. glutamicum*.
Methods: Single and multiple *rrn* deletion mutants of *C. glutamicum* CR099 with up to five deleted *rrn* operons have been constructed via homologous recombination using the suicide vector pK18*mobsacB.* The complete set of mutants was tested for growth characteristics on complex and minimal media with glucose, maltose or acetate as carbon source. These experiments were performed in 50 ml shaking flasks and in a parallel fermenter system with 600 ml culture volume.
Results: The final optical densities of all single and multiple *rrn* deletion mutants were roughly identical to that of the reference strain *C. glutamicum* CR099 in all media and under all conditions tested. In contrast, the growth rates were significantly lower (down to 30 %) with those strains lacking four or five of the *rrn* operons. Interestingly, the degree of growth rate decline of the quadruple and quintuple deletion mutants was dependent on the type of medium and on the carbon source.
Conclusion: Our experiments revealed that an impact of *rrn* deletions on the growth of *C. glutamicum* CR099 is only obvious when four or five of the six *rrn* operons have been deleted. These results show that *C. glutamicum* can compensate the loss of up to four *rrn* operons by so far unidentified mechanisms. Furthermore, the results indicate that growth conditions have an influence on the compensation for deleted *rrn* operons.
Acknowledgement: This work was supported by the BMBF grant 031A302E.

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BTP23

Development of biosensors for the detection of precursors involved in plant polyphenol production with *Corynebacterium glutamicum*

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Question: Plant-derived polyphenols such as resveratrol are of great biotechnological interest due to their diverse beneficial and health-promoting properties. Since classical extraction from plants can have several drawbacks, *e.g.* low yields, microbial production of these substances is highly desired. In this context, biosensors represent powerful tools to shorten the time needed for the construction of suitable production strains. Biosensors can be designed based on bacterial transcription factors that specifically bind target compounds and promote expression of a reporter gene, which results in a fluorescence signal. Here we aim at the design, construction and characterization of biosensors for the detection of precursors involved in polyphenol production using the biotechnological platform organism *Corynebacterium glutamicum* as production host.
Methods: Constructed biosensors were evaluated by fluorescence measurements in a microbioreactor system. FACS (fluorescence-activated cell-sorting) was employed for single-cell analyses of engineered strains carrying sensor plasmids.
Results: Two transcriptional regulators were employed to design biosensors for the detection of polyphenol building blocks: I) repressor PhdR from *C. glutamicum* involved in phenylpropanoid degradation for sensing phenylpropanoid CoA-thioesters [1] and II) the repressor FapR from *Bacillus subtilis* for sensing malonyl-CoA [2]. The constructed sensor plasmids were tested and characterized in *C. glutamicum* strains. As a result, increased fluorescence signals, correlating with the amount of effector added, could be detected. Increased intracellular fluorescence in response to the effector molecules on the single-cell level was confirmed by FACS measurements.
Conclusions: Biosensors for intracellular detection of phenylpropanoid CoA-thioesters and malonyl-CoA were constructed and characterized and will be applied for the development of superior *C. glutamicum* strains with increased precursor supply.

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BTP24

Optimization of Sec-dependent heterologous protein secretion in *Corynebacterium glutamicum*

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Question: So far, *Corynebacterium glutamicum* is used in industry almost exclusively for the production of amino acids and other low-molecular weight compounds. Besides this, recent findings have indicated that *C. glutamicum* also has a great potential as a host organism for the Sec-dependent secretory

production of heterologous proteins [1]. Various previous studies have shown that the secretion efficiency of a given protein via the bacterial Sec pathway is affected by different parameters such as growth medium composition, culture conditions, or the properties of the vector that is used to drive the expression of the target protein. Importantly, previous studies in *B. subtilis* have shown that the choice of the correct signal peptide that is required for Sec-dependent membrane transport is probably the most critical factor that ultimately determines the amount of a desired target protein that is secreted into the culture supernatant [2].
Using a cutinase from the fungus *Fusarium solani pisi* as a heterologous model protein, we here asked whether heterologous protein secretion by *C. glutamicum* can be optimized by signal peptide variation and whether the relative ranking of different signal peptides with respect to cutinase secretion is affected when different cultivation conditions and/or different strain backgrounds are used.
Methods: Cutinase fused to 5 different signal peptides was expressed in *C. glutamicum* wild-type and a *C. glutamicum* ΔhtrA mutant strain and the corresponding cells were grown in a BioLector microreactor cultivation device (m2p-labs) under different cultivation conditions. Subsequently, the amounts of cutinase secreted into the respective culture supernatants were determined.
Results: Our results clearly show that nature of the signal peptide that is used to drive the Sec-dependent translocation of the cutinase across the *C. glutamicum* cytoplasmic membrane is a major factor that determines the ultimate yields of cutinase in the culture supernatant. Furthermore, our results also show that the relative quality of different signal peptides is influenced by the tested culture conditions and/or the chosen strain background.
Conclusions: Heterologous protein secretion by *C. glutamicum* can be optimized by signal peptide and strain background variations. Furthermore, our data showing an interdependence of various secretion optimization parameters indicate that process optimization for a given heterologous target protein should be done as close as possible to the envisaged final production conditions.

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BTP25
Development of a platform for engineering the sensory properties of the histidine sensor kinase CitA

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Question: Bacterial two-component signal transduction systems (TCS) are required for sensing environmental stimuli and adapting the organism's metabolism [1,2]. Recently, these systems also emerged as promising tools for engineering novel couplings between sensory functions and gene expression [3]. Here, we employed the CitAB-TCS of *C. glutamicum* to construct a platform which enables easy manipulation and characterization of its ligand-induced activation.
Methods: A triple mutant of *C. glutamicum* which lacks *citAB* as well as genes enabling citrate uptake (*citH, tctCAB*) was constructed. This strain was transformed with different "sensor plasmids" encoding *citAB* and the *eyfp* gene under transcriptional control of promotors activated by CitB (either P_{tctC} or P_{citH}). Moreover, the native ribosome binding sites were replaced by optimized variants derived from the expression vector pET-16b. To complement the analysis of citrate-induced CitA activation *in vivo* the citrate affinity of the PAS domain was determined *in vitro* by Microscale Thermophoresis and Isothermal Titration Calorimetry.
Results: Expression of plasmid encoded *citAB* provided a functionally active CitAB-TCS in each of the four reporter strains as judged by citrate inducible eYFP expression. In addition, single cell analysis revealed homogenous expression of the reporter gene. The concentration dependence of the fluorescence output correlates to the affinity determined *in vitro* by biomolecular interaction analysis.
Conclusion: Based on the homogeneity and concentration dependence of the eYFP fluorescence the four reporter strains represent ideal platforms for altering the sensory functions of the CitA histidine kinase. This system is complemented by our experimental setup for characterizing the ligand binding properties of the sensory domain *in vitro*.

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BTP26
Metabolic engineering of *Corynebacterium glutamicum* for the synthesis of 2-methyl-1-butanol and 3-methyl-1-butanol

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Question: The aim of this work was the construction of *Corynebacterium glutamicum* strains for microbial production of 2-methyl-1-butanol and 3-methyl-1-butanol from the keto acids 2-keto-3-methylvalerate and 2-ketoisocaproate, respectively. These keto acids, both intermediates of the branched-chain amino acid metabolism, were converted to the desired alcohols through decarboxylation by a ketoacid decarboxylase and subsequent reduction by an alcohol dehydrogenase.
Methods: The *C. glutamicum*-based pentanol production strains were constructed by genetic engineering and characterized in shake-flask cultivations. HPLC and GC methods were employed for precursor and product quantification.
Results: L-Isoleucine-producing *C. glutamicum* strain K2P55 [1] and 2-ketoisocaproate-producing *C. glutamicum* strain MV-KICF1 [2] served as basis for the strain construction. *C. glutamicum* K2P55 was converted to a 2-keto-3-methylvalerate producer by a START codon exchange in the *ilvE* gene coding for the branched-chain amino acid transaminase. Subsequently, codon-optimized synthetic genes for ketoacid decarboxylases (*kivD* from *L. lactis*, *thi3* and *aro10* from *S. cerevisiae*) and alcohol dehydrogenases (*adh2* from *S. cerevisiae*, *adhA* from *C. glutamicum* and *yqhD* from *E. coli*) were cloned into plasmid pEKEx2 for the heterologous expression in these *C. glutamicum* strains. For the evaluation of the performance of these genes during pentanol production, different combinations of these heterologous genes were expressed in both *C. glutamicum* ketoacid producing strains. The resulting strains were grown aerobically in shake flasks for cell mass formation and subsequently cultivated under anaerobic (or microaerobic) conditions for pentanol production. The pentanol accumulation was followed over time in cell-free supernatants by gas chromatography analyses.
Conclusion: The constructed strains represent the first *C. glutamicum*strains for the production of 2-methyl-1-butanoland 3-methyl-1-butanol from glucose. Future strain engineering and optimization of cultivation and production conditions currently conducted in our lab will further improve the overall production performance of these strains.

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BTP27
Screening for fungal enzymes as novel biocatalysts for the production of chiral β-amino acids

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Chiral β-amino acids are valuable building blocks for pharmaceuticals and fine chemicals (Pilsl and Reiser 2011). Thus this work focuses on the development of a modified hydantoinase process using racemic dihydropyrimidines as educts for the production of chiral β-amino acids. The process is to be based on two enzymes. A cyclic amidase will be used for hydrolytic cleavage of the dihydropyrimidine ring followed by the reaction of a linear amidase able to decarbamoylate *N*-carbamoyl β-amino acids.
In previous work it was demonstrated that hydantoinases can hydrolyze racemic 6'-substituted dihydropyrimidines to the corresponding *N*-carbamoylated β-amino acids (Engel *et al.* 2012). However, up to now no enzyme for the subsequent decarbamoylation of aromatic *N*-carbamoylated β^3-amino acids to β^3-amino acids is described.
In order to find novel enzymes able to catalyze this decarbamoylation reaction the screening of several fungi is planned. They are to be cultivated in minimal medium containing the model substrate *N*-carbamoyl β-phenylalanine as sole source of nitrogen. It is hypothesized that fungi able to growth under these conditions use linear amidases (e.g. β-ureidopropionases) to exploit the model substrate as nitrogen source. These fungi are to be further investigated and the responsible enzymes are to be identified, characterized and applied for the modified hydantoinase process.

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BTP28
Chemoenzymatic synthesis of aromatic β-amino acids
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Hydantoins and related compounds are promising substrates for the synthesis of optically pure amino acids. In particular, the production of D-amino acids as side chains for semisynthetic beta-lactam antibiotics via the so-called "hydantoinase process" is well established on industrial scale [1].
A modified hydantoinase process was proposed for the synthesis of β-amino acids, which are gaining importance in the pharmaceutical industry. The enantioselective conversion of substituted dihydropyrimidines to *N*-carbamoyl-β-amino acids was successful using whole-cell biocatalysis. [2, 3]
A novel substrate, 6-(4-nitrophenyl)dihydropyrimidine-2,4(1*H*,3*H*)-dione (pNO$_2$PheDU), was chemically synthesized. The hydantoinase from *Arthrobacter crystallopoietes* DSM20117 was chosen to prove the enzymatic hydrolysis of this substrate. Whole cell biotransformations with recombinant *Escherichia coli* expressing the hydantoinase showed degradation of pNO$_2$PheDU [4].
Additionally, the corresponding *N*-carbamoyl-*β*-amino acid (*N*Carb$p$NO$_2$*β*Phe) was chemically synthesized, an HPLC-method with chiral stationary phases for detection of this product was established and thus *(S)*-enantioselectivity toward pNO$_2$PheDU has been shown. Consequently this novel substrate is a potential precursor for the enantiopure *β*-amino acid *para*-nitro-*β*-phenylalanine (pNO$_2$*β*Phe).

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BTP29
β-Amino acid production by a lipase/transaminase enzyme cascade_1 – screening the best fitting lipase
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Enantiopure β-amino acids represent highly valuable building blocks for peptidomimetics and the production of bioactive compounds [1].
Their efficient synthesis still poses a major challenge and can be achieved by coupling lipase and transaminase activity, starting from stable β-keto esters [2].
Here we describe the comparison of screening procedures for enzymes to be used for the hydrolysis of β-keto esters [3].
To choose the most efficient approach for screening, we assessed three different assays with statistical methods (classical Z'-factor, strictly standardized mean difference (SSMD), the Kolmogorov-Smirnov-test, and t-statistics). Based on our data we discuss the explanatory power of different statistical measures.
Finally, we successfully employed the most suitable assay to identify the *Rhizomucor miehei* lipase (RML) as the best fitting enzyme for the hydrolysis of the tested set of aromatic β-keto ethyl esters. By means of molecular modeling, we were able to suggest an explanation of the significantly higher activity of this lipase compared to another hydrolase tested.

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BTP30
β-Amino acid production by a lipase/transaminase enzyme cascade_2 – Increasing the stability and enabling recyclability of a β-transaminase.
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Transaminases are a powerful tool for the synthesis of chiral amines and amino acids due to their wide substrate spectrum and their high enantioselectivity [1].
The potential application of these enzymes for the production of enantiopure β-amino acids was discussed earlier [2]. The problem of using instable β-keto acids as substrates can be circumvented by coupling lipase and transaminase activity, starting from stable β-keto esters [3].
A remaining challenge is the suitability of the β-transaminases discovered so far for application in technical processes with respect to long-term stability and recyclability. We demonstrated a strong increase in long-term and storage stability by immobilization on functionalized magnetic beads. A recycling of the immobilized β-transaminase was possible without significant loss of activity during the first seven reaction cycles. Moreover, the use of magnetic beads turned out to be notably useful in the purification of the enzyme from the crude extract: We finally succeeded in coupling purification and functional immobilization to gain a ready-to-use and recyclable β-transaminase in one single step [4].

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BTP31
Covellite (Bio)-Leaching with Amino Acids
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Covellite is one of the main copper sulfides found in Kupferschiefer [1]. Besides that there are also indications of secondary covellite formation during leaching processes of related sulfides [2]. Therefore, understanding of covellite dissolution in the context of Kupferschiefer bioleaching is of great importance. However, conventional bioleaching employing acidophilic microorganisms is impeded due to its high acid neutralizing capacity. The use of neutrophilic bacteria secreting metabolites capable of metal interactions may circumvent this problem. Thus, we synthesized an artificial covellite [3] and subjected it to both chemical and biological leaching. Parameters considered important for covellite dissolution involve e.g. type and concentration of leaching agent, pH, and temperature, presence of other metals or solid phases with adsorptive capacities. First results from batch assays revealed that amino acids might be suitable leaching agents. Physicochemical parameters, which determine leaching success, differ between different amino acids. We found that covellite dissolution is strongly pH dependent for glutamic acid, aspartic acid and arginine, whereas for glutamine and asparagine this could not be shown. The different behavior is currently attributed to the differences in stability of the formed copper-amino acid complexes. In addition pH determines chemical speciation of the leaching agent which in turn is a key factor in complex formation. Growth of bacteria sometimes is heavily influencing pH and thus leaching success. Knowledge of these crucial parameters and how they interact allows for optimization of the leaching process. Furthermore, it is planned to produce amino acids in-situ, by e.g. *Corynebacterium glutamicum,* to ensure an economical feasible process.

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BTP32
Protein evolution to improve biocatalysts for use in thermophiles using random mutagenesis
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Though cloning and expression in well-studied organisms such as *E. coli* or Yeast has become common practice in academic and industrial settings, their field of industrial application is physically limited due to their physiological bias. These organisms deal poorly with harsher reaction conditions which can be encountered in industrial processes, especially high temperatures. Enzymes from extremophiles or engineered variants from mesophilic sources have been established as a means to deal with these challenges, offering an improvement of existing processes or the possibility to establish new pathways to bio-based products. We recently reported the use of a thiamin-pyrophosphate dependent decarboxylase in an extremophile host for the production of isobutanol.[1] However, the production of isobutanol at elevated temperatures (>50 °C) was hampered due to the limited stability of the enzyme, thus we used directed evolution to improve the thermostability of the decarboxylase while maintaining its activity. Initial screen design enabled us to screen directly at elevated temperatures, thus minimizing the detection of false positive variants. Subsequently random mutagenesis clone libraries were constructed and

about 2000 clones of the target enzyme were screened for enhanced thermostability. The screening assay was based on the consumption of NADH thus correlating the activity of the particular KIVD variants with an optical readout. In order to further investigate the stability and activity of improved variants in a more direct fashion, HPLC analysis was performed quantifying the product formation at 60 °C. In summary, we were able to find single amino acid mutations which contributed markedly to an enhanced stability without compromising the enzymatic activity. Furthermore, the variants also had a much higher stability compared to the wildtype when performing reactions at lower temperatures. The *in vivo* analysis of these enzyme variants is currently underway.

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BTP33

yTREX – update of a synthetic biology tool for effective gene cluster cloning and secondary metabolite production

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Questions: Microbes produce a great diversity of secondary metabolites which often possess high-value bioactivities, such as antibiosis, cytotoxicity or immunosuppressive activity. Biotechnological access by heterologous expression of the respective biosynthetic genes is a highly promising approach but typically impeded by various factors. Limitations are associated with cloning, transfer, stable maintenance and especially with the functional expression of all pathway genes. In addition, the microbial host provides a critical background for successful metabolite production, for example in offering appropriate precursor supply and tolerance toward the produced compound. Hence, synthetic biology tools are required that enable the effective cloning of large gene clusters and comparative evaluation of different pathway/host combinations.
Methods: We present here a synthetic biology tool named yTREX which is a modified version of the TREX (pathway transfer and expression) system[1] offering additional features enabling straightforward cloning of large or multi-part gene clusters within *Saccharomyces cerevisiae via* homologous recombination. The TREX system itself consists of two DNA cassettes which comprise different genetic elements allowing the conjugational transfer of the entire TREX-labeled gene cluster into a broad range of bacterial expression hosts and its stable integration into the host chromosome *via* transposition. Subsequently, expression of all pathway genes is accomplished either *via* random insertion of the transposon at a highly transcribed chromosomal locus[2] or by convergent T7 RNA polymerase-mediated transcription[1].
Results: Applying yTREX, we successfully TREX-labeled different secondary metabolite gene clusters, including those for prodigiosin and violacein, ranging from 6.4 kb to 21 kb in size, by straightforward one-step yeast recombination. This enabled the fast generation of various *Pseudomonas putida* expression strains carrying the gene clusters stably integrated in their chromosome. Among these, we could identify efficient production strains accumulating the respective metabolites, demonstrating the value of the yTREX tool and the chosen bacterial host.
Conclusion: yTREX is the first toolkit that combines the benefits of homologous recombination in yeast for gene cluster cloning with a system for gene transfer, randomized genome integration and bidirectional expression, thus applicable for the activation of complex gene clusters in various bacterial hosts. Consequently, yTREX allows the reconstitution of pathways within a number of different and metabolically versatile screening hosts in a plug-and-play fashion which offers new perspectives in the fields of genome mining and synthetic biology.

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BTP34

Functionalization of magnetosomes from *Magnetospirillum gryphiswaldense* by *in vivo* surface display of functional groups and reporter enzymes

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Magnetosomes of magnetotactic bacteria represent membrane-enveloped nanoparticles of Fe_3O_4 and have a number of unprecedented properties, such as high crystallinity, strong magnetization, and uniform shapes and sizes. Thus, magnetosomes have an enormous potential for applications e.g. as contrast agents for magnetic imaging techniques (MRI, MPI). In addition, both their crystal morphologies and the composition of the enveloping membrane can be manipulated by genetic means.
Recently, we started to systematically explore genetic functionalization of the magnetosome membrane. Using an optimized expression system, the most abundant proteins (MamC/A/F/G) of the magnetosome membrane were tested as anchors for the expression of various peptides and reporters as genetic fusions to magnetosome proteins. In order to modify the shell size and properties we expressed artificial peptides (e.g., poly-Asp or poly-Ser) by fusion to MamC/F/G. This resulted in an increased hydrodynamic diameter of the magnetosome shell and altered the surface charge. Furthermore, in addition to EGFP, we investigated the β-glucuronidase GusA as a potential reporter and to explore immobilization of enzyme proteins. Magnetosome-bound GusA activity followed Michaelis-Menten kinetics and was slightly increased compared to the non-immobilized enzyme, and remained active after repeated cycles of freezing and thawing.
By taking advantage of the inherent capability of native magnetosomes, we previously succeeded in chemical silica encapsulation of magnetic particles [1]. By variation of conditions, the thickness of silica shells could be varied, and suspensions of single silica-encoated MNP particles or encoated nanorods could be generated. Fluorescence of GFP-expressing magnetosomes was not only preserved during encapsulation, but coating significantly increased resistance against e.g. denaturation. Thus, silica-coated GFP-magnetosomes will be promising as future bimodal magneto-fluorescent contrast agent for magnetic imaging (e.g. as tracers for tumors).
In addition, we will explore expression of nanobodies (i.e., functional fragments derived from full-length camelide antibodies) as versatile molecular connectors [2], enzyme proteins and other peptides (e.g. for organic coatings). Thereby we expect the generation of tailored particles with improved biocompatibility and tuneable characteristics optimized for biomedical and biotechnological applications.

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BTP35

Biotransformation of *n*-octane to 1-octanol at the expense of molecular oxygen and hydrogen

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Questions: Cytochrome P450 monooxygenases (CYPs) have the extraordinary capability to introduce oxygen into non-activated C-H bonds in a regio- and stereoselective manner, which is still a challenging task for synthetic catalysts. CYP153A from *Polaromonas* sp. JS666 converts regioselectively *n*-octane into 1-octanol using O_2 as the oxidant [1]. This reaction requires continuous supplementation of the reduced cofactor NADH. The utilization of the O_2-tolerant NAD^+-reducing hydrogenase (SH) from *Ralstonia eutropha* and H_2 as reductant has proven to be an atom-efficient method for the regeneration of NADH [2,3,4]. We aim to design a synthetic pathway in *Pseudomonas putida* in order to convert *in vivo n*-octane to 1-octanol at the expense of molecular oxygen and hydrogen.
Methods: The NADH-dependent CYP153A monooxygenase from *Polaromonas* sp. JS666 and the NAD^+-reducing hydrogenase from *R. eutropha* were heterologously synthesized in *P. putida* KT2440. Biotransformations were performed with resting cells in the presence of 15 % (v/v) *n*-octane. The headspace of gas tight flasks was filled with a

gas mixture of 20 % H_2 in air. Control experiments were carried out under air. Aliquots of the biotransformation suspensions were analyzed using GC-FID.

Results: Addition of H_2 to the recombinant cell cultures resulted in an approximately 3-fold increase in the amount of 1-octanol. The hydrogenase sustains H_2-driven NADH cofactor regeneration even in the presence of O_2, the co-substrate of monooxygenase [5].

Conclusion: Our study shows that the SH is capable of overcoming possible bottlenecks of cofactor supply in whole-cell systems. Moreover, H_2 represents a viable alternative to carbon-based reductants currently used for *in vivo* cofactor recycling strategies. Thus, our H_2-driven *in vivo* cofactor regeneration system holds considerable potential for application in other cascades reactions that rely on sustainable supply of NAD(P)H as the reducing agent.

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BTP36

Optimization of the brasilicardin biosynthesis in actinomycetes Heterologous expression and boarder identification of the brasilicardin biosynthetic gene-cluster

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Brasilicardin, produced by *Nocardia terpenica,* is a terpenoid which is known to have an immunosuppressive activity (1). Compared to today´s standard drugs tacrolimus and cyclosporine, this natural product shows the advantage of being less toxic and having a higher potency (2).

Currently, the development is halted due to supply issues. The producer strain shows only a low production titer and is furthermore categorized as biosafety level 2 organism. Both facts make the production of the original brasilicardin by classic fermentation expensive and elaborate. In addition, despite considerable efforts, it is neither practical nor reasonable to generate the molecule by total synthesis.

Therefore, heterologous expression of the immunosuppressive and optimization of the brasilicardin biosynthesis should be performed in a biosafety level 1 organism. Since actinomycetes are known to be suitable heterologous hosts, certain actinomycetes strains were selected for heterologous expression of brasilicardin. For this reason a fosmid library was constructed and the gene-cluster encoding the immunosuppressive compound could be isolated. Via conjugation the fosmid containing the brasilicardin gene-cluster was transferred in the heterologous hosts. Considering that the boarders of the brasilicardin gene-cluster are not defined in detail, shortened versions of the fosmid were constructed in order to narrow down the genes required for brasilicardin biosynthesis. The brasilicardin production in the heterologous host was than evaluated with HPLC/MS.

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BTP37

Steroid side chain degradation in *Rhodococcus rhodochrous* DSM43269

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Rhodococci are soil dwelling bacteria with an enormous diversity of catabolic pathways enabled by their large genomes and great gene multiplicity. Among a large range of compounds Rhodococci are able to use steroids as carbon and energy sources for growth. This degradation pathway gives rise to a diversity of steroid intermediates, some of which are of interest as precursors of bioactive steroids for the pharmaceutical industry[1]. Bacterial cholesterol degradation has gained strong interest in recent years after the discovery that several related pathogenic bacteria, among which the causative agents of the disease tuberculosis (TB) *Mycobacterium tuberculosis* in humans and *Rhodococcus equi* in foals, have a conserved cholesterol degradation pathway[2,3]. Several cholesterol catabolic genes have since then been identified and are essential for pathogenicity of *M. tuberculosis* and *R. equi*. Enzymes of this pathway are therefore promising targets for TB drug development. In this study we focus on identification/ characterization of steroid side chain acting enzymes in *Rhodococcus rhodochrous* DSM43269. The two main events in steroid catabolism, steroid ring degradation and side chain catabolism, occur simultaneously. As a consequence, the steroid polycyclic structure can be degraded even when side chain catabolism is blocked, preventing proper analysis of accumulated intermediates of cholesterol side chain catabolism. In our lab we have been able to construct a *kshA* null mutant of *Rhodococcus rhodochrous* fully blocked in steroid ring degradation by deletion of 5 *kshA* genes[4]. In this study we used unmarked gene deletion to introduce additional deletions of potential side chain catabolic genes in strain RG32. We analysed the effects of the gene deletions on steroid catabolism and we biochemically characterized the encoded enzymes.

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BTP38

Transition from Aerobiosis to anaerobiosis – deciphering the adaptation of *Corynebacterium glutamicum* to various oxygen availabilities

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Questio: Bacteria encounter varying oxygen concentrations in manifold situations e.g. in their natural habitat and especially in large scale industrial processes evoking viability and production deficiencies (1). These fluctuations range from aerobic via microaerobic to anaerobic conditions. To adapt to the changing environment bacteria have to remodel their entire metabolism (2, 3). Despite its relevance for pathogenicity and pharmaceutical and bio-based production processes, the molecular events during these transitions are poorly understood. To address this question, we systematically investigate the adaptation of the industrially relevant *Corynebacterium glutamicum* to such altering conditions.

Method: A "triple-phase" batch bioprocess with *C. glutamicum* was established that depicts the three successive phases (aerobiosis, microaerobic interface and anaerobiosis) in a single bioreactor. Throughout the process, samples were withdrawn and analyzed for substrate consumption and organic acid production and additionally used for whole transcriptome analysis by RNA-sequencing.

Result: A definition of the three phases was directly feasible by the bacterium's physiological changes, i.e. a decreasing growth rate with increasing oxygen limitation. Furthermore, L-lactic acid, succinic acid and acetic acid were the main fermentation products secreted to the culture supernatant, interestingly in a manner, that their respective differential product yields clearly bordered each process phase. A closed carbon balance indicated that all significant products were analyzed. RNA-Sequencing analysis revealed differential expression of an abundance of genes of the central and peripheral metabolism matching the expectations of previous work (4) but also delivered novel insights into the regulatory regime required for adaptation.

Conclusion: The established bioprocess is an elegant approach for the systematic understanding of *C. glutamicum*'s adaptation to a progressive oxygen deprivation. Deeper analysis of the RNA-sequencing data especially focusing on regulatory networks and their hierarchy could reveal novel targets for strain optimization or pharmaceutical targets. A comprehensive understanding of the molecular events during the transitions might be also projected to other organisms and applications.

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BTP39

Metabolic engineering for directed evolution – enhancing allocation of oxaloacetate as crucial precursor for L-lysine production in *Corynebacterium glutamicum*

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Question: *C. glutamicum* is an established microorganism in white biotechnology and well-known for the industrial production of large quantities of amino acids, in particular L-lysine. To improve L-lysine formation in *C. glutamicum*, we aimed to enhance the allocation of the crucial L-lysine precursor molecule oxaloacetate by applying an evolutionary approach.
Methods: This was accomplished by deletion of the genes *ppc* and *pyc*, encoding the oxaloacetate-forming anaplerotic enzymes phosphoenolpyruvate carboxylase and pyruvate carboxylase, respectively. The resulting strain *C. glutamicum* Δ*ppc* Δ*pyc* was cultivated in minimal medium containing 40 g l^{-1} glucose and 1 g l^{-1} yeast extract and sequentially transferred for 14 days including concomitant screening for faster growing mutants. Subsequently, comparative whole genome (re-)sequencing (WGS) was performed to identify relevant mutations.
Results: In contrast to the initial strain *C. glutamicum* Δ*ppc* Δ*pyc*, which showed a growth rate of 0.17 h^{-1}, three independently evolved mutants yielded growth rates of about 0.32 h^{-1}, indicating mutational events improving growth and probably oxaloacetate supply. Interestingly, the intersection of the genomic alterations obtained by WGS revealed isocitrate dehydrogenase (ICD) as consistent target in these strains.
Conclusion: The evolutionary acquired point mutations might lead to reduced ICD activity, thereby activating isocitrate lyase and malate synthase of the glyoxylate shunt which both are typically repressed during growth on glucose as sole carbon source[a]. An active glyoxylate shunt might provide a surplus of oxaloacetate for improved growth of the evolved mutants. The identified mutations in ICD will be re-engineered in *C. glutamicum* Δ*ppc* Δ*pyc* and in the L-lysine producer *C. glutamicum* DM1800[b] and tested for the suitability to improve L-lysine production with *C. glutamicum*.

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BTP40

Validation of assays for detection of relevant pathogenic microorganisms in a grass-silage biogas process

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Grass and corn silage, dung, green and bio waste are used as substrates for biogas processes. Infected substrate might contaminate the whole biogas plant, including the whole material circuit including the use of digestate for agricultural use. Also biological markers are still missing as parameter for control of biogas processes [1], but are part of the network project "BiogasMarker". Thus, a rapid detection and reproducible quantification is a necessity in order to evaluate the risk of pathogens during a biogas process. Therefore, the import through substrate, the potential to survive the whole process and the recovery in the digestate are analysis points. During the project "Pathogen diagnostic within biogas reactors" the development of qPCR based detection systems for some selected relevant pathogens was established. Besides some already available detection systems [2, 3, 4] the focus was especially on phyto- and human pathogenic microorganism which had been selected by a risk priority analysis [5] such as *Xanthomonas translucens, Clostridium difficile*, *C. sordellii, Listeria ivanovii und L. monocytogenes.* In addition to the mentioned established assays the preparation of an appropriate plasmid vector for the detection system of both pathogenic *Listeria* species, *L. ivanovii und L. monocytoges,* was initially performed. An inter-laboratory assay revealed the need of an optimization and validation of all protocols [6]. The associated protocols had to be validated and an experimental determination of the LOD and LOQ were performed. An internal amplification control was also included in all multiplex PCRs. Spiking experiments did check for interferences.
The validation was successfully performed for extraction and quantification of biogas samples from different biogas plants. All established multiplex assays were able to comply with the requirements and give now the possibility to identify some relevant "key" species within biogas plants. This might enable the definition of biological qPCR parameters for further regulations to control safety of biogas processes.

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BTP41

Improving ectoine production by ^{13}C metabolic flux analysis

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Ectoine is a cyclic amino acid derivative branching off from a common precursor (aspartic semialdehyde) within the biosynthetic network of the aspartic acid family and one of the best studied compatible solutes. Due to its remarkable properties, ectoine is marketed as an active ingredient in cosmetic, health care and life science products, and is presently produced at an annual scale of several tons, employing the moderate halophilic γ-proteobacterium *Halomonas elongata* [1, 2]. In order to improve production rates and identify potential biosynthetic bottle-necks (in particular for future heterologous production) we decided to elucidate the carbon fluxes by specific ^{13}C-labelling of substrates. Drainage of the intermediate oxaloacetate of the central TCA cycle for ectoine biosynthesis requires the action of efficient anaplerotic reactions, most likely either by carboxylation of pyruvate/PEP or via the glyoxylate cycle, as suggested by Pastor *et al.* for *Chromohalobacter salexigens* [3]. As natural ectoine producers have adjusted their metabolic fluxes in an optimized way, we can take advantage of the existing solutions to optimize industrial production strains by resolving the underlying carbon fluxes.
In this work the substrate pyruvate, specifically labelled at 1-^{13}C, 2-^{13}C or 3-^{13}C, was fed to *H. elongata*, allowing for the first time an insight into the flux distribution of this industrial ectoine producing strain. The resulting labelling pattern of ectoine revealed that both of the above mentioned basic anaplerotic reactions are employed, but against expectations some labels could not be explained by these standard pathways.

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BTP42

Targeting the toxin-antitoxin complex MazEF - an antibacterial approach

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Bacterial infectious diseases, especially nosocomial infections with multiresistant pathogens are on the rise and cause at least 20.000 fatalities in the European Union every year. EMEA and WHO call for efforts to fight the spread of bacterial resistance e.g. by developing antibiotics with new modes of action or by controlling the spread of resistance genes[1]. Plasmids are supposed to be an important cause for the fast spread of resistance genes and are one possible target for novel antibiotics. Clinical important plasmids like pRUM or pS345RF in vancomycin-resistant enterococci [2,3] do harbour toxin antitoxin systems.
Toxin antitoxin complexes act as plasmid addiction systems that stabilize a plasmid within a population by killing off plasmidless daughter cells. The mechanism is an automated activation of the toxin upon halt of translation. The toxin is more stable than the antitoxin, so an arrest in their synthesis frees the toxin as the antitoxin is degraded faster. The toxin then takes up its function, thereby killing the plasmidless cell.
Artificial activation of the toxin by preventing the assembly of the toxin-antitoxin complex or its disruption would possibly kill resistant populations. Such substances could represent a new class of antibiotics, which would combat the growing problem of resistance and stop the advance of life-threatening bacteria.

We developed an *in vivo* model assay system for the toxin-antitoxin complex MazEF from *Escherichia coli* which is one of the best-researched systems. The system MazEF consists of the stabile toxin MazF and the labile antitoxin MazE. The toxin MazF is a ribonuclease, cutting mRNA at the sequence ACA. It is inactivated by the antitoxin MazE, which is degraded by the protease ClpAP.
Due to the fact that the toxin is a ribonuclease which cuts mRNA at ACA motifs we developed an assay based on an mRNA which codes for a non-sense protein but when cut by the mazF ribonuclease turns into a functional luciferase. This approach makes it possible to screen libraries for compounds which are able to disrupt or affect the MazEF complex *in vivo*.

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BTP43

Structural basis and stereochemistry of 3,4- and 3,6-dehydrogenation by cyclic acyl-coenzyme A dehydrogenases

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Question: The FAD-containing acyl-coenzyme A (CoA) dehydrogenase (ACAD) family comprises a large number of biologically important enzymes that catalyze the anti-1,2-elimination of a hydride and proton from carboxylic acid CoA thioesters.[1] Proton abstraction from the C1 is accomplished by a highly conserved glutamyl-residue in the active side. During syntrophic fermentation of cyclohex-1-ene-1-carboxylic by the Deltaproteobacterium *Syntrophus aciditrophicus* an unorthodox ACAD has recently identified that catalyses both, the unusual 3,6-dehydrogenation of cyclohex-1-ene-1-carboxyl-CoA (Ch1CoA) to cyclohex-1,5-diene-1-carboxyl-CoA (Ch1,5CoA) and the 3,4-dehydrogenation of the latter to benzoyl-CoA.[2] Most importantly, the conserved glutamyl residue is missing in this enzyme. We studied the structural basis and the stereochemistry of Ch1CoA DH.
Methods: ChCoA CH was crystallized in the presence and absence of the individual substrates and the structure was solved to a maximal resolution of 1.6 Å; molecular variants with exchanged amino acids in the active site were kinetically characterized. The stereochemistry of the reaction was analyzed by high-frequency NMR-spectroscopy.
Results: The high resolution X-ray structure together with kinetic studies of molecular variants provide the molecular basis for the 3,4- and 3,6-dehydrogenation with an catalytic aspartyl-residue, present only in Ch1CoA DH serving as catalytic base. NMR analysis elucidated the stereochemical course of the unusual dehydrogenation reaction.
Conclusion: Ch1CoA DH is the only member of the acyl-CoA DH that misses the conserved glutamyl-residue and uniquely catalyses 3,4- and 3,6 dehydrogenation of a cyclic CoA-ester substrate. The results obtained expand our knowledge of the functional capabilities of acyl-CoA DH and offer previously unknown biocatalytic options.

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BTP44

Single-cell adaption upon carbon-shift of *Escherichia coli* and *Corynebacterium glutamicum* populations

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Introduction: Fast metabolic adaptation is crucial for the survival of bacterial populations under changing environmental conditions. Standard bulk approaches, e.g. the measurement of the optical density or transcriptome analyses, obscure the complex phenotypic pattern of isogenic microbial populations. To monitor adaptation to changing carbon source availability, the aim of this study was the analysis of the adaption process at the single-cell level using *Escherichia coli* and the important industrial amino acid producer *Corynebacterium glutamicum* as model organisms.
Methods: In first experiments, we established a staining protocol using a lipophilic, fluorescent dye which enabled the observation of cell division in shake flaks experiments via flow cytometry [1]. After staining, the dye is diluted by every cell division and remains stable over a long experimental time (>120 hours). In standard batch cultivations rapid environmental changes by carbon source shift experiments were performed, e.g. switching from glucose to gluconeogenic growth on acetate or succinate. Single-cell adaptation was monitored using appropriate genetic reporter circuits in combination with the lipophilic fluorescent dye indicative for the growth of the particular cell. Further, in-depth analysis of adaptation and growth was performed using microfluidic chip devices in combination with time-lapse fluorescence microscopy [2].
Results: Upon switches in carbon source availability, *C. glutamicum* cells displayed continued growth. The particular reporter constructs revealed a specific and homogeneous response to the carbon source availability. In contrast, *E. coli* cells displayed metabolic adaptation after a glucose-gluconeogenic carbon shift. Whereas approximately 5 % of the population continued to grow upon shifting to succinate as carbon source, the majority of the population entered a persistent state. The analysis of microfluidic chip experiments are in line with a positive correlation growth and reporter output. Stochastic switches in the biosensor output are occasionally observed when *E. coli* cells are growing on glucose as sole carbon source.
Conclusion: Altogether, these data highlight significant differences in metabolic adaptation capacities of these two important model species and provide detailed insights into adaption processes based on the analysis of single-cell behaviour.

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BTP45

Concepts for the development of new biosorbents on the base of microbial constituents

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The world's growing demand for high-tech metals together with a simultaneously deteriorating availability is one of the central challenges of our modern society. Thusly, the development of new and innovative processes for a more efficient extraction of raw materials as well as economic methods for recycling is needed. Established methods for reclaiming production residues often include the chemical treatment with concentrated acids or alkalis, and are also polluting and energy-intensive. To overcome existing deficiencies and disadvantages of such methods emphasis is increasingly placed on biological alternatives. Thereby, biosorptive materials are prevalent for the recovery of dissolved chemical species. They are inexpensive and manufacturable in large quantities and often have excellent binding properties as compared to synthetic materials. Microorganisms are particularly in focus for biosorption processes because of their ubiquity and their enormous variability. A number of microbial cell structures and metabolites have been developed evolutionarily in direct interaction with toxic or essential elements, including heavy metals. Mediated by a variety of functional groups combined with the perfect structural fit these molecules are able to bind such elements partially highly selective and specific.
In our group we are investigating the potential suitability of biomolecules such as siderophores, short peptides, and S-layer proteins as biosorptive compound. Our presentation discusses the usability of these compounds for the development of novel, selective binding filter materials for removing toxic elements and the recovery of valuable metals from aqueous solutions.

BTP46

Engineering the citric/isocitric acid overproduction by *Yarrowia lipolytica*

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Question and Methods: Functionalized carboxylic acids are highly versatile chemical species with a wide range of applications (e.g. as co-

polymers, building blocks, acidulants). Therefore, they are of special interest as biotechnologically available targets.
The yeast *Yarrowia lipolytica* secretes high amounts of organic acids, like citric and isocitric acid (CA/ICA) under conditions of growth limitation from a carbon source excess. Depending on the carbon source, *Y. lipolytica* produces a characteristic CA/ICA ratio, on carbohydrates or glycerol of 90:10 and on sunflower oil or n-alkanes of 60:40.
To examine, whether this CA/ICA product ratio can be influenced isocitrate lyase (*ICL1*), aconitase (*ACO1* or *ACO2*), NADP- (*IDP1*) or NAD- (*IDH1, IDH2*) isocitrate dehydrogenases gene-dose-based overexpressing strains were constructed (using integrative multicopy vectors) containing multiple copies of these genes alone or combinations of them.
Results: The *ACO1* [1] (but not in case of *ACO2*) or the *IDP1* overexpression and a combination of them resulted in a product pattern shift in direction of ICA, reducing the undesired CA for ICA production. On sunflower oil the ICA proportion increased from 35-55 % to 65-72 % of total acid produced in shaking flasks experiments. Strains with increased copy numbers of both *ACO1* and *IDP1* showed the highest ICA selectivity up to maximally 75-80 % in bioreactor experiments. Otherwise, overexpression of only one NAD-isocitrate dehydrogenase subunit genes (*IDH1* or *IDH2* to decrease IDH enzyme activity) resulted only in a moderate ICA-increase.
Conclusions: By using wild-type or engineered *Y. lipolytica* strains the enantiomerically pure form of D-*threo*-isocitric acid, currently available as a speciality compound, can be produced now in large amounts and used as a building block for organic synthesis [2].

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BTP47
Screening of protease producing microorganisms from biogas plants
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Proteolytic enzymes are ubiquitous in occurrence in all living organisms and of high commercial value. Especially extracellular proteases find multiple applications in various industries. Although there are already many microbial sources available for producing proteases, there is still a demand for improved and novel technical enzymes. Especially the 'black box' of the biogas plant offers an enormous potential for new enzymes as proteases which play an important role in the disintegration of biomass in the hydrolysis phase of the biogas process.
The microbial community of an industrial biogas plant is mostly unknown and highly dependent on the substrate used. The composition of organisms is able to adapt to nearly every kind of different organic substrates. In this project, the aim was to search for microorganisms that are able to produce novel proteases in a biogas plant, which was fed with protein rich substrates. The project is split into two parts, the microbial screening for protease producing microorganisms and the PCR-based identification of subtilisin-like-protease gene fragments.
Microbial screenings were performed to isolate protease producing species. Since several Bacillus species are well known and industrially established protease producers, a first screening was restricted to aerobic spore forming microorganisms. At different points in time, samples were taken from the digesters and pasteurized before plating on skim-milk agar at different pH values. Strains with significant clearing zones were isolated and preliminarily characterized. Different strains of the genera *Bacilli* and *Virgibacilli* were identified by 16S rDNA sequencing. Additionally, MALDI-TOF was applied to discriminate various *Bacillus* strains by fingerprint analysis.
Furthermore, isolated DNA samples taken from several digesters were used as templates in a PCR amplification of an internal subtilisin-like-protease gene fragment. Primers were derived from highly homologous regions as published by *Cheng et al.* (2011). The obtained fragments were cloned, sequenced and subjected to BLAST analysis. A first glance at the partial protease gene sequences derived from the biogas plants revealed a high diversity of subtilisin-like-proteases. Present studies focus on selected novel proteases, which will be characterized in detail.

Cheng, X., Gao, M., Wang, M., Liu, H., Sun, J, Gao, J., Curr. Microbiol. (2011) 62, 1542-49

BTP48
Tailor-made generation of glycolipids in *Ustilago maydis*
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The generation of high-value products by microbial bioconversion of inexpensive renewable substrates is a major goal in bioeconomy. Biosurfactants exemplify such substances that can deal as bio-based alternatives to surfactants produced from petroleum precursors. The corn smut fungus *Ustilago maydis* is a eukaryotic model organism that produces two different biosurfactants: cellobiose glycolipids (ustilagic acids) and mannosylerythritol glycolipids (MELs). The enzymes required for biosynthesis of these glycolipids are encoded in gene clusters that are induced under nitrogen starvation conditions and the underlying biosynthesis pathways are very well characterized. However, the composition and properties of glycolipids are limited by the respective metabolic pathways in the natural production strains. Hence, we here aim to extend the natural repertoire of produced biosurfactants by combining genetic engineering of both synthesis pathways and feeding of artificial lipids to substitute the native hydrophobic side chains.

BTP49
Modification of microfluidic devices by immobilized laccase-hydrophobin fusion protein
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Laccases are blue copper-containing enzymes that perform oxidation of aromatic and nonaromatic substrates. Because of their high redox potential and their ability to use non-toxic molecular oxygen as final electron acceptor, laccases are of great interest for biotechnology. In particular, laccases can be used in enzymatic biofuel cells (EBFC) to improve cathode performace (Sané *et al.*, 2013). Biofuel cells allow the direct conversion of renewable biomass like lignocellulose or biowaste into electricity and can be potentially used in implants, bioreactors and recycling. A laccase from the white-rot basidiomycete *Pycnoporus sanguineus* has been reported to show high performance on the cathode, even using crude culture supernatant (Fokina *et al.*, 2014). However the efficiency of the laccase on the cathode depends on the unspecific interaction between the enzyme and the cathode material. Immobilization of the laccase on the cathode surface would allow stable interaction between the partners and reduce the amount of enzyme needed. Also modification of microfluidic devices to add enzymatic functions to *lab-on-a-chip* technologies are of great scientific interest. Storage of a wide range of enzymes by immobilization would greatly increase application versatility of microfluidic chips.
One of the possibilities to immobilize proteins on the surface presents a class of small amphiphilic proteins produced by fungi, hydrophobins. They can interact with both hydrophobic and hydrophilic surfaces and self-assemble into monolayers on interfaces. Here we present fusion proteins of laccases from the ascomycete *Aspergillus nidulans* and the basidiomycete *P. sanguineus* fused to hydrophobins that perform electron transfer from substrate and can be immobilized on different surfaces in active form.

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Fokina O, Eipper J, Winandy L, Kerzenmacher S, Fischer R (2015). Improving the performance of a biofuel cell cathode with laccase-containing culture supernatant from *Pycnoporus sanguineus*. *Bioresour Technol* 175: 445-453

BTP50
Electrode assisted acetoin fermentation in *Escherichia coli*
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Through metabolic engineering *E. coli* is capable of synthesizing acetoin in an unbalanced fermentation. Acetoin is a value chemical with a well-established application in industrial food production. Further it is the direct metabolic precursor of 2,3-butanediol, an important platform chemical, which may be processed to aircraft fuel. The biotechnological production however does not reach global demands, due to the simultaneous

production of several end products, potentially pathogenic organisms used as biocatalysts and a difficult process technology.
Our goal is to overcome these limitations by establishing a new technology based on an unbalanced fermentation process in *E. coli*. This promising technique's key feature is the possibility of gaining fermentation products more oxidized than the substrate. The residual electrons are transferred to a non-depletable acceptor, a carbon electrode. The heterologous expression of *c*-type cytochromes from *Shewanella oneidensis* in presence of a soluble redox mediator empowers *E.coli* to interact with the electrode.
A previously generated fermentation deficient strain accumulates pyruvic acid as the sole end product during anaerobic metabolism. This was achieved by successively blocking *E. coli's* fermentation pathways at crucial points through genetic knock-outs. Originating from pyruvic acid an acetoin pathway was added. Necessary are codon optimized genes for the acetolactate synthase and the acetolactate decarboxylase.
This composite biocatalyst consisting of an *E. coli* production strain interacting with a carbon electrode is capable of producing acetoin with high carbon recovery rates.

BTP51
Development of succinate production in *Clostridium autoethanogenum*
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There is an increased need to generate fuels and platform chemicals in a more sustainable manner. One of the chemicals believed to have potential in a bio-based, circular economy is succinic acid. Already used in food and pharmaceutical market, it also functions as C4 building block and can therefore supply the basis for high value-added derivatives with applications in the technical and chemical industry.
Using the acetogenic bacterium *Clostridium autoethanogenum* as a microbial chassis, the proposed research aims to combine the utilisation of exhaust and waste fumes with the fermentative production of succinic acid. A prerequisite for this is a thorough understanding of the existing native metabolic route(s) to succinate, which is already generated by the organism in low amounts, as well as interconnecting pathways. This will be achieved through a combination of enzymatic studies, ^{13}C labelling experiments and gene inactivation/overexpression analyses.
Interestingly, provision of exogenous fumarate, a metabolite which other bacteria can convert to succinate acid in a single step, considerably increased growth of the organism without increasing the amount of succinate produced. However, this increase was only observed in the presence of other carbon and energy sources: addition of fumarate alone could not sustain growth. NMR analyses were therefore initiated to clarify the metabolic fate of fumarate. Investigations are still ongoing, but first results supported by these NMR analyses suggest a clear decrease in the culture supernatant accompanied by an increase in intracellular fumarate, suggesting that the compound is indeed taken up and co-metabolised in the presence of other carbon and energy sources.

BTP52
Impact of salt on the production of organic compounds in the cyanobacterium *Synechocystis* sp. PCC 6803
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Introduction: Cyanobacteria became increasingly attractive as cellular fabrics for the production of valuable organic compounds. Due to their photoautotrophic lifestyle, they can fix CO_2 on the expense of solar energy in mineral media. Thus, they promise a CO_2-neutral production, which is not competing with classical agriculture used for human nutrition. However, freshwater is becoming a limiting resource on Earth, which can be replaced by seawater in cyanobacteria-based production.
Objectives: The aim of the study was to analyze, how high salinity influences the amount of organic compounds produced in engineered cyanobacterial hosts.
Methods: Strains of *Synechocystis* 6803 were engineered, which express specific genes or in which competing reactions were deleted by mutation, to allow the production of ethanol, isoprene or sucrose. The corresponding strains were cultivated under low or high salt conditions and the product yield was compared.
Results: Ethanol-producing strains were obtained by the co-expression of a pyruvate decarboxylase from *Zymomonas mobilis* and the alcohol dehydrogenase of *Synechocystis*. To establish isoprene synthesis in *Synechocystis*, the isoprene synthase *from Pueraria montana* (kudzu vine) was selected and introduced. Sucrose accumulation was optimized by the mutation of the genes for competing glucosylglycerol biosynthesis and sucrose degradation via invertase. The resulting strains produced considerable amounts of three organic compounds. Ethanol as well as isoprene production was slightly lower in cells grown at 4 % of NaCl compared to low salt media, despite the expression of production genes was rather stimulated than decreased under saline conditions. The sucrose accumulation was clearly stimulated under salt-containing compared to salt-free conditions.
Conclusion: Further work is necessary for the development of efficient production systems under saline conditions to replace freshwater by seawater for the mass cultivation of cyanobacterial strains.

BTP53
Systematic molecular optimization of heterologous protein production in the halophilic *Halomonas elongata*
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The natural producer of the amino acid derivative ectoine, the moderately halophilic gamma proteobacterium *Halomonas elongata,* is not only relevant for the production of this compatible solute at industrial scale [1], but may also be of special interest as an expression system for recombinant proteins. It could be shown that compatible solutes (chemical chaperones) can have positive effects not only at whole cell level, but also on conformation and activity of proteins exposed to a variety of stress factors [2, 3]. Therefore, they can support the functional expression of (recombinant) proteins [4]. The broad salt tolerance of halophilic bacteria like *H. elongata* combined with the ability to synthesize or accumulate compatible solutes accordingly enables us to create a unique stabilizing environment for recombinant proteins. Until now, several approaches to optimize heterologous protein expression in *H. elongata* have been addressed, as for example the establishment of a vector for salinity-controlled protein expression [5] or the directed transport of recombinant proteins into the periplasm [6].
Possibilities to analyze, simulate and optimize metabolic pathways, genetic organization and gene expression *in silico* have increased rapidly. In the course of our investigations to further optimize recombinant protein expression in *H. elongata*, we focused on a web tool developed by Salis *et al.* [7], named the RBS Calculator. This bioinformatic tool can be used to predict and/or optimize the translation initiation rate of a protein, considering the specific gene sequence and the expressing organism.
In this study, a vector for salinity-controlled protein expression was constructed with a number of sequences for suitable recombinant proteins in combination with corresponding synthetic ribosome binding sites, calculated by the RBS Calculator and adapted to *H. elongata* as expression host. Thus we were able to demonstrate the aptitude of the RBS Calculator and the suitability of a systematic molecular approach for the optimization of heterologous protein expression in the halophilic *H. elongata*.

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BTP54
Protein production driven by artificial promoters in *Thermus thermophilus*
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Recently there has been a growing interest in the use of non-standard (not *E. coli* or Yeast) organisms in biotechnology. However, in order to understand and engineer such organisms and to establish them as 'chassis' for synthetic biology and biotechnology, new tools have to be developed in order to monitor processes on the molecular level and inside the cell. Especially the detailed understanding and optimization of promoters in order to fine tune protein expression requires quantification on the single cell level. The standard reporter gene GFP and also its more stable derivatives displayed no detectable fluorescence when analyzed *in vivo* in *Thermus thermophilus* HB27 at elevated temperatures in our hands.
We thus developed a thermostable esterase into a reporter protein. The protein is active at a broad range of environmental conditions, it is

monomeric, does not need maturation or cofactors and can be applied as an *in vivo* reporter. Furthermore, it offers the advantage of signal amplification due to its enzymatic activity, which results in a low limit of detection. As prove of concept different promoters were cloned and tested in *Thermus thermophilus* HB27 and the protein production was quantified using a set of different esterase substrates. Intravital whole cell measurements now open the way to engineer promoters inside extremophile hosts, since a high-throughput selection of improved mutants can be performed at the single cell level. We will show examples of artifical promoters and their use in *T. thermophilus*. In general the proposed reporter protein will enable the molecular analysis of fundamental biological questions regarding the lifestyle of thermophiles and other extremophiles and open the way to utilizing these organisms as whole cell catalysts.

BTP55 – withdrawn

BTP56

A novel strain of *Y. lipolytica* as a platform for value-added products synthesis from glycerol

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Growing world population requires progressively more energy sources, therefore the production of biodiesel increased. Glycerol is an important renewable feedstock as it is the main side-product of the biodiesel production process, which is nowadays applied on a large commercial scale. Moreover, glycerol is produced by several others industries, such as fat saponification and alcoholic beverage production units. Despite the high contamination, crude glycerol might be easily utilized by yeast *Yarrowia lipolytica*. Oleaginous yeast *Y. lipolytica* is an interesting host for converting a diverse range of feedstocks, such as waste glycerol, into value-added products such as erythritol (sweetener) or citric acid. Glycerol in *Y. lipolytica* cells is assimilated by phosphorylation pathway, the substrate first is phosphorylated to 3-P-glycerol by a glycerol kinase (GK) and subsequently is dehydrogenated to dihydroxyacetone phosphate by glycerol-3-P dehydrogenase (GDH). To enhance glycerol assimilation we over-expressed the *GUT1* (*YALI0F00484g*) gene coding GK and *GUT2* gene (*YALI0B02948g*) encoding GDH. The modified strains have been tested for glycerol consumption rate and erythritol and citric acid synthesis. Erythritol is a natural sweetener, a four-carbon sugar alcohol, occurring in fruits, such as grapes or melons. Because of its low energy value and non- insulin stimulant properties, erythritol is used as a non-caloric sweetener and pharmaceutical excipient. Citric acid is mainly produced by the mycelial fungus *Aspergillus niger*, but in the past few years much consideration has been focused on the potential use of the yeast *Yarrowia lipolytica.* The engineered strains might be used as a starting platform for further modification for broad-range value-added products biosynthesis.

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BTP57

Impact of ammonia and cyanide on fermentation of (crude) syngas

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The bioliq® pilot plant at the KIT covers the complete process chain required for producing customized fuels from dry lignocellulosic biomass. For energy densification of the biomass, fast pyrolysis is applied. The liquid pyrolysis oil and solid char obtained can be processed further in the entrained flow-gasifier to tar-free, low-methane raw synthesis gas. Prior to chemically catalysed fuel synthesis a multistep cleaning of raw synthesis gases is performed: Particles, alkaline salts, HCl, H_2S, COS, CS_2, NH_3, and HCN are removed to avoid catalyst poisoning during fuel synthesis. The pilot plant is equipped with an innovative hot-gas cleaning system.
Acetogenic bacteria are able to ferment syngas to a variety of organic acids and alcohols. In contrast to the catalysts used in the Fischer-Tropsch process, these biological catalysts can process a broad range of syngas compositions and deal with impurities like sulphur compounds or CO_2.
To assess industrial large scale applicability of acetogenic bacteria it will be necessary to determine the performances of the strains with crude syngas, as each gas purifying step will decrease the economy of the process.
The influence of one of two of the main impurities of crude syngas, cyanide and ammonia, were examined during growth of *Clostridium ljungdahlii* on either CO, CO_2 + H_2 or syngas (CO + H_2 + CO_2) in our multiple 2 L bioreactor setup with product analysis and online gas measurement.

BTP58

In vitro* analysis of xylose repressor XylR from *Bacillus megaterium

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Within the last years Gram-positive *Bacillus megaterium* was systemically developed as an expression system for the production of recombinant proteins using the strong xylose-inducible promoter. This system is based on a multicopy plasmid containing the functional elements of the system namely the gene encoding the xylose repressor XylR with its corresponding promoter and the promoter P_{xylA} followed by a multiple cloning site. It is postulated that in the absence of xylose XylR binds to the operator and prevents expression of the following genes, while in its presence xylose binds to XylR and causes structural changes. XylR loses its affinity for the operator and expression is possible. Nevertheless, so far this model was not confirmed using *in vitro* analyses with purified XylR.
For a deeper understanding, $XylR_{Bmeg}$ with and without bound xylose was recombinantly produced and purified. Native PAGE analysis revealed two different conformations depending on the presence or absence of xylose. Electrophoretic mobility shift assay (EMSA) showed two different mobility complex bands indicating a lower oligomeric state of protein/DNA-complex with bound xylose compared to a higher one without xylose. This result manifested the occurence of two distinct DNA/protein-complexes obviously resulting from different multimeric forms of XylR. So, for the first time it was shown, that also XylR with bound xylose binds to the same DNA-fragment as the XylR without xylose. This leads to two working models - (1) the different oligomeric forms of XylR bind to the same operator sequence causing different DNA organization or (2) they bind to different sequence motifs close to each other. In order to gain more information about binding behaviour footprinting assays were performed using XylR with or without bound xylose. These analyses showed that XylR is able to bind to the operator region independently of xylose but clearly indicated a different binding behaviour of both forms. This could lead to different DNA organization like loop structures causing repression of genes in the absence of xylose.
In summary, these results indicated that the postulate model of DNA-bound XylR without xylose and free XylR with xylose needs to be adapted.

BTP59

FMN-binding fluorescent proteins as versatile *in vivo* reporters

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Introduction: Fluorescent proteins have been proven to be versatile tools in molecular biology that enable the analysis of complex cellular structures and processes such as gene expression, protein localization or protein-protein interaction. GFP and derivatives are widely known proteins that are used as fluorescence reporters. However, they do not mature under anaerobic conditions and their fluorescence brightness is affected by acidic pH. In contrast, FMN-binding fluorescent proteins (FbFPs), which were engineered from Light Oxygen Voltage (LOV) proteins found in plants and bacteria[1-2], are not affected by these environmental factors. Here, we present novel FbFP derivatives and FbFP-based biosensors that can be used for new microbial applications.
Methods: We constructed and evaluated new FbFP derivatives as reporters applicable for *in vivo* analysis of bacterial secretion processes. Furthermore, Förster resonance energy transfer (FRET)-based biosensors were constructed, consisting of FbFPs as a FRET donor and different enhanced yellow fluorescent protein (EYFP) variants as FRET acceptor domains that can be used for ratiometric determination of intracellular pH values.
Results: Comparative expression studies revealed that in contrast to YFP, FbFPs can be used in *E. coli* as *in vivo* reporter for protein secretion *via* the general secretion (Sec) and twin-arginine translocation (Tat) pathway. Using different EYFPs with pK_a-values of 5.7, 6.1 and 7.5 as FRET

acceptor domains we have developed a novel FbFP-based pH biosensor platform (FluBpH) for the measurement of pH values from 5 to 9 with a superior sensitivity. A similar design has recently been used to determine the concentration of molecular oxygen inside of bacterial cells *in vivo* (FluBO)[3].

Conclusion: The development of FbFP-based reporters and biosensors opens up new approaches for the non-invasive analysis of cellular processes in living cells. These *in vivo* analysis tools therefore provide important information for the optimization of biotechnological production processes.

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BTP60

Chain length and isomer determinants in actinobacterial olefin biosynthesis

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Aliphatic hydrocarbons are the predominant components of petroleum-based fuels. Certain bacteria produce such compounds naturally and are therefore regarded as a promising source of enzymes capable of converting saccharification products to aliphatic hydrocarbons. One of the pathways by which bacteria synthesize long-chain hydrocarbons involves the head-to-head Claisen condensation between two fatty acyl-CoA molecules. This type of alkene biosynthesis is well documented in many representatives of the *Actinobacteria* phylum. Members of this group produce primarily *anteiso*- and *iso*-branched, long-chain (C25 to C29) alkenes. The first reaction of the ole pathway is catalyzed by enzymes of the thiolase superfamily and it is currently debated if and to what extent these proteins (termed OleA) determine the nature of the condensation products formed by an organism.

In order to better understand the role of the OleA enzymes in determining the type of olefins formed, we initiated a detailed study of the alkene profiles, *oleA* genes and free fatty acids composition of a collection of 23 isolates belonging to the *Micrococcus, Kocuria* and *Kytococcus* genera. GC-MS analysis of hexane extracts showed that all tested strains were capable of producing olefins and displayed a substantial variation in the olefin amounts, the distribution of chain lengths and of isomers. It can be presumed that the variations in the chain lengths and isomer composition could be determined by the fatty acid composition of each strain, by the substrate specificity of the Ole proteins or by other proteins or combinations of these factors. To investigate this, we cloned selected *oleA* genes or *ole* gene clusters from our strain collection in an engineered *Micrococcus luteus* strain lacking its native *ole* genes. This allowed us to probe the role of different OleA proteins in shaping the product profile under the same conditions (growth conditions, fatty acid precursor pools etc.). The results from these experiments highlighted strong selectivity differences between OleA proteins from different sources. Several *oleA* genes conferring characteristic olefin profiles were selected for cloning in *E. coli* in order to obtain recombinant proteins for further structural studies which will address the relations between Ole protein structure and substrate specificity.

BTP61

Towards the optimization of salt-free heterologous ectoine production in *Escherichia coli* by means of carbon sources variation and supply of potential precursor molecules

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The compatible solute ectoine is accumulated by halotolerant and halophilic microorganisms to obtain osmotic equilibrium at fluctuating salinities. Besides osmoprotection, ectoine has pronounced stabilizing effects on biomolecules and whole living cells exposed to physical stresses and is therefore of great interest for biotechnological applications [1]. At present the natural halophilic producer *Halomonas elongata* is used for the industrial production of ectoine [2]. However, the need to separate ectoine from salt results in a time-consuming and costly purification process. The non-halophilic *Acidiphilium cryptum* DSM 2389^T exhibits a biosynthetic gene cluster for ectoine, which seems to be a promising candidate for salt-free heterologous production [3].

We have generated a heterologous production system for ectoine under low salt conditions in *Escherichia coli* using the biosynthetic gene cluster from *A. cryptum* under the control of an inducible *tet* promoter [4]. Our production strain synthesized and excreted high amounts of ectoine into the medium, while almost no disturbing by-products (e.g. glutamate) were detectable via HPLC. We further optimized the heterologous ectoine production in *E. coli* by analysing the impact of different carbon sources on growth rate, final concentration of product and productivity. It was shown that glycerol represents a suitable substrate for a high product yield under low salt conditions. Inspired by the work of He *et al.* (2015) we also examined the potential of externally supplied precursor aspartate for enhanced low-salt ectoine production [5].

In conclusion our salt-free heterologous production system for ectoine operates very well and, in view of the above improvement strategies, appears to have great potential for simplifying the ectoine production and purification processes.

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BTP62

Engineering *Corynebacterium glutamicum* for a fast production of L-Lysine and L-pipecolic acid

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Corynebacterium glutamicum, a Gram-positive soil bacterium, is a model organism widely used in industrial fermentative processes for the production of amino acids. The world production of L-lysine has surpassed 2 million tons per year [1]. In this study we engineered the glucose uptake and utilization of the lysine producing *C. glutamicum* strain *GRLys1* in order to increase the L-Lysine productivity. In addition, the designed strains used for the production of the non-proteinogenic amino acid pipecolic acid (L-PA), a precursor of immunosuppressants, peptide antibiotics or piperidine alkaloids.

Glucose is taken up into the *C. glutamicum* cell by the phosphotransferase system PTS. The PTS can be replaced by a permease and a glucokinase [3]. Glucose utilization was accelerated when the genes for the endogenous inositol permease IolT2 and a glucokinase were overexpressed (pEKEx3-IolTBest) in addition to the PTS. This plasmid was used to transform the lysine producing *C. glutamicum* strain *GRLys1*. Further modifications resulted in strain *GSLI*(pEKEx3-IolTBest), which produced 40 % more L-Lysine than *GRLys1* (pEKEx3) with a 30 % increased volumetric productivity.

L-lysine can be converted to L-PA by L-lysine 6-aminotransferase and pyrroline-5-carboxylate reductase [2]. To enable production of L-PA, a synthetic operon containing the L-Lysine dehydrogenase gene (*lysDH*) from *Silicibacter pomeroyi* and the pyrroline 5-carboxylate reductase gene (*proC*) from *C. glutamicum* was developed. Transformation of *C. glutamicum GSLI* with this vector allowed for production of L-PA in the millimolar range.

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BTP63

Towards understanding synergistic interactions in co-cultures of *Pseudomonas aeruginosa* PA14 and *Enterobacter aerogenes* for applications in microbial fuel cells

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Question: Microbial fuel cells (MFC) are new biotechnological devices for bioelectricity generation based on the microbial oxidation of substrates e.g. wastewater at an anode. Especially for these undefined substrates a defined pure-culture microbial catalyst is not suitable and quickly a complex microbial mixed culture biofilm establishes on the anode surface. The ecological relationships among different microorganisms in an anode biofilm tremendously influence electric current generation. Recently, a synergistic interaction regarding current production in a defined co-culture of the phenazine redox mediator producer *Pseudomonas aeruginosa* (providing the electron shuttles) and the sugar fermenter *Enterobacter aerogenes* was observed[1]. The central goal of our work is to gain a thorough understanding of the inter-microbial interactions in defined

microbial co-cultures of *P. aeruginosa* PA14 together with *E. aerogenes* for application in MFCs.
Method: To evaluate the full extent of mediator-based synergism in MFCs, we conduct highly controlled co-culture experiments by applying electrochemical techniques. The co-culture is physiologically characterized including metabolite analysis by HPLC and species quantification via fluorescence measurement of tagged strains. Experimental parameters are varied to enhance co-culture performance. Further, the influence of different *E. aerogenes*`s fermentation products on *P. aeruginosa* pure cultures is evaluated by the use of alternative carbon sources. The mode of action of *P. aeruginosa*`s phenazines in different ecological contexts is elucidated via knockout studies.
Results: Preliminary results of the cultivation of *P. aeruginosa* PA14 with 2,3-butanediol as the substrate revealed a significant increase in current generation due to enhanced phenazine production. On the other hand, the barely electroactive organism *E. aerogenes* exhibited electron transfer to the electrode when provided with synthetic phenazines. Targeted gene deletions in the phenazine pathway of PA14 were accomplished to analyze the influence of the diverse phenazines on the mixed culture. A mixed culture comprising PA14 and *E. aerogenes* produced higher currents compared to pure cultures of both organisms. The co-culture performance could be strongly increased by optimization of experimental parameters. Dissolved oxygen concentrations and pH were identified as influential factors.
Conclusion: Overall we demonstrate a potential of optimizing co-cultures of *P. aeruginosa* to enhance performance of MFC. Further optimization and exploration of the factors underlying the synergistic interactions will provide information on how to tap the benefits of this ecological phenomena for MFC applications.

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BTP64
Tailoring *Clostridium ljungdahlii* for electroreduction of itaconic acid
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In 2004, the U.S. Department of Energy (DoE) identified itaconic acid as one of the top twelve sugar-derived building blocks with a high biotechnological potential. Since this, the research interest in fungal fermentation of biomass to itaconic acid highly increased. Furthermore, new synthetic chemical reactions for the conversion of this biomass feedstock to new fuels or fuel additives like 2-Methyl-1,4-butanediol, 3-Methyltetrahydrofuran or 3- and 4-γ-Butyrolactone were identified. The main disadvantage of these processes are the high amount of process energy that is used. To overcome this hurdle the use of microbial electrocatalysis for the production of biofuels from itaconic acid seems to be an interesting opportunity.
During the last few years, a lot of basic research in this area has been performed and some new electroactive microorganisms have been identified. One recent discovery was the microbial electrosynthesis from carbon dioxide by several homoacetogenic bacteria (e.g. *Moorella thermoacetica*, *Sporomusa ovata* or *Clostridium ljungdahlii*). The genetic modification and optimization of these electroactive microbes is a possible opportunity to expand their variety of potential bioconversions. Due to the available genome sequence and genetic engineering tools *Clostridium ljungdahlii* seem to be an interesting candidate for first biocatalytic approaches.
In this work, we report the first results for the bioconversion of itaconic acid to biofuels by using a genetically modified *Clostridium ljungdahlii* strain. The first necessary step of the conversion is an activation of itaconic acid. Therefore the genes coding for a Succinyl-CoA:coenzyme A transferase as well as a Succinyl-CoA ligase were cloned into shuttle vectors. After an, electroporation positive strains were confirmed via PCR and Plasmid rescue. Currently, first conversion experiments with fructose as carbon source and itaconic acid as co-substrate are on the way.

BTP65
Production of glutamate derivatives in *B. methanolicus* MGA3
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The use of methanol as a carbon source in the biotechnological processes has recently become an issue of great interest due to its relatively low price, abundance, purity, and the fact that it is a non-food raw material. *Bacillus methanolicus* is a Gram-positive thermotolerant methylotroph considered to be a suitable candidate for methanol-based production of amino acids and their derivatives (1). Although lately there has been a great progress in research of the physiology of *Bacillus methanolicus*, there is a need of further studies of its metabolic pathways especially with respect to methylotrophy and production of industrially relevant substances (2, 3).
The aim of the project is to establish the production of glutamate derivative γ-aminobutyric acid (GABA). GABA is a component of drugs and functional foods and is used as monomer for production of the biodegradable plastic polyamide 4. It can be synthesized from L-glutamate in a single step by decarboxylation. *Bacillus methanolicus* is a natural L-glutamate producer; however, it does not produce GABA. To enable synthesis of γ-aminobutyric acid (GABA) in this organism the gene encoding glutamate decarboxylase (Gad) was heterologously overexpressed.
B. methanolicus MGA3 is a suitable candidate for GABA production due to its high tolerance to GABA (IC_{50} of 70mM) and lack of catabolic pathways of this compound in the genome. In the proof-of-concept experiment the 0.3mM titer was achieved. This result was further optimised by change of cultivation conditions and media composition leading to full conversion of the precursor and final titer of 4 mM in the flask experiments. To improve the productivity we focused on development of the existing molecular tools.
The RNAseq data (3) was used to identify highly expressed genes, their promoter sequences and genomic organisation. GFPuv fluorescence was analysed by FACS to compare the influence of different: plasmids, promoters, stop and start codons, and presence of terminator on the level of gene expression.
This way the production of GABA was achieved in methanol-based system for the first time and then further optimised. Moreover, the existing molecular biology tools were improved which enables further development of *B. methanolicus* GABA producing strains.

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BTP66
Dirigent proteins as versatile tool for biotechnology
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Dirigent proteins convey selectivity to oxidative coupling reactions like laccase catalyzed reactions and can be developed into a versatile tool at the interface of lignin degrading processes and bio-sustainable fine chemical production. Until now little is known of the class of dirigent proteins (reviewed in Pickel *et al.*, 2013).
The proposed project aims to identify new dirigent proteins from plants by means of bioinformatics and express them functionally in yeast. Therefore a palette of expression vectors were constructed first, harboring the two host sequence harmonized genes coding for the model dirigent proteins FiDIR1 (Davin *et al.*, 1997) and AtDIR6 (Pickel *et al.*, 2010; Kim *et al.*, 2012) from *Forsythia x intermedia* respective *Arabidopsis thaliana*. Yeasts used as hosts for the production of the dirigent proteins include different strains of *Saccharomyces cerevisiae*, *Pichia angusta*, *Kluyveromyces lactis* and *Yarrowia lipolytica*.
Finally, these dirigent proteins shall be used as bio-sustainable tools at the direct technological interface of lignin degrading processes and fine chemical production.
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BTP67

Production and secretion of a Protein G derived ligand for equine antibody purification with *Bacillus megaterium*

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Normally the foal is born with an intact immune system but with a general absence of immunoglobulins to provide protection against infection. The essential immunoglobulins (Ig) are received with the first mare´s milk (colostrum). Consequently, for generation of artificial foal nutrition purified horse antibodies are required. Conventional horse antibody purification is performed via affinity materials using protein G, an Ig-binding surface protein found in *Staphylococci* strains, as a ligand. However, the purification efficiency is limited by a rather low binding capacity. So far, protein G ligands were derived from clinical *Streptoccus sp.* isolates such as strain GX7809. As an attempt to find a ligand with increased affinity for horse immunoglobulins a blast search was performed identifying a protein G homolog from the horse pathogen *Streptococcus equus*. An amino acid alignment of the two Ig-binding domains of protein G, originated from *Streptococcus sp.* GX7809 ($B1_{S.sp.}$, $B2_{S.sp.}$) and *Streptococcus equus* ($B1_{S.equus}$, $B2_{S.equus}$), showed a sequence identity of 67.3 % and 76.4 %, respectively. Protein G based affinity material is expensive since production and particularly purification of the protein G derived ligands is time consuming and costly. One possibility to simplify the purification protocol is to secrete the proteins directly into the supernatant. For this purpose the Gram-positive soil bacterium *Bacillus megaterium* was employed as it offers the enormous advantage to possess a more efficient secretion machinery over the common protein production host *E. coli*. The sequence of the binding domains was first adapted to the codon usage of *B. megaterium* and then successfully cloned into a shuttle vector in frame with the sequence of a signal peptide (SP_{LipA}). The ligands were then produced and secreted by *B. megaterium* up to 10 mg per 1 litre culture medium. Finally, their general binding functionality regarding horse immunoglobulins were tested and compared via western blot and dot blot experiments.

BTP68

Systems metabolic engineering of *Escherichia coli* for the production of tryptophan-based high value chemicals

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Escherichia coli is one of the major workhorses in industrial biotechnology and recognized as safe for the production of compounds used for human treatment [1]. It has a natural high capacity to produce tryptophan [2], an important industrial amino acid with global annual production of 4,000 t [3]. It is used as a dietary supplement, antidepressant and feed additive. Interestingly, tryptophan is also the building block for a broad range of other high value products, including for example antibiotics and antitumor drugs such as rebeccamycin, staurosporine, violacein, among others.
Here, we streamlined tryptophan production in *E. coli* by systems metabolic engineering We optimized the central metabolism of *E. coli* by engineering of the pentose phosphate pathway, the entire biosynthesis up to the level of chorismate and the tryptophan route. We also enhanced the supply of serine, eliminated tryptophan repression/attenuation, and degradation. Finally, heterologous pathways towards the antitumor therapeutics violacein and deoxyviolacein were integrated into the optimized producers. This enabled biotechnological production of the valuable molecules at the gram scale [5]. At present, we aim to extend the product portfolio to other tryptophan derived high value chemicals.

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BTP69

Enhanced production of lipids from glycerol by the engineered yeast *Yarrowia lipolytica*

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Glycerol is an important renewable feedstock as it is the main side-product of the biodiesel production process, which is nowadays applied on a large commercial scale. According to the European Biodiesel Board, in 2011 the total European biodiesel production capacity reached 22 million tones. It was estimated that approximately 1 kg of crude glycerol is generated for every 10 kg of biodiesel produced. Furthermore, glycerol is produced by several other industries, such as fat saponification and alcoholic beverage production units. Taking into consideration the huge quantities of glycerol produced by above mentioned industries and its low costs, it is of urgency to find an alternative ways to convert this substrate into value added products. Unfortunately, crude glycerol may contains many impurities that significantly decrease its value and the purification process of crude glycerol is time and energy consuming. Despite the high contamination, crude glycerol might be easily utilized by yeast Yarrowia lipolytica, a well-known oleaginous yeast. *Y. lipolytica* is one of the most extensively studied "non-conventional" yeasts due to its biotechnological potential. This unconventional yeast, which has been classified as a GRAS organism, is able to metabolize this renewable feedstock and has a huge biotechnological potential to produce citric acid and other organic acids (pyruvic, α-ketoglutaric), single cell oils (SCO), erythritol, mannitol, coca-butter-like lipid or proteins. To enhance production of SCO from glycerol we over-expressed the *GUT1* (*YALI0F00484g*) gene coding GK and to direct carbon flow into lipid production additional we over-expressed the *SCT1* (*YALI0C00209g*) gene encoding G3P- acyltransferase. The modified strains have been tested for glycerol consumption rate, lipid content, fatty acid composition and biomass production. Subsequently, to improve the SCO production, we additionally over-expressed *DGA1* (*YALI0E32769g*) gene encoding DAG-acyltransferase involved in the last step of triglycerides synthesis and. All genes were under control of a TEF promoter and enhanced TEF promoter (with 16 times repeated upstream activation sequence). Finally, we achieved strain of *Y. lipolytica* with enhanced production of lipids from glycerol.This work was financially supported by the Ministry of Science and Higher Education of Poland - project no. IP2012 008972.

BTP70

Expanding and establishing synthetic biological tools for *Chlamydomonas* chloroplasts

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Question: Photoautotrophic organisms fix carbon dioxide into organic molecules using sun light as an energy source. This process is the basis for all food production on Earth. Increases in global population and rising greenhouse gasses in the atmosphere both contribute to an increased interest in optimizing and harvesting the efficiency of biological carbon dioxide fixation. In eukaryotic organisms, CO_2 fixation is located in the chloroplast, which represents an interesting target for bioengineering. Due to its longstanding use as a model organism for photosynthesis, chloroplast genetics, and biofuel production research, the single chloroplast containing eukaryotic algae, *Chlamydomonas reinhardtii,* has become an important model system and an emerging biotechnological platform. However, synthetic biological tools for the manipulation of the chloroplast are still underdeveloped in *C. reinhardtii*.
Methods: *C. reinhardtii* is a long established model organism for photosynthesis due to its ease of growth and genetic manipulation, as well as its capacity for heterotrophic, photoautotrophic, and mixotrophic growth. In order to bioengineer the chloroplast, the current molecular tools must be further improved to allow for tunable expression of multiple genes and complete operons. We are developing and establishing a suite of plug and play genetic elements for tunable expression in *C. reinhardtii* chloroplasts.
Results: An extensive genetic toolbox is being developed for chloroplast genome modification and tunable expression of complex operons. In these initial expression studies we are utilizing routinely used fluorescent probes to study expression levels.
Conclusion: The construction of an extensive genetic toolbox for chloroplast transformation will give us the platform necessary to begin introducing and controlling new functions in the *C. reinhardtii* chloroplast.

This will allow scientists to introduce novel solutions to solve some of the most important issues of today including meeting food and energy demands and decreasing atmospheric carbon levels.

BTP71

Population analysis of nitrifying bacteria enriched in a fermenter of a toximeter

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Nitrification is a step in biological wastewater treatment where nitrifying bacteria oxidize ammonia to nitrite, and then to nitrate. It is a sensitive microbial process, which can be disturbed by toxic substances, and leads to inhibition of bacterial respiration. A failure of nitrification in wastewater treatment plants can cause increased discharge of ammonia into rivers and lakes. A toximeter developed by a company enables monitoring for toxicity of incoming loads, and protects the nitrifying biology of wastewater treatment. This toximeter measures oxygen consumption of the bacterial fermenter community, and hence the toxicity. However, the bacterial community inhabiting in the fermenter is almost unknown and poorly understood. Furthermore, the sensitivity of nitrifying bacteria for toxic compounds ought to be increased.
The community dynamics and composition within the fermenter were monitored over a few days by fluorescence *in situ* hybridization (FISH) combined with confocal laser scanning microscopy (CLSM), using specific probes targeting ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB). The amount of total cells was determined by DAPI (4′,6-diamidino-2-phenylindole) staining to evaluate AOB und NOB content. In addition, high throughput Illumina MiSeq analysis of 16S rRNA gene was applied to reveal the community composition in two fermenters during different growth stages.
FISH results showed the majority of the bacterial population belonged to AOB. Only single cells of NOB could be detected. Compared to total cell counts, AOB percentages varied from 65 % to 80 %. These findings were quite consistent over a period of time and in different growth stages within the fermenter. MiSeq analysis of 16S rDNA verified the FISH results and showed *Nitrosomonas* as AOB was the main group. Depending on growth stages, 20 % up to 40 % of the bacteria belonged to heterotrophic genera within *Bacteroidetes* phylum. Phylogenetic analysis of 16S rDNA sequences revealed 99 % similarity to *Nitrosomonas stercoris* strain KYUHI-S [1]. For this recently described species, a specific newly primer set was designed to monitor their role during different growth phases and toxicity measurements.
This study suggested that the first step of nitrification is stable over a period of time and in different growth stages within the fermenter. The bacterial sensitivity to toxic compounds may be increased by lowering the percentage of heterotrophic bacteria. Therefore, the application of the new primer set in qPCR analysis is a simple approach to monitor the bacterial population composition, depending on technical and growth conditions within the fermenter.

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BTP72

Online biomass monitoring of plant cell suspension cultures based on conductivity measurement

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Background and aim: Increasing interest is spent to produce various pharmaceuticals with plant *in vitro* cultures. In contrast to whole plants these cultures can produce these substances independently from environmental influences. To establish industrial production processes using plant cell cultures product yields have to be increased. To achieve this, the knowledge of the cultivation progress is required. One of the most interesting parameters is the development of biomass over the time course of a cultivation. A real-time determination of the current biomass in a bioreactor is therefore essential.
Methods: Various measuring methods, using different principles like acoustics, laser light scattering, fluorescence, nuclear magnetic resonance spectroscopy, calorimetry, dielectric spectroscopy and conductivity have been investigated to accomplish this goal [1,2,3,4]. Plant cells in suspension cultures tend to form agglomerates, which makes optical methods quite inaccurate. Conductivity measurements are easy to implement in a bioreactor and an inverse correlation with biomass increase has been proved [5]. Using this fact a computer algorithm was created that converts online data from a conductivity sensor into biomass-data in real-time.
Results: A culture specific coefficient α was defined, which represents the decrease of conductivity during biomass increase [6]. To calculate the current biomass concentration, the value for the biomass concentration at the beginning of the cultivation needs to be known. Conductivity changes were multiplicated with α and then added to the biomass concentration at the start of the fermentation. The algorithm, based on an MS Excel macro, and the data processing method is presented. The routine was tested on a plant cell suspension culture of *Salvia fruticosa.* Raw data of conductivity and semi-online as well as offline (gravimetrically) biomass data from a batch cultivation are compared. Advantages and limitations will be discussed.
Conclusion: The implementation of a conductivity sensor for the semi-online determination of biomass is a helpful monitoring tool for cultivations of e.g. plan cell suspension cultures.

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BTP73

A new strain of docosahexaenoic acid (DHA)-rich Thraustochytrid from Indonesian mangrove habitat

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High amount of docosahexaenoic acid (DHA) in Thraustochytrids has attracted many researchers to isolate new thraustochytrids strain from marine environments. Until 2015, the publication(s) regarding the isolation of new thraustochytrids from marine environments are still available. Thraustochytrids, that now established candidates for commercial production of DHA, are marine heterotrophic protists, included within the phylum Heterokonta within the kingdom Chromophyta (Stramenopiles). Docosahexaenoic acid (DHA) is important for brain and retinal vision of infants, prevent several diseases in adults, particularly coronary ailments. Our new isolate from Indonesian mangrove habitat, LR52, was considered to be an *Aurantiochytrium* based on its morphology, fatty acid profile and 18S rRNA gene sequences. Based on nile red fluorescence microscopy, LR52 contain high amount of neutral lipid/ oil production. Fatty acid profile of *Aurantiochytrium* sp. LR52 revealed that this strain has only DHA as prominent polyunsaturated fatty acid (PUFA). Cultivation of *Aurantiochytrium* sp. LR52 in TubeSpin 600 with salt ocean water (SOW) concentration 14.4 g/L and total glucose consumption 225 g/L, yielded DHA content 19.4 g/L in 5 days. The DHA production rate of 162 g/(Lh) is the highest value reported for any DHA producing microorganisms in shake flask scale.

BTP74

Investigating behavioural differences amongst high-performing *Pseudomonas* spp. surfactants

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Question: Bacteria produce a range of bio-surfactants with differing surface activities and behaviours in air-water and oil-water mixtures. Surfactants which reduce the surface tension of water the most are generally selected for testing in a range of biotechnology applications, but it is unclear how much structural and behavioural variation exists amongst these high-performing compounds. In order to investigate this question, a collection of surfactant-expressing *Pseudomonas* spp. isolates producing a limited range of very low surface tensions (24 - 28 mN/m) were examined.
Methods: Surfactant behaviours were assessed for 25 surfactant-expressing and 5 non-expressing control isolates using foam stability and oil-film displacement assays. For the latter, three oils (mineral, vegetable and used lubricating oil) with NaCl and pH alterations were tested to reflect a range of biotechnological applications and aqueous conditions.

Replicate data were assessed by Analysis of Variance (ANOVA) and Hierarchical Cluster Analysis (HCA) using the Ward method.
Results: Analysis of the foam stability indices and oil-film displacement diameters suggest that there are significant differences in surfactant behaviour between the 25 surfactant-expressing isolates ($P < 0.05$). This data was also used to construct a constellation dendrogram in which isolates were grouped according to similarities in surfactant behaviour (driven by inspection of the HCA scree plot and resulting in all control isolates clustering together). Critically, this resulted in more groups (≥ 5 groups) than could be explained by differences in the surface tensions (≤ 2 groups as previously determined by ANOVA and Tukey Kramer HSD, *alpha* = 0.05). We interpret this to mean that this collection of *Pseudomonas* spp. isolates are expressing a number of structurally-different surfactants (i.e. different types) with varied air-water and oil-water behaviours.
Conclusion: This analysis demonstrates significant behavioural variation within a collection of high-performing surfactants expressed by *Pseudomonas* spp. isolates. The constellation dendrogram produced using data from simple behavioural assays provides a useful tool to choose surfactants for future structural characterisation and testing.

BTP75
Arylmalonate decarboxylase-catalyzed asymmetric synthesis of both enantiomers of optically pure flurbiprofen
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The bacterial arylmalonate decarboxylase (AMDase) catalyzes the enantioselective decarboxylation of prochiral arylmalonates with high enantioselectivity. While this reaction would provide a highly sustainable synthesis of active pharmaceutical compounds like flurbiprofen or naproxen, competing spontaneous decarboxylation has prevented so far the catalytic application of AMDase. We report on reaction engineering and an alternate protection group strategy for the synthesis of these compounds that successfully suppresses the side-reaction and provides pure arylmalonic acids for subsequent enzymatic conversion. Protein engineering increased the activity in the synthesis of the (*S*)- and (*R*)-enantiomers of flurbiprofen. These results demonstrated the importance of synergistic effects in the optimization of this decarboxylase. The asymmetric synthesis of both enantiomers in high optical purity (>99 %) and yield (>90 %) can be easily integrated into existing industrial syntheses of flurbiprofen, thus providing a sustainable method for the production of this important pharmaceutical ingredient.

BTP76
Stepwise error prone PCR and gene shuffling changes the pH optimum and product specificity of a cyclodextrin glucanotransferase
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Question: Cyclodextrins (CD) are used in versatile applications in industrial processes due to their ability to form inclusion complexes with guest molecules. CD are produced enzymatically by cyclodextrin glucanotransferases (CGTases). An optimization of the enzymes to enhance their product specificity and CD yields is investigated.
Methods: A synthetic CGTase gene was cloned into the expression vector pET20b+ and used as template for two rounds of error prone PCR, followed by gene shuffling. Mutagenic constructs were expressed in *E.coli* BL21(DE3). Screening for gamma-CD-synthesizing activity was performed with congored-agar plates. Selected clones were purified by Ni-NTA affinity chromatography. The purified variants were used for CD synthesis reactions with soluble starch as substrate. Synthesized CD were analyzed by HPAEC-PAD.
Results: Two error prone rounds followed by gene shuffling resulted in up to 9 single amino acid substitutions within the whole protein sequence. 15000 clones were screened for the formation of gamma-CD and the 21 clones with the highest activity were further characterized. Five variants showed altered pH activity spectra in a range between pH 4 and pH 11. Furthermore, variants with increased product specificity and cyclization rate for gamma-CD were obtained.
Conclusion: By using directed evolution strategies, the pH activity range and product specificity of a CGTase derived from an alkaliphilic *Bacillus* could be modified. CGTases with these properties are of interest for the industrial production of CD since they can be readily adapted to the reaction conditions of the synthesis process yielding CD of a desired size.

BTP77
Malic acid production from renewable sources by *Aspergillus oryzae*
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Question: L-malic acid is a C4 dicarboxylic organic acid and considered as a promising chemical building block. It can be applied as food preservative and acidulant, in rust removal because of its chelator properties and as polymerization starter unit due to its bifunctionality. Up to now it is produced chemically from crude oil via maleic anhydride. The mould *Aspergillus oryzae* produces malic acid in large quantities from glucose and other carbon sources. The microbial production of organic acids from renewable sources has the potential to be a sustainable alternative to petroleum and to reduce greenhouse gases as CO_2 fixation is involved in microbial biosynthesis.
The potential of malic acid production from renewable resources is evaluated. Therefore, different carbon sources based on lignocellulosic biomass, e.g. fractions of pyrolysis oil and hemicellulosic sugars, are tested.
Methods: *A. oryzae* was cultivated in preculture medium in shaking flasks for 17 hours. Due to nitrogen limitation and an excess of glucose in the production medium the fungus started to produce malic acid. A bioreactor process using glucose as carbon source was already established[1] and used as basis for the evaluation of alternative carbon sources. Organic acid concentration was measured by HPLC.
Results: In our experiments several promising carbon sources based on pretreated biomass for growth and malic acid production were identified and evaluated. These carbon sources include different sugars from hemicellulosic fractions as well as components from pyrolysis products.
Conclusions: *A. oryzae* proved to be a promising natural host for malic acid production with the potential to use several renewable carbon sources.

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BTP78
Calcium binding site engineering of the polyester hydrolase TfCut2 from *Thermobifida fusca* increases protein stability and polyethylene terephthalate degradation efficiency
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The polyester hydrolase TfCut2 from *Thermobifida fusca* is able to degrade polyethylene terephthalate (PET) films and fibers. Ca^{2+} bound to the Ca^{2+} binding site of TfCut2 increases the thermal stability of the protein. A similar effect could be achieved by a salt bridge substituting the Ca^{2+} binding site, however to a lesser extend. Since a high thermal stability is required to degrade PET at its glass transition temperature around 70 °C, a further thermal stabilization was required to exploit the full degradation potential of TfCut2. Molecular dynamics simulation experiments resulted in the identifation of hotspots in the protein structure affecting its stability. The melting point of the resulting variants constructed by amino acid substitutions was remarkably increased compared to the wild type protein. Concomitantly, the half-inactivation temperature and the PET degradation performance at 70 °C were also higher than in the Ca^{2+}-stabilized wild type enzyme. The most active variant showed a temperature optimum between 75 °C and 80 °C. By removal of the Ca^{2+} dependence of the enzyme by protein engineering, a highly stable biocatalyst with high PET degradation activity could be created.

BTP79
Tracking the pleiotropic role of RNA binding protein Hfq from *Clostridium acetobutylicum*
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Questions: The RNA-binding protein Hfq acts as one of the central players of RNA metabolism and posttranscriptional network in bacteria. The

importance of Hfq was highlighted by the discovery of pleiotropic effects of *hfq* gene inactivation in several bacteria, including e.g. increased stress sensitivity, reduced growth rate, cell elongation, and altered motility. Furthermore, Hfq influences stability of small noncoding RNAs (sRNAs) and facilitates binding between these sRNAs and their target mRNAs. This interaction leads to a negative or positive regulation of gene expression at the level of translation.
Here, we focus on Hfq from the Gram-positive *Clostridium acetobutylicum.* This solvent-producing anaerobic bacterium is of importance as it naturally produces butanol, an important bulk chemical as well as biofuel. The aim of this work was the functional characterization of the *hfq* gene from *C. acetobutylicum*.
Methods: Complementation of a *hfq* deficient *E. coli* strain was achieved using *hfq* from *C. acetobutylicum* and adequate *E. coli hfq* promoter sequence. The respective complementation strain was analyzed by microscopy and growth experiments under different stress stimuli. Moreover, the *C. acetobutylicum hfq* overexpression mutant was analyzed regarding growth and solvent production. RT-PCR analysis was performed with RNA from *C. acetobutylicum* to determine whether *hfq* transcription starts within the neighboring *miaA* coding region or further upstream.
Results: As *E. coli* wild type, the *E. coli* complementation mutant producing Hfq from *C. acetobutylicum* showed a higher maximal optical density, a decreased generation time and a reduced cell length compared to the *E. coli hfq* deficient mutant. The *C. acetobutylicum hfq* overexpression mutant showed a delayed and decreased solvent production compared to *C. acetobutylicum* wild type. The organization of *hfq* in the genome of *C. acetobutylicum* has been identified.
Conclusion: As the RNA-binding protein Hfq from *C. acetobutylicum* is able to replace Hfq-specific functions in *E. coli,* both proteins might accomplish the same functions. Moreover, the delayed solvent production of *C. acetobutylicum hfq* overexpression mutant indicates that *C. acetobutylicum* is able to tolerate acidic stress over a longer period of time than the wild type. These results emphasize for Gram-negative and Gram-positive bacteria a necessity of Hfq, when living in a stressfull environment.

BTP80
Optimisation of bioflocculants produced by three marine bacteria belonging to the genera *Alcaligene* and *Bacillus* isolated from Sodwana Bay in the Kwazulu-Natal Province of South Africa
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We assessed the bioflocculant producing potentials of three marine bacteria isolated from the sediment samples of Sodwana Bay in the KwaZulu-Natal Province of South Africa. Analysis of the partial nucleotide sequence of the 16S rDNA of the bacteria revealed their identities as *Alcaligene faecalis*, *Bacillus subtilis* and *Bacillus pumulis*. Cultivation condition studies for *Alcaligene faecalis* revealed that bioflocculant production was optimal with an inoculum size of 1 % (v/v), initial pH of 9.0, K^+ as the metal ion, and maltose as the carbon source. Metal ions, including Li^+, and Ca^{2+} stimulated bioflocculant production resulting in flocculating activity of above 70 %. On the other hand, bioflocculant production by *Bacillus subtilis* was optimal when fructose (96 % flocculating activity) and urea (91 % flocculating activity) were used as carbon and nitrogen sources respectively; inoculum size was 1 % (v/v); initial pH 10; and Fe^{2+} as coagulant aid. However bioflocculant production by *Bacillus pumulis* was optimal when maltose (94 % flocculating activity) and casein (79 % flocculating activity) were used as carbon and nitrogen sources respectively; inoculum size was 1 % (v/v); initial pH 10; and Fe^{3+} as cation.

BTP81
Engineering *Planctopirus limnophila* –towards a synthetic planctomycete with artificial organelles
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Planctomycetes are very unusual yet ubiquitous distributed and environmentally important bacteria. In contrast to "normal" bacteria, some planctomycetal species contain membrane enclosed "organelles" which are employed to convert nitrite and ammonium via **an**aerobic **am**monium **ox**idation into nitrogen with hydrazine as toxic intermediate that would normally poison the cell. Due to this trait anammox Planctomycetes are already used in wastewater treatment. The concept of confined membrane sealed environments for toxic reactions is usually limited to complex eukaryotic cells.
However, since so far the broad biotechnical application of Planctomycetes is often hampered by their slow division rates, taking up to a month for one cell division cycle, and lack of genetic tools to functionally analyze the genes involved. Here we present an approach based on our planctomycetal model organism *Planctopirus limnophila,* which has a doubling time of approximately six hours. In addition, we constructed the transposon TnCJ003 to demonstrate heterologous expression of the green fluorescent protein (GFP) in *P. limnophila* cells, showing that heterologous gene expression in *P. limnophila* is feasible. Moreover, recently a synthetic magnetotactic bacterium was constructed that uses a confined membrane surrounded organelle -the magnetosome- to produce magnetite crystals. Thus, the construction of synthetic microbes with subcellular compartments that perform defined chemical reactions became possible. Now we aim to construct a defined compartment in *P. limnophila* that hosts a modified version of the anammox reaction. In a first step, we will express known key proteins involved in the process of anaerobic ammonium oxidation of the anammox Planctomycetes in our model bacterium with both a constitutive and an inducible promoter and study the functionality of such proteins in individual assays. The genetic tools developed for *P. limnophila* to reach this goal are instrumental for other applications as well. Recently we showed Planctomycetes to harbor several novel secondary metabolite gene clusters. However, secondary metabolite related genes and clusters are frequently silent under laboratory conditions, these tools could be applied to express such genes in *P. limnophila* to enhance production under defined growth conditions.
These tools will allow us to further unearth the planctomycetal repertoire of biotechnological interesting compounds and to improve application systems like waste water treatment.

BTP82
Quantification of ribosome degradation in *E. coli*
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Question: Ribosome degradation in *E. coli* is commonly associated with starvation such as lacking of carbon, nitrogen or phosphate. However, the principal mechanisms which result in degradation of stable RNA *in vivo* and *in vitro* are still discussed [1]. Reliable estimations of ribosome concentrations are indispensable in order to follow rRNA degradation processes, compare rRNA turnover rates and to identify possible degradation pathways.
Methods: To date the most routine method of RNA analysis is agarose or polyacrylamide gel electrophoresis where degradation is comprehended by comparison of relative intensities of electrophoresis bands. The current work demonstrates the absolute quantification of ribosomes *in vitro* and *in vivo* by measuring the 16S and 23S rRNA applying capillary gel electrophoresis with laser-induced fluorescence detection (CGE-LIF).
Results: We demonstrate the quantitative documentation of ribosome degradation *in vitro* under high and low Mg-concentrations. We further observe the quantitative dynamics of ribosome in growing cells (*in vivo*) where ribosomes are supposed to be stable during exponential growth and are degraded when the culture enters the stationary phase.
Conclusion: CGE-LIF enables the reliable measurement of 16S and 23S rRNA in a quantitative manner. In particular, this technique allows the quantitative analysis of ribosome dynamics during growth processes (*in vitro*) and in cell-free systems.

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BTP83
Altering substrate specificity of soluble, NAD^+-reducing hydrogenase for H_2-driven cofactor regeneration in cytochrome P450-catalyzed selective oxidations
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Question: The application of oxidoreductases as biocatalysts in chemical synthesis of chiral compounds is increasingly gaining importance. A dominant group comprises cytochrome P450 monooxygenases (CYP) that are able to catalyze O atom insertion into non-activated C-H bonds[1]. The activation of O_2 by these enzymes requires reducing equivalents in form of NAD(P)H. A stoichiometric supply of these cofactors is highly costly, and therefore an alternative strategy for providing reducing agents is favorable. Continuous

cofactor recycling by the O_2-tolerant NAD^+-reducing hydrogenase (SH) from *Ralstonia eutropha* appears to be well suited for such an approach as it only uses H_2 and does not produce any interfering side products[2,3,4]. As there are many oxidoreductases that require the phosphorylated cofactor NADPH and only few $NADP^+$-reducing enzymes available for cofactor regeneration, we aimed at changing the NAD^+ binding pocket in the SH into a site that also accepts and converts $NADP^+$.

Methods: Specific amino acid exchanges designed by rational mutagenesis led to SH derivatives with synthetic $NADP^+$-reducing activity. The resulting variants were characterized biochemically as well as electrochemically by protein film voltammetry. Based on these methods the kinetic parameters K_M, K_{cat} and K_{cat}/K_M were used as indications of changes in substrate specificities.

Results: Remarkably, we generated a variant with higher affinity for the new substrate $NADP^+$ than for its natural substrate NAD^+. The applicability of this SH variant in H_2-driven NADPH supply to CYP102A1 monooxygenase from *Bacillus megaterium* was successfully tested as the selective oxidation of octane with safe H_2/O_2 mixtures was fully accomplished in presence of a cofactor concentration of 1.5 mM.

Conclusion: With this work we show that it was possible to convert a hydrogenase to an attractive candidate for NADPH recycling. Specificity and affinity of the enzyme could be altered according to the need for NADPH regenerating biocatalysts in central biotechnological processes e.g. alkane oxidation by P450 monooxygenases in the context of flavor/fragrance industry.

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BTP84

Screening of metallophors from bacteria and fungi for metal-affinity

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Question: Under iron-restricted conditions many bacteria and fungi produce metal chelating molecules called metallophores for scavenging iron (therefore also called siderophores) or other metal ions from the environment. This low-molecular-weight compounds possess a high affinity and selectivity for ferric iron ($K_f > 10^{30}$). Some of them are able to effectively bind other metals as well. In order to identify metallophores, which are able to bind strategically important metals like Gallium and Vanadium we screened numerous bacteria and fungi for metallophore production and metal-affinity.

Methods: A general and widely used method for the detection of metallophores is the CAS-assay from Schwyn & Neilands, based on the utilization of chrome azurol S [1]. We used a low phosphate minimal growth medium and a liquid CAS assay variant. The test has been optimized regarding to different metals, metal concentrations and pH values in order have an applicative and useful screening method for different types of microorganisms.

Results: We established the test for Gallium, Aluminium and Vanadium in microtiter plates and calibrate these metal CAS assay solutions as well as the available solutions for iron [2] and copper [3] with desferrioxamine B (DFOB) to calculate DFOB-siderophore-equivalents. Low quantities up to 1 µM DFOB was successfully measured. Using these assay variants, we screened more than 35 stains of different bacteria and fungi for metallophores and their affinity to Fe, Al, V, Ga and Cu ions.

Conclusion: The colorimetric assay with different metal CAS solutions and culture supernatants in microtiter plates was found to be a rapid and easy-to-perform method to test different strains for the production of metallophores and their affinity to different metal ions. The screening of various strains gave an interesting insight into the metal specificity of different siderophores. Indeed we found strains, which do produce Vanadium binding metallophores. *Petromyces alliaceus* is one of the most promising candidates for producing such a metallophore.

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BTP85

Electro-acoustical method for bacteriophages detection in liquid phase

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Question: Recently various approaches such as microbiological and biochemical tests, genetic engineering techniques and immunological methods are used for viruses' identification. The development of the new methods of bacterial viruses' detection for obtaining correct precise results in a short time (express-methods) is actual problem, which is in the focus of attention of researchers in microbiology. One of the promising methods for achievement of such goal is the electro-acoustic method of analysis based on registration of bio-specific reactions in liquid suspension that contacts with the piezoelectric material surface.

Methods: All experiments for registration of the changes in the mechanical and electrical properties of microbial cell suspensions due to biospecific interaction with microbial cells were carried out with a help of specially manufactured sensor. Sensor was based on a piezoelectric resonator with a lateral electric field in the frequency range of 6-7 MHz. This resonator was made of X_cut lithium niobate plate with thickness of 0.5 mm.

Results: For the first time the possibility of detection of bacteriophages by the use of the electro-acoustic method of analysis was demonstrated on the example of the interaction of bacteriophages ΦAl-Sp59b with microbial cells *Azospirillum lipoferum* Sp59b. As a biological sensor, a piezoelectric lateral electric- field- excited resonator containing a liquid container with volume of ~ 1 ml was used. It has been found that the frequency dependencies of the real and imaginary parts of the electrical impedance of the resonator loaded by suspension of viruses with microbial cells significantly differ from the dependencies of the resonator with the control virus suspension without microbial cells. It has been shown that the detection of bacteriophages ΦAl-Sp59b by means of microbial cells is possible also in the presence of other viral particles and extraneous microbial cells.

Conclusion: The proposed method allows to reliably determine the type of the test virus after 5 minute of its interaction with the culture cells. At that the minimum concentration of virus is 5 viral particles per cell. As a whole obtained results demonstrate the possibility of detecting the specific interaction of bacteriophages with microbial cells and provide a basis for the development of a biological sensor for quantitative detection of viruses directly in liquid phase.

BTP86

Systems metabolic engineering of *Corynebacterium glutamicum* for bioproduction from xylose

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Bio-based production promises a sustainable route to chemicals, materials and fuels. With regard to eco-efficiency, its future success strongly depends on a next level of bio-processes using raw materials beyond glucose [1]. Such renewables, i.e., polymers, complex substrate mixtures and diluted waste streams, often cannot be metabolized naturally by relevant industrial cell factories [2].

Hemicellulose, one of the most abundant biomass compounds on earth, represents a promising alternative substrate for sustainable bioproduction. Its major constituent xylose is, however, naturally not utilized by *C. glutamicum* - a major work horse in biotechnology [1]. For making xylose bio-available for *C. glutamicum,* the two *E. coli* genes *xyl*A and *xyl*B, encoding xylose isomerase and xylulokinase, respectively, were expressed in *C. glutamicum* strains streamlined for diaminopentane production [3]. Integration of metabolic flux analysis with *in silico* pathway modelling and systems-wide transcriptome profiling was then used to identify additional metabolic engineering targets for improving xylose-based production [4]. In fed-batch process, the engineered diaminopentane producer was approaching industrial level performance with higher titer (103 g L^{-1}) and yield (0.32 g g^{-1}) [4]. Initial studies on direct utilization of hydrolysates of the polymer hemicellulose for production are highly

promising for future application as yield substantially increased by 50 % [3]. At present, the production portfolio from xylose based processes is extended to other industrially relevant compounds.

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BTP87

Food protection against *Alternaria brassicicola* contamination using lactic acid fermentation with polyols

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Alternaria brassicicola is a mould species which is known as one of major plant pathogens, which causes infectious plant diseases, and common allergens in humans. The preliminary studies suggest that some of lactic acid bacteria protect food from fungi and presence of polyhydroxyalcohols (polyols) in bacterial medium has an beneficial impact on their antifungal effect.
Antagonistic activity of sixty *Lactobacillus* sp. strains in the presence of 1 % (w/v) of polyols - erythritol, xylitol, lactitol, maltitol, mannitol and sorbitol - was examined using double layer method. First layer of medium was MRS for cultivation of lactobacilli, second one - Sabouraud for moulds. Additionally, we estimated the fungistatic effect of various supplementation of cell-free supernatants after lactic acid fermentation of chosen *Lactobacillus* sp. strains in the presence of the above polyols in fungal growing medium using poisoned media method. We evaluated two parameters: a linear growth index of fungi and a fungistatic activity of bacterial supernatant according to Abbot's formula described by Glen and Boliglowa (2011).
Majority of examined *Lactobacillus* sp. bacteria shown poor or moderate antifungal activity in the control sample, without polyols. However, supplementation of the medium with 1 % of sorbitol, xylitol or mannitol enhances fungal inhibition by lactic acid bacteria. The results of fungistatic activity for specified bacterial strains confirm this trend. We observed strong correlation between presence of xylitol in growing medium and inhibition of tested moulds on sweet cherries as well. Fruits threatened by cell-free supernatant after lactic acid fermentation were resistant to *Alternaria brassicicola* contamination.
Polyols in growth medium change the metabolism of most tested *Lactobacillus* bacteria to produce antifungal compounds. We observed strong correlation between an antagonistic activity of investigated *Lactobacillus* sp. strains and composition of medium.

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BTP88

Discovery of enzymes and natural products in the moss microbiome

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Sphagnum mosses are colonized by highly diverse and species-specific microbial communities. Mosses are known to produce plenty of bioactive substances and the antimicrobial activity of their inhabiting microorganisms has also been reported (1). Recently we elucidated the Sphagnum metagenome by Illumina-sequencing and *de novo* assembly (2). The *Sphagnum* microbiome harbors highly specific genetic features that distinguish it significantly from comparable microbiomes. Abundant functions support abiotic stress protection, communication and interaction between microorganisms. Based on this findings we explored the Sphagnum microbiome as a source of industrially interesting enzymes and natural products for biotechnological and biomedical applications. Different gene targets such as esterases, phosphatases, decarboxylases, polyketide synthases (PKS) and non-ribosomal peptide synthetases (NRPS) were selected for screening employing a metagenomic fosmid clone library. NRPS and PKS genes are involved in the production of several microbial bioactive secondary metabolites. The study of NRPS and PKS sequences in the *Sphagnum* metagenome by *in silico* data mining revealed a high level of diversity. Furthermore, PCR-amplification screening of the fosmid clone library led to identification of thirteen novel NRPS-related sequences with identities ranging from 48 % to 91 % to annotated sequences that belong mainly to the phyla *Proteobacteria*, *Actinobacteria*, and *Cyanobacteria* (Müller *et al.*, 2015). The novel NRPS sequences are putatively involved in production of microbial metabolites such as siderophores, phytotoxins, and antibiotics. Selected clones containing NRPS and esterase genes are currently being evaluated to discover new enzyme functions and activities.
Our study highlights the potential of plant-associated microbiomes found in extreme natural ecosystems for the discovery of biocatalyst and natural products with biotechnological potential.

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CMP01

Regulation of motility and phototaxis in a model cyanobacterium

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Many prokaryotes are able to actively move using different motility machineries. The regulation of motility enables the cells to make decisions about lifestyle and to actively search for suitable environmental conditions for live. Light is the most important factor for photosynthetic organisms. Although light-controlled movement of prokaryotes was observed and have been described for a long time the basic biophysical and molecular mechanisms are poorly understood and investigated. The well characterized model cyanobacterium *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*) shows phototactic responses and light-induced motility on surfaces using type IV pili. The movement of *Synechocystis* cells is controlled by quantity and quality of light. The inhibition of movement towards blue light was shown to be mediated by the second messenger c-di-GMP via the unique photoreceptor Cph2 [1]. The Cph2 protein contains c-di-GMP synthesis and degradation domains in an unusual combination with two photosensory modules which perceive red/far-red or green/blue light, respectively.
To understand how altered c-di-GMP levels control motility from a mechanistic point of view and what other processes are regulated by this second messenger we first started with analyses at the level of gene expression.
Changes in blue-light dependent c-di-GMP levels were achieved by inactivation of the *cph2* gene and the transcriptome of the wild type and the Δ*cph2* mutant were compared using microarray analysis. Several genes known to be involved in phototaxis were differentially expressed in the Δ*cph2* mutant. However the functions of many of these gene products are not known and their role within phototaxis remains unclear. Furthermore, several of these Cph2-controlled genes are also known to be regulated by the *Synechocystis* RNA chaperone Hfq and cAMP related signalling pathways. The binding of Hfq to the pilus base was shown to be essential for the motility of *Synechocystis* [2].
Since *Synechocystis* does not contain proteins harboring the PilZ domain or any other known c-di-GMP binding effector domain, we further started to search for novel putative c-di-GMP binding proteins and analyzed their binding characteristics. These analyses will help us to enlighten the c-di-GMP dependent regulation at the level of proteins.
Our results suggest a highly complex regulatory network for the motility of *Synechocystis* including transcription factors, photoreceptors, second messengers and sRNAs.

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CMP02
3D ultrastructure and motility of *Pyrococcus furiosus*
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Question: The organotrophic, hyperthermophilic Euryarchaeum *Pyrococcus furiosus* serves as a unique model system for numerous studies of archaeal molecular cell biology. The slightly irregular cocci exhibit 50 or more archaella, 10 nm in diameter and several µm in length. They are directly involved in cell motility, adhesion to various surfaces and formation of cell-cell connections, resulting in a network of interconnected *P. furiosus* cells (Näther et al, 2006).
As *P. furiosus* can swim very fast and is able to withstand harsh environmental conditions, its cellular architecture, like the structural organisation of the archaella bundle and the architecture of the S-Layer, is of great interest for us. In particular, our focus is on variations in cellular ultrastructure and extent of flagellation, as observed for naturally occurring mutants (Näther-Schindler *et al.*, 2014).
Methods: We are in the process of analysing these cells using various electron microscopical methods (Rachel et al, 2010), in combination with proteome analysis. In particular, 300 nm and 600 nm sections of high-pressure frozen and resin-embedded *P. furiosus* cells are analysed using TEM and STEM tomography. STEM tomography datasets of 600 nm sections are useful in particular in order to reach a higher content of information, as the focus gradient is eliminated, due to a great depth of focus in STEM mode. At the same time, a considerably larger cell volume is visualised (Yakushevska et al, 2007). Datasets were reconstructed using 3Dmod / SIRT and visualized in AMIRA. We extend our studies towards naturally occurring lab mutants, and using other techniques like proteome analyses.
Results: The tomography datasets turned out to be rich source for detecting new features in the architecture of intact *P. furiosus* cells. We unravelled yet undescribed features, like the partial disintegration of the S-Layer in certain areas, or structures associated with the cell wall, inside and outside of the cell.
Conclusion: Combined ultrastructural and biochemical analyses are necessary in order to fully understand the complexity of *P. furiosus* cells; we aim to combine our data with FIB-SEM datasets, in order to obtain an overview of the total architecture of several intact cells, in parallel. We plan to complement our studies using cryo-tomography, to be performed in the near future.

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CMP03
Public goods of *Bacillus subtilis* – How public are they really?
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The question of how cooperation could evolve is still not solved in evolutionary biology. Several studies show that in microbial populations cooperation can determine the community structure and can increase the overall fitness of the community although being costly to the individual cells. One aspect of cooperation is the production of public goods i.e. products that are secreted into the environment so that not only the producing cell but also non-producers can benefit from it. The maintenance of cooperation can be explained for example by the microbial colonization of spatially heterogeneous environments where the spatial structure stabilizes cooperation. This might particularly be important for the colonization of surfaces e.g. the formation of colony biofilms or sliding communities on agar (Grau *et al.*, 2015) where the production of public goods can be crucial. *Bacillus subtilis* is a Gram-positive bacterium that was previously used for studies on the stability of cooperation in colony biofilms (van Gestel *et al.*, 2014). However, there is less information about the influence of public goods in expanding communities such as sliding. Therefore, this study aimed to analyze the importance of different public goods produced during sliding of *B. subtilis* and to expose how well they are shared in the expanding population. Competition experiments were performed under sliding promoting conditions with different combinations of the wild-type and mutant that lacks a certain public good production. Further, the metabolic cost of production was determined for each public good. Being important for sliding, we analyzed the impact of surfactin, the amphiphilic protein BslA and extracellular polysaccharides as public goods. Competition experiments revealed that while certain public goods can be exploited by non-producers, others are kept private benefiting only the producer linage. Our experiments demonstrate that the tested public goods are not shared equally and thus have varying influence on the population structure during sliding.

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Grau R, de Oña P, Kunert M, Leñini C, Gallegos-Monterrosa R, Mhatre E, Vileta D, Donato V, Hölscher T, Boland W, Kuipers OP, Kovács ÁT (2015). A duo of potassium-responsive histidine kinases govern the multicellular destiny of *Bacillus subtilis*. mBio 6(4):e00581-15.

CMP04
Visualizing flagellar filament behavior of *Shewanella putrefaciens*
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Flagellum-driven motility is the most common way of moving in liquid media or on semi-solid surfaces among bacteria. Although it is not essential for cell viability, this motility provides crucial advantages under hostile environments or nutrient limitation. In concert with the chemotaxis system, flagellated cells can propel themselves towards favorable conditions. Bacterial motility is also a topic of biomedical interest since many human pathogenic bacteria must pass the mucosal lining of the respiratory, gastrointestinal or reproductive tracks to invade their host. We use *Shewanella putrefaciens* CN-32 as a model organism for flagella-mediated motility. This species possesses a polar flagellum to efficiently move through liquid, semi-solid, or viscous media. Under high nutrient conditions, both in planktonic growth and in semi-solid medium, *S. putrefaciens* can grow additional lateral flagella which are expressed from a different genetic locus. Those flagella can help spreading in semi-solid media and might also give an advantage in highly viscous environments, such as mucus. Here, we address the behavior of the flagellar filaments of *S. putrefaciens* during swimming in various conditions, with an emphasis on the interplay between the polar and lateral flagella. The flagellar filaments were visualized by attaching fluorescent dyes and fluorescence microscopy. An increased number of cells with a single polar and several lateral flagella were detected in the outer edge of a colony spreading in semi-solid medium. Fluorescence microscopy analysis revealed that for those cells the polar flagellum seems to be the main driver of swimming direction but the lateral ones might still contribute to swimming torque or maneuvering. Although the lateral filaments appear to actively rotate, they never form a bundle unlike the filaments of peritrichously flagellated *E. coli*. Taken together, the study reveals potential mechanisms of how lateral flagella effect bacterial swimming.

CMP05
Swimming motility of *Phaeobacter inhibens* DSM 17395 and the regulation of the archetypal flagellum of Rhodobacteraceae
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Question: Swimming motility is the prerequisite for the swim-or-stick-lifestyle of roseobacters. Recent analyses identified three different conserved flagellar operons, designated *fla1, fla2* and *fla3* (Frank et al, 2015) *Phaeobacter inhibens* DSM 17395 contains a single flagellar superoperon encoding the archetypal and most abundant type-1 flagellum (*fla1*). The flagellar composition has been extensively studied for decades. However, the function of four *fla1* genes universally conserved among *Rhodobacteraceae* remains still unclear. In this study we exhaustively screened transposon mutants of *P. inhibens* in order to identify the crucial components for flagellar movement of this model organism, which should be representative for the whole roseobacter group.
Methods: High throughput transposon mutagenesis with a calculated genome saturation of 95 % was performed using EZ-Tn5 the transposome kit of epicentre. The mutants were phenotypically screened for the lack of motility by incubation on selective soft-agar with kanamycin for two days. Transposon insertions were determined by arbitrary PCR as described by O'Toole and Kolter (1998). The most promising mutants were genetically complemented by transformation of plasmids containing the wildtype genes.

Results: Screening of 12,100 *Phaeobacter inhibens* DSM 17395 transposon mutants on soft agar plates allowed to identify 180 mutants with abolished or severely constricted motility. More than half of the mutants carry transposons inserted into flagellar genes. However, we could also identify 75 mutants with an insertion in non-flagellar genes. Accordingly, we identified all genes of the CckA-ChpT-CtrA phosphorelay, the nitrogen regulator NtrX and a LuxR type transcriptional activator. The most conspicuous phenotype has been observed for the mutant of an alternative ECF sigma factor and a peptidase that showed a "swarming-like" swimming zone.
Conclusion: Our systematic screening of transposon mutants for motility defects, allowed us to identify four universally conserved genes of the *fla1* type operon, which were not characterized yet. Mutations in ctrA phosphorelay genes resulted in the loss of motility as previously predicted for *Dinoroseobacter shibae*. The regulatory network of transcription factors and the role of the ECF sigma factor have to be elucidated.

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CMP06
Characterization of chemoreceptors and chemotaxis in *Thermococcus kodakarensis*

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Question: *Thermococcus kodakarensis* was isolated from a solfatara on the shore of Kodakara Island, Japan. The hyperthermophilic archaeon is obligate heterotrophic and grows on organic compounds in the presence of elemental sulphur, at an optimal temperature of 85 °C [1]. Previous studies have shown that *T. kodakarensis* possesses polar flagella, and five annotated chemoreceptors, but up to now, no data are available which chemical compounds can be sensed by *T. kodakarensis* and direct its swimming towards more favourable conditions. Therefore the influence of different media and growth conditions on the presence and number of these chemoreceptors and flagella and how this affects the swimming behaviour should be determined in this study.
Methods: The expression of chemoreceptors in different media and under different conditions, e.g. changes in temperature, oxygen concentration or growth phase, was analyzed via Western Blot using polyclonal antibodies against one cytoplasmic and one out of four transmembrane receptors. For analyses on the swimming behaviour, thetemperature gradient-forming device described by Mora *et al.* [2] was used. This device enables swimming studies of the hyperthermophilic organism under anaerobic condition at temperatures up to 110 °C. In parallel, cells were fixed with glutardialdehyde and negatively stained for electron microscopy to determine the number of flagella depending on the different conditions tested.
Results: Different growth conditions, such as temperature, NaCl-concentration and the reduction of organic compounds, were shown to influence the expression of chemoreceptors in Western Blot analyses. Particularly, increasing of unfavourable growth conditions lead to an increasing amount of expressed chemoreceptor proteins. Furthermore a change in the presence and number of flagella at different condition could be observed and, interestingly, correlated with the ability to swim. Initial studies of the chemotactic response of *T kodakarensis* identified complex organic compounds like yeast extract to have a positive effect on its swimming direction.
Conclusion: The positive or negative influence of different parameters and chemicals on the expression of chemoreceptors and flagella in *T. kodakarensis* and their impact on its motility could be demonstrated here. Hence, we suggest *T. kodakarensis* as a model organism to study chemoreceptors and chemotaxis in Archaea to better understand the link between sensing and movement.

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CMP07
The archaellum – analyzing the role of archaellin-like proteins FlaG and FlaF in *Sulfolobus acidocaldarius* and *Pyrococcus furiosus*

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Questions: Among Archaea motility is exclusively mediated by archaella, formerly known as archaeal flagella. Archaella are unique structures since they share structural similarities with type IV pili, while generating thrust by rotation like bacterial flagella. [1].
Periplasmic archaellum components have yet to be identified. The small, monotopic membrane proteins FlaG and FlaF are conserved among all archaellated species [1] and harbor an archaellin domain, making them good candidates for periplasmic localization. The objective of the project was to confirm periplasmic localization of FlaG/FlaF and further characterization of a potential periplasmic complex.
Methods: Heterologous produced soluble domains of FlaG (sFlaG) and FlaF (sFlaF), from *S. acidocaldarius* and *P. furiosus*, were used for biochemical characterization. Oligomeric protein species were analyzed using gel filtration and chemical crosslinking. Microscale Thermophoresis was performed to prove and quantify protein-protein interactions. Protein structures were assessed by small-angle X-ray scattering (SAXS) and solving the crystal structures. Finally, point mutated proteins were used for complementation *in trans* and their effect on *S. acidocaldarius* motility was tested employing *in vivo* swimming assays.
Results: Purified soluble domains of Saci FlaG and Saci FlaF are highly stable at pH 3 and interact with a K_D of 14 - 16 µM. Individually, both proteins crystallized as dimer and show β-sandwich fold. sFlaF structurally resembles S-layer proteins from other species and binds to the *S. acidocaldarius* S-layer [2]. Point mutations in the sFlaF dimer interface disrupt the native dimer and result in monomeric sFlaF that does not interact with sFlaG anymore *in vitro*. Furthermore monomeric FlaF leads to non-motile cells [2]. However, the sFlaG/sFlaF co-crystal structure revealed that the FlaF dimer interface is also the interaction interface with sFlaG. Analytical gel filtration, including also the *Pyrococcus* proteins, suggests that FlaG and FlaF are forming a membrane bound, high molecular weight complex.
Conclusions: Given the facts that Saci FlaG and FlaF are very stable at pH 3, harbor a predicted archaellin-domain and interact with the S-layer, a periplasmic localization is highly probable. Additionally, the lack of a class III signal peptide and the gel filtration data suggest that the proteins form a large, membrane-bound complex. This complex could represent a periplasmic archaellum complex which anchors the archaellum in the cell wall and allows the archaellum filament to passage through the rigid S-layer.

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EMP01
Models on the kinetics of phenol utilization in ammonium phosphate supplemented phenol laden refinery effluent

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Growth of mixed cultures of phenol utilizers which included *Pseudomonas sp.tww, Bacillus sp.rww., Citrobacter sp.tww, Klebsiella sp.rbww* and *Staphylococcus sp.rww* were monitored in batch cultures of phenol laden refinery effluents supplemented with different concentrations of ammonium phosphate. The nutrient supplement $(NH_4)_3PO_4$) encouraged cell growth and phenol reduction in a concentration dependent manner with optimum values observed at higher concentrations. After 21 days of treatment, $(NH_4)_3PO_4$) concentration of 0.05 %, 0.2 %, 0.5 % and 1.0 % reduced phenol concentration of 103ppm to 4.4 %, 3.7 %, 0.88 % and 0.83 % respectively with a corresponding cell growth of 6.3×10^9, 1.1×10^{10}, 3.0×10^{10} and 2.6×10^{10} cfu/ml respectively. As phenol concentration increased beyond 203ppm, cell growth and phenol reduction rate decreased at all levels of nutrient concentration. In the effluent treatment control, the inoculated process waste water showed no appreciable cell growth or phenol reduction at 51.65ppm of phenol. Phenol concentration of 103ppm was reduced to 50.8 % with a maximum cell growth of

9.8×10^5cfu/ml after 28 days. The uninoculated process waste water showed no cell growth or phenol reduction. The experimental data obtained from this study was modelled with the first order differential equation ($dx/dt = \pm\mu x$) describing the rate of depletion of phenol (d phen I/d (time) = µp phen I) and growth of biomass d(THCI)/d(time) =µtTHCI) for the different nutrient concentrations. At a known ($(NH_4)_3PO_4$) concentration and time, phenol depletion and biomass growth rate was predicted using the model.

EMP02

Microbial communities in Mississippi sediments under alternating redox conditions

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Soil microbial communities play a key role in biogeochemical processes. Flooding events alter the redox potential of the soil and therefore lead to changes in microbial community structure. However, up to date it is largely unknown how flooding events change composition and amount of these soil microbial communities. To investigate soil microbial community dynamics, an automated biogeochemical microcosm system was used to simulate flooding of the soil in the laboratory. This system allows establishing pre-defined redox conditions in soil suspensions by flushing them with nitrogen (N_2) or oxygen (O_2). Samples were taken at these pre-defined redox potential levels from anoxic (-56 mV) to oxic (468 mV). To assess the microbial community structure and abundance, phospholipid fatty acids (PLFA) of the samples were analyzed. In addition, the bacterial community was investigated via denaturing gradient gel electrophoresis (DGGE) of the 16S rRNA gene PCR products. Some of the appearing and disappearing bands of the DGGE analysis were excised and sequenced to identify the according species.
The varying redox conditions had a considerable effect on the microbial community structure, indicated by a differing PLFA profile. Correspondingly, DGGE analysis showed a changing bacterial community over the course of a shifting redox potential. Comparison of the untreated sample with the microcosm samples showed varied banding patterns. While obligate aerobe bacteria were only found in the initial sample, low redox potential led to the occurrence of sporogenous bacteria preferring anaerobic conditions. Summarizing, flooding events can have significant effects on soil microbial community composition and therefore play an essential role in changing of biogeochemical properties of the soil.

EMP03

Pedobacter glucosidilyticus* DD6b genome-specific properties and common features compared with the publicly available type strains genomes of the genus *Pedobacter

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Phosphorus is one of the five vital elements and occurs in bio- and geochemistry mainly as phosphates, where the central P atom is in its higher oxidation state [+V]. Some recent reports show that phosphorus occurs on Earth in reduced forms, with central P atom in [+III] or [+I] oxidation state. Providing that P is one of the growth limiting factors mainly, but not only in water ecosystems, the ability of bacteria to metabolize reduced P compounds gains in interest.
We present the genome sequence of *P. glucosidilyticus* DD6b, an aerobic phosphite oxidizer, its specific properties and analysis of the common features with all publicly available whole genome sequences of *Pedobacter* species.
The genomic DNA of the strain was used for the preparation of shotgun libraries. Sequencing resulted in 12,380,618 paired-end Illumina reads of 112 bp., trimmed with Trimmomatic 0.32 to discard adaptor sequences and those with quality scores lower than 20 (Illumina 1.9 encoding). For the initial *de novo* assembly of the 4,150,000 reads resulting in in 93 contigs larger than 0.5 kb SPAdes 2.5 software was used, and Prodigal for automatic gene prediction. rRNA and tRNA were identified with RNAmmer and tRNAscan, respectively. An automatic annotation with the IMG-ER system and manual curation by employing BLASTP, Swiss-Prot, TrEMBL, and InterPro databases, were performed.
The genome size is 3,876 Mb, containing 3,352 predicted genes of which 3,311 protein-encoding and 41 RNAs genes (3 rRNA and 38 tRNA). Overall GC content is 34.74 mol %. Protein-encoding genes with a putative function were 2,610 (77.86 %) and 701 (20.91 %) annotated as hypothetical proteins. The pairwise genome comparison of *P. glucosidilyticus* DD6b and *P. glucosidilyticus* DSM 23534 revealed the presence of a complete and specific DNRA pathway in DD6b. It assimilates phosphite under phosphate starvation via a sec-dependent periplasmic alkaline phosphatase, analogously to *E.coli*. The whole genome analysis of all sequenced *Pedobacter* type strains and DD6b allowed defining the size of the core (1,398) and the pan genome (9,962) orthologous groups in *Pedobacter* species.
Our work indicates higher level of genomes plasticity amongst *Pedobacter* species.

EMP04

Peptidyl-prolyl *cis*/*trans* isomerase FkpA from *Corynebacterium glutamicum* improves biomass yield at increased growth temperatures

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Question: Peptidyl-prolyl *cis*/*trans* isomerases (PPIases) catalyze the rate-limiting protein folding step at peptidyl bonds preceding proline residues and were found to be involved in several biological processes, including gene expression, signal transduction, and protein secretion. In *C. glutamicum*, a workhorse in industrial biotechnology, an FK-506 (tacrolimus) binding protein (FKBP)-type PPIase FkpA is predicted to be encoded directly downstream of *gltA* encoding citrate synthase (CS). This gene cluster is also present in other *Actinobacteria*. Here we asked for the physiological relevance of the predicted FkpA protein for *C. glutamicum*, analyzed the enzymatic activity and studied the influence of FkpA on parameters of a lysine producer model strain.
Methods: *In vitro*, chymotrypsin-coupled PPIase activity assays and thermal aggregation assays were performed to determine kinetic parameters of the PPIase activity and to assess the chaperone activity. For *in vivo* studies, an *fkpA* deletion mutant was constructed to test the influence on CS activity, on global gene expression and on growth properties of *C. glutamium*.
Results: *In vitro*, FkpA indeed shows typical PPIase activity parameters with artificial substrates and is inhibited by FK-506. FkpA also delays the thermal aggregation of CS demonstrating chaperone activity. Surprisingly, FkpA has a positive effect on the activity and temperature range of CS *in vitro*. Deletion of *fkpA* causes a 50 % reduced biomass yield compared to that of the wild type at increased growth temperature (37 °C). *In vivo* data suggest that CS is not the limiting factor for the growth defect under this condition. Comparative transcriptome analysis using DNA microarrays revealed 69 genes which exhibit >2-fold mRNA level changes giving insight into the transcriptional response upon mild heat stress when FkpA is absent.
Conclusions: Predicted FkpA from *C. glutamicum* indeed exhibits PPIase activity and also chaperone activity [1)]. The absence of FkpA causes strongly reduced biomass yields at increased growth temperatures and is therefore physiologically relevant for *C. glutamicum* to cope with such conditions [1)]. FkpA may be exploited for improved product formation in biotechnical processes.

1) Kallscheuer *et al.* (2015) Appl Environ Microbiol 81(22): 7839-7850

EMP05

Shinella* sp. strain DD12 – report on the first genome sequence of a member of genus *Shinella

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Phosphorus occurs in bio- and geochemistry mainly as phosphates, with a central P atom in its higher oxidation state [+V]. Recent reports show, that phosphorus occurs on Earth in reduced forms, with central P atom in [+III] or [+I] oxidation state. Providing that P is one of the growth limiting factors in water ecosystems, the ability of bacteria to metabolize reduced P compounds attracts the interest.
Here we report the first draft genome sequence of a member of genus *Shinella* - *Shinella* sp. strain DD12, a novel phosphite assimilating bacterium, isolated from homogenized guts of starved zooplankton *Daphnia magna*.

From genomic DNA of the strain were prepared shotgun libraries. Sequencing resulted in 7,118,226 paired-ends Illumina reads of 112 bp and a 72.54-fold coverage. Reads were trimmed using Trimmomatic 0.32 software to remove sequences with quality scores lower than 20 and remaining adaptor sequences. The initial hybrid *de novo* assembly employing the SPAdes 2.5 software resulted in 236 contigs larger than 0.5 kb. For automatic gene prediction YACOB and GLIMMER software tools were used, whereas RNAmmer and tRNAscan for identification of rRNA and tRNA genes. Functional annotation of the predicted protein-coding genes was carried out with the IMG/ER system with subsequent manual curation by using Swiss-Prot, TrEMBL, and InterPro databases.
The genome size is 7.678 Mb with an overall GC content of 63.40 mol %. It harbours 7,555 putative genes, of which 7,505 are protein-encoding and 50 RNAs (2 rRNA and 48 tRNA, including those for selenocystein incorporation). 6,241 (82.61 %) protein- encoding genes and 1,264 (16.73 %) genes encoding hypothetical proteins were identified. The majority of the protein-encoding genes were assigned to COG categories.
The genome analysis of strain DD12 revealed the presence of two complete operons encoding:1) dissimilatory nitrate reduction to ammonia and 2) assimilative nitrate reduction to L-glutamine, and L-glutamate. *Shinella* sp. DD12 cannot fix nitrogen. The genome encodes three complete pathways for assimilation of phosphonates, which indicates the relatively broad abilities of the strain to utilise phosphonates as P- and/or C- and N-sources, compared to the remaining genomes of *Rhizobiaceae* members and even to *Alphaproteobacteria* as a whole.

EMP06

Molecular characterization and activity measurements of the ammonia oxidizing community in a pre-alpine freshwater lake (Lake Constance)

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Microbially driven ammonium oxidation to nitrite is the rate limiting step in nitrification and as such an important part of the global nitrogen cycle. This process has been extensively studied in marine and terrestrial soil environments but is not yet well understood in freshwater ecosystems. Although nitrification does not directly change the inventory of fixed N in freshwater ecosystems, it changes the quality of N available for assimilation by plants, phyto- and bacterioplankton and thus directly impacts primary production. Our primary goal was to characterize the ammonia oxidizing microbiota, which typically consists of both ammonia oxidizing archaea (AOA) and bacteria (AOB), in Lake Constance as a model for large oligotrophic freshwater lakes. The ammonia oxidizing microbiota was followed throughout the annual cycle of plankton succession at four distinct depths which span from the epilimnion through the metalimnion to the hypolimnion. T-RFLP-based screening and cloning of bacterial and thaumarchaeotal*amoA* genes (coding for the alpha-subunit of the ammonia monooxygenase) indicated that both clades are present in Lake Constance throughout the water column, but surprisingly at very limited diversity. Only one species-level operational taxonomic units (OTU) was detected for AOA, with *Nitrosopumilus maritimus* as next cultured relative (92 % DNA sequence identity), and only one major and one minor OTU were detected for AOB, both being distantly related to *Nitrosolobus multiformis* as next cultured relative (77 % and 81 % DNA sequence identity, respectively). Incubations of lake water samples in the presence of 20 µM ammonium (up to 1.4 µM natural concentrations) revealed potential activities of these ammonia oxidizers in the range of 1.0-4.2 µmol NH_4^+ L^{-1} day^{-1}. Extending these activity measurements to gross nitrification rates determined by a $^{15}NO_3^-$ isotope pool dilution technique revealed maximum activities of 15.9 µmol L^{-1} day^{-1} at a depth of 85 m. Our results provide evidence for active nitrification in the water column of a pre-alpine lake that is driven by a low-diversity ammonia-oxidizing community.

EMP07

Chasing microorganisms involved in cryptic sulfate reduction in sediments of a large pre-alpine lake, Lake Constance

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Microbial sulfate reduction in aquatic sediments is a process of high relevance for the global carbon and sulfur cycle. In contrast to marine sediments, sulfate concentrations in freshwater sediments are very low. It was therefore generally assumed that sulfate reduction in freshwater environments has a minor role for element cycling. However, this is contradicted by the high sulfate reduction rates (SRR) regularly observed in lake sediments and other freshwater habitats. To explain such high SRR despite the prevailing low sulfate concentrations, a cryptic sulfur cycle was proposed to occur in freshwater habitats (Pester, Knorr *et al.* 2012). Lake Constance is a large oligotrophic lake, where high sulfate reduction rates of up to 2000 nmol L^{-1} day^{-1} have been observed (Bak and Pfennig 1991). Building upon this observation, we setup anoxic sediment microcosms in the presence and absence of small periodic sulfate amendments (200 µM). Individual microcosms were incubated in the presence of one of the following typical organic matter degradation intermediates (ca. 200 µM): formate, acetate, lactate, propionate, butyrate, and no-substrate control. To discern the effect of sulfate amendment on the degradation of individual organic substrates, we followed the production of methane and carbon dioxide as well as the turnover of sulfate, and short-chained fatty acids/lactate. Methanogenesis was reduced by 18-57 % in sulfate-amended microcosms while there was no effect on carbon dioxide production and substrate degradation products. Currently, we follow changes in bacterial and archaeal 16S rRNA and 16S rRNA genes in the individual setups using next generation amplicon sequencing. The combination of these microbial community responses and the obtained activity profiles will help us to identify microorganisms actively involved in cryptic sulfate reduction in Lake Constance sediments.

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EMP08

Defining unknown members of the *Roseobacter* group in marine sediments

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The *Roseobacter* group is a marine bacterial lineage representing a significant part of pelagic and benthic microbial communities. It is the largest group within the *Rhodobacteraceae* comprising 70 genera and app. 180 species. Although around 20 % of the cultured representatives were isolated from marine sediments, there is still a lack of knowledge about the role and distribution of benthic roseobacters. To get deeper insights in the diversity of the *Roseobacter* group in sediments, a comprehensive dataset comprising 14 sampling sites with different biogeochemical background was analyzed. Pyrosequencing of bacterial 16S rRNA gene transcripts resulted in around 90,000 sequences from all sampling sites. After classification using the rdp database, 5952 sequences (0.7 % of all transcripts) could be assigned to the *Rhodobacteraceae.* While ~ 700 sequences were affiliated to known genera, 88 % were associated to so far unknown *Rhodobacteraceae.* Especially for the *Roseobacter* group, only 7 % of the sequences could be affiliated to described genera. Based on this finding, we performed an extensive phylogenetic study to define new clusters of benthic roseobacters. An ARB database containing the *Rhodobacteraceae*-affiliated sequences was set up and phylogenetic trees were calculated using two different algorithms. New clusters of benthic roseobacters were defined when more than 10 sequences branched together in the neighbour joining as well as in the maximum likelihood tree. Thus, around 100 new clusters were identified as monophyletic branches within the *Roseobacter* group covering ~ 4000 sequences. Consequently, 65 % of all *Rhodobacteraceae*-affiliated sequences were defined within the new clusters. Half of theses sequences formed ~ 20 clusters, comprising more than 50 sequences, each. This extended phylogenetic description now allows a more detailed and statistically valid community analysis of the *Roseobacter* group within our comprehensive dataset from marine sediments. Additionally, specific primers and probes were designed for the newly defined clusters of benthic roseobacters to detect and quantify them within enrichment cultured and environmental samples. These tools will help to find new species affiliated to so far uncultivated roseobacters and to obtain a holistic picture of their distribution and ecology in the marine environment.

EMP09

Occurence and diagnosis by PCR and culture for American foulbrood disease (*Paenibacillus larvae subsp. larvae*) in honey bee colonies in Algeria

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The American foulbrood in one of the most serious diseases that may affect brood of larvae and pupae stages, which cause economic losses and biological hazards in a large beekeeping sector in several countries across the world in general and Algeria in particular. The causative organism is a Gram-positive bacterium *Paenibacillus* larvae. The objective of this study was to determine the prevalence of this disease in some regions of Algeria. The diagnostic method used is based on sampling of honey and detection of bacteria using microbiological methods, microscopic and biochemical. The QIAamp DNA Mini kit is used to identify the DNA of *Paenibacillus* larvae. The study was conducted on 87 samples of adult honey bees of various origins, harvested directly from the hive in 2014 and from different parts of northern Algeria. The results of the research *Paenibacillus* larvae at our study have shown notable variability between samples of honey bee from different regions examined. Several factors may explain this variation in the prevalence of the disease. The average infection rate for all regions is 16 %. American foulbrood is a serious disease that affects our bees. This bacterium is the second most serious threat after the mite *Varroa destructor*.

EMP10

Physiological and genetic studies on an isolated strain of *Magnetospirillum* from a Planted Fixed Reactor (PFR)

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Magnetospirillum is a genus characterized majorly by some magnetic strains and their ability to break down aromatic compounds, such as toluene and phenol (Shinoda *et al.*2005), under microaerophilic and anaerobic conditions. Currently most of the studies on this genus have been focused on their magnetosomes and there is a lack of information about at what extent these bacteria can metabolize toxic compounds, such as toluene, under denitrifying anaerobic conditions. Furthermore only a few studies have identified and analyzed the genes involved in the toluene catabolic pathway.

In this work, microcosm samples were taken from a Planted Fixed Reactor (PFR), planted with *Juncus effusus* and fed with 40mg/L of toluene for 4 years. The predominant group of bacteria were isolated and physiologically characterized. A whole genome sequencing approach was carried out and genes related to toluene and benzoate degradation pathways for anaerobic conditions, were detected and analyzed.

Through *16s rDNA* analysis, our results showed that the strain were closely related to *Magnetospirillum* AMB-1. However, contrary to the *Magnetospirillum* AMB-1, magnetosomes organelles were absent in our strain as reported for other strains, such as CC-26 (Shinoda *et al.*, 2000). Furthermore, the isolated strain was able to degrade toluene up to a concentration of 50 mg/L in liquid cultures and to grow in the presence of other aromatic compounds such as p- and m-cresol, with nitrate as the electron acceptor under anaerobic and aerobic conditions. The toluene concentration at which half of the population was still viable (EC_{50}) was at 0.6 mM and the minimal inhibitory concentration (MIC) was at 1.6 mM. Genes related to toluene degradation, specifically *bssA* (benzylsuccinate synthase) *bcrC* (benzoyl-CoA reductase) and *bamA* (6-oxocyclohex-1-ene-1-carbonyl CoA hydratase) were detected by genome sequencing analysis and successfully amplified.

Matsunaga, T., Okamura, Y., Fukuda, Y., Wahyudi, A. T., Murase, Y., & Takeyama, H. (2005). Complete genome sequence of the facultative anaerobic magnetotactic bacterium Magnetospirillum sp. strain AMB-1. *DNA Research: An International Journal for Rapid Publication of Reports on Genes and Genomes*, *12*(3), 157-66.

Shinoda, Y., Sakai, Y., Ué, M., Hiraishi, A., & Kato, N. (2000). Isolation and characterization of a new denitrifying spirillum capable of anaerobic degradation of phenol. *Applied and Environmental Microbiology*, *66*(4), 1286-91.

EMP11

***Delftia acidovorans*, a potential degrader in Constructed Wetlands**

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Constructed Wetlands (CWs) are technologies suitable as a fourth step in treating industrial wastewater. In these systems, the main role in the transformation and mineralization of organic pollutants is played by microorganisms present in the rhizosphere. Dimethylphenols (DMPs) are toxic compounds and they are known as the main constituents of effluents from petro- and carbochemical industry. Therefore, understanding the bacterial degradation processes that inhabit Constructed Wetlands (CWs) is an important approach for further improvements of these treatment systems. In order understand the DMPs degradative catabolic pathways in CWs, as a first step, it is necessary to isolate and characterize potential DMPs bacterial degraders, followed further investigations concerning their contribution and activity in CWs bacterial communities. Therefore, in this study, a selective DMP enrichment was done. Thus, an outflow water sample from a horizontal sub-surface CW, fed with contaminated groundwater containing phenols, was inoculate in liquid medium M9 with a 70 mg/L of a equimolar ratio of 2,6-; 3,4- and 3,5-DMP as sole carbon and energy source. Enrichments were incubated at 30 °C for three weeks, and isolates were purified by streaking on M9 agar and carbon sources, as mention above. Furthermore, selected bacteria were taxonomically identified by sequencing of 16s rDNA, followed by the evaluation of DMPs degradation, DMPs co-metabolism and degradation of other simple aromatics. Finally, the strain was assessed for the presence of catabolic genes involved in aromatic aerobic degradation. From isolates, the β-proteobacteria *Delftia acidovorans*, was the strain able to completely metabolize o-xylenols, 3,4-DMP and 2,3-DMP as singles isomers. Whereas, the accompaniment of 3,4-DMP with others non-metabolized DMPs isomers showed to have an effect on its transformation by *D. acidovorans*. The isomers 2,5 and 3,5- DMP were significantly depleted in the presence of 3,4-DMP, while 2,4-DMP slightly decreased. On the contrary 2,6-DMP did not have any co-metabolic effect. In addition, the mix of the two metabolized isomers, 3,4- and 2,3-, exhibited synergistic interactions due to the improvement in degradation time and bacterial growth. Amplification of catabolic aerobic genes, revealed the presence of genes of multi-component mono-oxigenases-like in *D. acidovoras*. Specifically, TMBD, TMOA and PHE, corresponding to the Subfamily 1 and 2 of α-subunits of hydroxylase and the α-subunit of phenol-methylphenol monooxygenase-like, respectively. In addition, the microorganism, exhibited the ability to degrade a wide range of simple aromatics. Therefore, *D. acidovorans* showed to be an optimal bacterium for the genomic reconstruction of the metabolic DMPs pathways. Additionally, further studies can be addressed to evaluate its contribution and performance within Constructed Wetlands for phenols-like wastewater treatment.

EMP12

Biochemical characterization and functional analysis of the iron responsive regulator RirA from *Dinoroseobacter shibae*

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Question: The rhizobial iron regulator RirA from *Dinoroseobacter shibae* belongs to the Rrf2- family of transcription factors and is supposed to coordinate a Fe-S cluster and thereby measure iron availability. The RirA protein of *D. shibae* contains four cysteine residues which are highly conserved in other RirA homologs and might be important for the cluster formation [1].

Methods: RirA fused with a Strep-tag was recombinantly produced and purified under anaerobic conditions. We used UV/ Vis and electron paramagnetic resonance (EPR) spectroscopy to study the nature of the Fe-S cluster. The Fe content of the protein was determined with the atom absorbance spectroscopy (AAS). Four cysteine residues of RirA were changed to alanine via site directed mutagenesis of the corresponding gene to determine their role in cluster formation. DNA binding of the anaerobically purified RirA wildtype and mutant proteins were analyzed using electro mobility shift assays (EMSA). The oligomeric state of the RirA protein and the mutant proteins was determined using the gel permeation chromatography (GPC).

Results: Using UV/ Vis spectroscopy an absorption maximum at 420 nm, typical of Fe-S cluster containing proteins, was observed for anaerobically purified wildtype RirA and missing for the four RirA cysteine mutants. Moreover, exposure of RirA to air drastically reduced the absorption, indicating an oxygen sensitive Fe-S cluster. By EPR spectroscopy a $[3Fe\text{-}4S]^{1+}$ cluster was identified. This finding was supported by AAS measurements of iron, resulting in a 3:1 ratio of iron per molecule RirA. Gel filtration experiments revealed a dimeric form for *D. shibae* RirA. The dimeric form was also found for the mutant proteins but with a slightly different migration, supposing a structural difference compared to the wildtype RirA. The *hemeB2* gene, encoding a TonB-dependent heme/hemoglobin receptor family protein was induced under iron-limited

conditions. Using EMSA analyses, binding of RirA to *hemB2* promoter sequences, containing a potential RirA binding site 5´-TGA-N_9-TCA-3´, was shown.
Conclusion: The rhizobial iron regulator protein RirA from *D. shibae* is able to measure iron availability by coordination of a $[3Fe\text{-}4S]^{1+}$ cluster as cofactor and regulates target genes in response to iron limitation.

[1] Bhubhanil, S, Niamyim, P, Sukchawalit, R, and S. Mongkolsuk (2013). Cysteine desulphurase-encoding gene *sufS2* is required for the repressor function of RirA and oxidative resistance in *Agrobacterium tumefaciens*. *Microbiology*, 160:79-90

EMP13
How to survive as a non-pathogenic or pathogenic microbe under high polyamines concentrations?
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Although naturally occurring polyamines are absolute required for cell growth, maturation and proliferation in virtually all organisms, they can be very toxic when in excess. These compounds can interact with DNA, RNA, proteins and potentially with other components of the cell leading to deregulation of the cell metabolism following lethal effect. Free living *Streptomyces sp*. and other non-motile actinobacteria have to cope with very high concentration of polyamines that are produced during the decomposition of animal bodies and are locally released from putrefying cadaver into the soil. Plant-pathogenic *Streptomyces* species are able to survive in the plant tissue despite very high polyamine concentrations which are produced by the defense system of the plant during infection. Almost all actinobacteria, including the important pathogens possess a set of *glnA*-like genes encoding non-functional glutamine synthetase-like enzymes. *In silico* analysis of all *glnA*-like genes (*glnA2*, *glnA3*, *glnA4*) across the actinobacteria genomes revealed that *glnA* has evolved to other genes encoding proteins that may play an important role in surviving and colonizing of many diverse habitats [1]. Our studies in *S. coelicolor* demonstrate that *glnA2*, *glnA3* and *glnA4* encode gamma-glutamylpolyamine synthetases (GPSs) responsible for the first step of the utilization of naturally occurring polyamines as an alternative *N*-source. These enzymes confer also resistance against toxic concentrations of polyamines thereby ensuring persistence in a host or surviving in a locally rich polyamine soil habitat. Since GPSs ensure both nutrients availability (*C*- and *N*-source) and resistance against high polyamine concentrations they represent a promising target for a new antibiotic drug development. Inhibition of the GPSs might be an effective therapeutic strategy since these kinds of enzymes do not naturally occur in eukaryotes.

[1] Rexer H.U., Schäberle T., Wohlleben W., Engels A. Investigation of the functional properties and regulation of three glutamine synthetase-like genes in *Streptomyces coelicolor* A3(2). *Arch Microbiol.* 2006 186(6):447-58.

EMP14
Influence of biotic and abiotic factors on intracellular calcium profile in the green unicellular alga *Chlamydomonas reinhardtii*
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Microalgae are an essential part of the food chain as they contribute significantly to the global carbon fixation [1]. To get a better understanding about this process, it is important to understand how microalgae respond to changes in environmental conditions. Earlier work on the marine diatom, *Phaeodactylum tricornutum* showed how this microalga responds to external signals such as temperature, osmotic stress and iron by altering its calcium homeostasis [2]. However, very limited information is available on the impact of such changes on freshwater microalgae. Using the freshwater model microalga *Chlamydomonas reinhardtii*, we have established a transgenic reporter line for measuring cytosolic calcium ion concentration. We subjected this reporter line to changes in several abiotic and biotic factors and measured the altered calcium concentration. In this poster, we present our preliminary work on the effect of these factors on *C. reinhardtii*. This work would be of great impact to understand the similarities and differences in the environmental sensing abilities of freshwater and marine microalgae as well as provide us with insights into mechanisms of interaction between microalgae and other microorganisms.

[1] Field, C.B., M.J. Behrenfeld, J.T. Randerson and P. Falkowski. 1998. Science 281:237-240.
[2] Falciatore, A., d'Alcalà, M. R., Croot, P., & Bowler, C. 2000. Science 288: 2363-2366.

EMP15
Isolation and characterization of a cellulose-degrading bacterium from soil samples of mixed woodlands
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Question: A non-motile, Gram-positive, cellulose degrading bacterium, which shows a filamented, branched cell shape, was isolated from soil of mixed woodlands. The isolated strain was identified as *Oerskovia enterophila* and designated as strain VJag. The aim of this work was to clarify the genetic and metabolic properties in terms of survival in forest soils of the isolated strain. Because of limited genomic information regarding the genus *Oerskovia*, the genome of the isolated strain was sequenced.
Methods: The isolated strain was identified using the 16S rDNA sequence. A phylogenetic tree based on 16S rDNA sequences of close relatives was created. Additionally, the consumption of carbon sources typically available in mixed woodlands, such as cellulose or starch were tested. Therefore, the isolated strain was cultivated on silica media plates with carboxymethylcellulose (CMC), stained with Kongo-red, cultivated in liquid media using soluble starch as carbon source, yeast extract or respective combinations. The genomic properties were identified through genome sequencing using the 454 GS-FLX TitaniumXL system (titanium GS70 chemistry, Roche Life Science, Mannheim, Germany) and the Genome Analyzer II (Illumina, San Diego, CA). Analysis of genome properties was performed using the Integrated Genomes and Metagenomes/Expert Review (IMG/ER) (Lawrence Barkeley National Laboratory).
Results: 16S rDNA analysis revealed that the isolated strain belongs to the species *O. enterophila* with an identity of 100 %. The G+C content amounts to 72.4 %. The created phylogenetic tree showed a close relationship between the isolated strain and members of the genera *Cellulomonas* or *Cellulosimicrobium*. Genomic analysis revealed that the isolate has all features to use starch and cellulose as carbon sources (presence of amylases and cellulases/endoglucanases). The consumption of CMC on silica plates stained with Kongo-red was proven by the formation of clear halos around the cell spots on the silica plates. Growth experiments in liquid media showed that the isolate was also able to use soluble starch as sole carbon source.
Conclusion: *O. enterophila* VJag is able to degrade typical carbon sources present in mixed woodlands via an enzymatic set of cellulases/endoglucanases and amylases.

EMP16
Analysis of membrane protein complexes of the marine sulfate reducer *Desulfobacula toluolica* Tol2 by 1D Blue Native-PAGE complexome profiling and 2D Blue Native-/SDS-PAGE
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Question: Sulfate-reducing bacteria (SRB) obtain energy from cytoplasmic reduction of sulfate to sulfide involving APS-reductase (AprAB) and dissimilatory sulfite reductase (DsrAB). These enzymes are predicted to obtain electrons from membrane redox complexes, i.e. the quinone-interacting membrane-bound oxidoreductase (QmoABC) and DsrMKJOP complexes. In addition to these conserved complexes, the genomes of SRB encode a large number of other (predicted) membrane redox complexes, the function and actual formation of which is unknown.
Methods: This study reports the establishment of 1D Blue Native-PAGE and 2D BN-/SDS-PAGE for analysis of the membrane protein complexome of the completely oxidizing, marine sulfate reducer *Desulfobacula toluolica* Tol2. 1D BN-PAGE-based complexome profiling was performed to allow for determination of the membrane protein complexome of a SRB on a whole cell level.
Results: Analysis of normalized score profiles of >800 proteins in combination with hierarchical clustering and identification of 2D BN-

/SDS-PAGE separated spots demonstrated separation of membrane complexes in their native form, e.g. ATP synthase. In addition to the QmoABC and DsrMKJOP complexes, other complexes were detected that constitute the basic membrane complexome of *D. toluolica* Tol2, e.g. transport proteins (e.g. sodium/sulfate symporters) or redox complexes involved in Na^+-based bioenergetics (RnfABCDEG). Notably, size estimation indicates dimer and quadruple formation of the DsrMKJOP complex *in vivo*. Furthermore, cluster analysis suggests interaction of this complex with a rhodanese-like protein (Tol2_C05230) possibly representing a periplasmic electron acceptor for DsrMKJOP.
Conclusion: This study demonstrates the benefit of combining 1D BN-PAGE complexome profiling with 2D BN-/SDS-PAGE separation of membrane complexes of SRB, by providing comprehensive insights into the proteomic backbone of membrane embedded processes on a whole cell level. This approach generated new hypotheses for future proteomic and biochemical studies, e.g. in case of the DsrMKJOP complex.

EMP17
Regulation of mono- and polyamine utilization clusters in *S. coelicolor*
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Actinobacteria include species that are major antibiotic producers, as well as various human, animal and plant pathogens. Non-motile, soil-dwelling *Streptomyces* are able to withstand and overcome unfavorable, rapid changing conditions despite constant confrontation with nutrient limitation and pollutants in the soil. Primary nitrogen compounds assimilated by actinobacteria include: ammonium, nitrate, amino acids, urea, amino sugars and peptides. Our studies revealed that *S. coelicolor* can utilize monoamines (ethanolamine) and polyamines (putrescine, cadaverine, spermine and spermidine) as a sole nitrogen source and is able to grow in the presence of toxic polyamine concentrations. Actinobacteria have developed multifaceted regulation mechanisms to control their nitrogen metabolism in a response to nitrogen availability and environmental conditions [1]. Although the regulation of the nitrogen assimilation in *S. coelicolor* was intensively studied [2], the regulation of polyamine utilization clusters was not investigated so far. Our studies show that these clusters in *S. coelicolor* are controlled by two transcriptional regulators PauR and PauRII. Target genes of the PauR and PauRII were identified using RT-PCR and EMSAs. Recent advances in the study of the metabolism of nitrogen with a focus on the monoamine/polyamine utilization in *S. coelicolor* will be presented.

[1] Rexer, H. U., Schäberle, T., Wohlleben, W., & Engels, A. (2006). Investigation of the functional properties and regulation of three glutamine synthetase-like genes in *Streptomyces coelicolor* A3 (2). Archives of microbiology, 186(6), 447-458.
[2] Reuther, J., & Wohlleben, W. (2007). Nitrogen metabolism in *Streptomyces coelicolor*: transcriptional and post-translational regulation. *Journal of molecular microbiology and biotechnology*, *12*(1-2), 139-146.

EMP18
Genome and catabolic subproteome of the marine, nutritionally versatile, sulfate-reducing bacterium, *Desulfococcus multivorans* DSM 2059
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Question: Sulfate-reducing bacteria (SRB) are key players of the carbon- and sulfur-cycles in the sediments of the world's oceans. Of prominent habitat relevance are members of the *Desulfosarcina-Desulfococcus* clade within the deltaproteobacterial family of *Desulfobacteraceae*. A metabolically versatile representative of this cluster is *Desulfococcus multivorans* DSM 2059 that is able to completely oxidize a variety of organic acids, including fatty acids up to C_{14}, as well as aromatic compounds under anoxic conditions.
In the present study proteomic-enhanced annotation was applied to comprehensively reconstruct the metabolic network of *D. multivorans*.
Methods: Manual revising of automatically predicted and annotated protein-coding sequences (CDS) was combined with proteomic data (2D-DIGE, shotgun, membrane protein-enriched fraction) based on cells adapted to 17 (6 aromatic and 11 aliphatic compounds) different substrate conditions to allow for improved functional prediction.
Results: The 4.46 Mbp genome of *D. multivorans* with 3942 predicted CDS resembles other sulfate-reducing deltaproteobacteria with respect to size, GC content and number of encoded proteins. The comprehensive proteogenomic dataset allowed for reconstructing a metabolic network of degradation pathways and energy metabolism that consists of 167 proteins (152 identified). Peripheral degradation routes feed via central benzoyl-CoA, (modified) b-oxidation and methylmalonyl-CoA pathways into the Wood-Ljungdahl pathway for complete oxidation of acetyl-CoA to CO_2. Dissimilatory sulfate reduction is embedded in a complex electron transfer network composed of cytoplasmic components (ETFs and electron bifurcating Hdr/Mvh and Nfn complexes) and diverse membrane complexes (Dsr, Qmo, Hmc, Tmc, Qrc, Nuo and Rnf).
Conclusions: While a high degree of substrate-specific formation of catabolic enzymes was observed, most protein complexes involved in transmembrane electron transfer appeared to be constitutively formed. Overall, the study underpins the value of proteomic analysis to enhance functional genomic predictions and represents another puzzle piece in understanding the proteogenomic basis of the habitat-relevance and -success of the deltaproteobacterial SRB family *Desulfobacteraceae*.

EMP19
Chemical warfare – Chemical defence mechanisms in *Vibrio* strains
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In marine systems, microbial communities, including bacteria, archaea and eukaryotic nano- and picoplankton, form a complex food web called the microbial loop. Within the microbial loop interactions of different trophic levels, e.g. heterotrophic bacteria, and predatory protest species, lead to extensive carbon cycling. However, the mechanisms of these interactions, in particular between unicellular predators and their bacterial and archaeal prey, remain poorly understood. Hence, we used a diversity of *Vibrio* strains as model organisms and challenged them with three predators to investigate their predation avoidance strategies. In particular, the focus was laid on chemical strategies to avoid predation, such as alteration of surface structures, or usage of secondary metabolites as chemorepellents. Differences in the structures of O-antigens and exudation of chemicals repelling or toxic to the predators may function as such chemorepellents. We used >200 fully sequenced strains of the genus *Vibrio* and three different heterotrophic protists as predators to establish a matrix of growth behaviours under predation. The growth of the *Vibrio* strains was measured using Illumina MiSeq sequencing of the hsp60 genes of the different strains from growth experiments. These experiments included a combination of several strains, while predation pressure exerted by one of the predators. Some strains showed higher, constant, or only minutely decreased growth, indicating predation avoidance strategies. First closer investigations of the candidates that showed decreased susceptibility to predation indicated the use of chemical strategies for predation avoidance. Further investigations of surface structures and exuded chemicals, such as indole or O-antigens, will help to elucidate the use of chemorepellents as predation avoidance strategies, and will shed light on survival strategies of the bacterial community towards eukaryotic predation within the microbial loop.

EMP20
Complete genome sequence and physiological characterization of starch degrading enzymes of the plant growth promoting *Paenibacillus riograndensis* SBR5^T
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Paenibacillus riograndensis is a plant growth promoting rhizobacterium (PGPR) isolated from the rhizosphere of wheat plants cultivated in Rio Grande do Sul, Brazil (Beneduzi *et al.*, 2010). To lay a foundation for characterizing its traits as PGPR, we aimed to determine its complete genome sequence.
Preparation of libraries, sequencing protocols and software used for genome assembly and annotation were described by Brito *et al.* (2015). Growth experiments were performed in MVcM minimal medium supplemented with different vitamin combinations. Genes from SBR5^T for starch degradation were heterologously expressed in the starch-negative *Corynebacterium glutamicum* ATCC 13032 and the starch degradation was assayed by the Lugol's iodine test.

We showed that the genome of SBR5^T consists of one chromosome with 7.893.056 bps, containing 6705 protein coding genes, 87 tRNAs and 27 rRNAs (Brito *et al.*, 2015). Biotin auxotrophy was demonstrated since growth of SBR5^T in MVcM minimal medium was not observed in the absence of biotin corroborating the finding that biotin biosynthesis genes are absent from its genome. Two of three putative amylase/pullulanase genes could be functionally expressed in *C. glutamicum* enabling this bacterium to degrade starch. This indicated that these genes may be involved in starch utilization by *P. riograndensis* SBR5^T.
Determination of the complete genome sequence of SBR5^T was an important step to further our understanding of the physiology of this bacterium and will be valuable for future studies involving SBR5^T as PGPR.

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EMP21
***Formosa* strain *B* as a model for polysaccharide degradation – *In situ* detection of a glycosyl-hydrolase 92 in *Formosa* strain B with direct geneFISH**
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Phytoplankton spring blooms are seasonal events in which autotrophic microorganisms massively grow for a short period of time. In the German bight these spring blooms are followed by successional blooms of heterotrophic bacteria. *Formosa* strain B is a flavobacterium isolated from seawater at Helgoland after the phytoplankton bloom. Genome analysis of its genome suggests that this strain is a mannan degrader and metagenomic analysis indicated that it is also highly clonal and recurrent in Helgoland waters. Here, we showed the direct link of the potential mannan degradation to *Formosa* strain B with direct geneFISH, a molecular tool that uses fluorescence *in situ* hybridization to enable the direct linking between taxonomy and potential metabolic function by simultaneously detecting the 16S rRNA and a gene of interest in the target organism. In this case the gene of interest was the glycoside hydrolase 92 (GH92) which codes for the protein alpha-mannosidase that is able to degrade mannan, a linear polymer of the sugar mannose serving as a storage polysaccharide in diatoms. The cell identity of *Formosa* strain B was detected with a clade specific probe (FORM_181B). Conducting research on *Formosa* strain B yields a deeper insight into the polysaccharide degradation mechanisms and its niche adaption after the annual spring bloom and improves our understanding of the microbial ecology of the coastal shelf seas.

EMP22
Towards population genomics of bacterial clades associated with phytoplankton blooms
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Spring phytoplankton blooms off the coast of Helgoland revealed a substrate-controlled succession of bacterioplankton populations[1]. To date the functional characterization of microorganisms which are not yet cultured is approached by metagenomics or single cell genomics, but these methods are either non-targeted or miss out groups of low abundance. Here, we present a protocol for the targeted retrieval of genomic information from phylogenetically defined bacterial clades. Fluorescence *in situ* hybridization (FISH) is combined with fluorescence-activated cell sorting (FACS) followed by whole genome amplification. We applied a recently developed hybridization-chain-reaction (HCR) -FISH protocol[2] which is less damaging to the DNA compared to the commonly used catalysed reporter deposition (CARD) -FISH. By using genus- or species-specific probes, even microorganisms with low abundances can be sorted and sequenced. The resulting genomic information enables the annotation of metabolic functions of the targeted bacterial clades and thus improves the understanding of their role in the breakdown of algal biomass after the spring phytoplankton blooms.

1 H. Teeling *et al.*, Science 336, 608 (2012)
2 T. Yamaguchi *et al.*, Environ. Microbiol., 17 (2015)

EMP23
Transpositional mutagenesis of a heavy metal resistant streptomycete strain
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Streptomycetes are largely known for their production of industrial and medical relevant substances. As a source of most antibiotics they are of vital importance for medical treatments, but these organisms can also be employed for other applications, such as bioremediation. Their ability to adapt to harsh environmental conditions and ubiquitous distribution in natural habitats makes them ideal candidates for the remediation of contaminated areas.
The former uranium mining site Ronneburg (Thuringia, Germany) is highly heavy metal contaminated, due to formation of acid waste waters and subsequent mobilization of metals. A streptomycete strain collection from this site was established, which contains mostly metal resistant strains. In this project an *in vivo* transposon mutagenesis approach is used to investigate the genes involved in the metal resistome of these isolates.
The highly nickel resistant *Streptomyces mirabilis* P16 B-1 was transformed *via* conjugation with *Escherichia coli* ET12567 pUZ8002. After establishing this plasmid delivery system, P16 was transformed with two plasmids, either pTNM or pHTM, which contain transposons (Tn) and corresponding transposases based on different transposon types: Tn5 or mariner. Subsequently, transposition was initiated and transformants screened for the appropriate antibiotic resistances, as well as sensitivity against nickel, in order to find heavy metal sensitive knock-out mutants. Southern Blotting was used for confirming and comparing integration sites between the transformants. The location of the Tn in the streptomycete genome was determined by plasmid rescue and sequencing.
Applying the intergeneric conjugation system, P16 was successfully transformed with either plasmids. Transposition of the Tn5-based transposon yielded transformants with identical integration sites: either a sigma factor or a *tetR* transcriptional regulator gene. Although some of these colonies had lost their nickel resistance, others performed as the wild type. Concluding that there had been an early transposition event after conjugation and the loss of resistance was due to stress during the procedure rather than a gene disruption, further work was carried out with the mariner Tn *Himar1*. In contrast to Tn5, *Himar1* transformants showed in Southern Blots different Tn integration sites. Therefore, they will be screened further for loss of heavy metal sensitivity.
Transpoitional mutagenesis in *Streptomyces* is a useful tool for creating knock-out mutants. The role of thereby identified candidate genes of the heavy metal resistome of *Streptomyces mirabilis* will be confirmed be gene disruption and complementation assays. Furthermore, the employed methodology will be adopted to other *Streptomyces* strains.

EMP24
Identification and characterization of an Ibuprofen degrading bacteria isolated from Constructed Wetland
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Micropollutants occur in the aquatic environment all over the world. Industrial chemicals and pharmaceutically active compounds have attracted significant attention of science and public during the last years. After being administered, human drugs are partially metabolized in the human organism, excreted and finally end up in the municipal wastewater. Physical, chemical, and biological processes are combined in the constructed wetlands to remove contaminants from wastewater. An understanding of these processes is fundamental not only to designing wetland systems but to understand the fate of chemicals once they enter the wetland system. Thus, further degrading studies of ibuprofen, a micropollutant discovered in groundwater 25 years ago, should be conducted. In this study, samples from a subsurface-flow water treatment wetland, located in the city of Langenreichenbach-Germany, were tested. From several ibuprofen enriched culture media, an uncategorized bacteria strain (LRB-1) have being isolated and studied. The strain *Sphingomonas* Ibu-2 is still the single species of ibuprofen degrading bacteria genetically and chemically studied. Our results demonstrate that the LRB-1 have similar growth rate and ibuprofen degradation effectiveness as the *Sphingomonas* Ibu-2. LRB-1 has the unusual ability to cleave the acid

side chain from ibuprofen and related arylacetic derivates, under aerobic conditions. The exact mechanism whereby LRB-1 degrades ibuprofen remains to be determined and further work will be required to elucidate this mechanism.

Murdoch, R. W.; Hay, A. G. (2013), Microbiology 159, p. 621-632

EMP25

Prevalence and antibiogram study of S*almonella* and *Staphylococcus aureus* in retail raw meats sold in Awka, southeasthern nigerian locality

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Rationale: Foodborne infections and diseases remain a major cause of morbidity and mortality in the general population, particularly in resource poor settings. Food plays a significant role in the transfer of antibiotic resistance. Antimicrobial resistance is a major challenge in the management of severe foodborne infections as antimicrobial use in animals selects for resistant foodborne pathogens that may be transmitted to humans as food contaminants.
Objectives: This study was designed to evaluate the presence of potential foodborne pathogens *Salmonella* and *S. aureus* in the retail red meat in Awka and equally to evaluate the antimicrobial sensitivity patterns of these pathogens to conventional antibiotics.
Methods: Foodborne pathogens *(Salmonella and S.aureus)* were isolated from raw meat and confirmed with the aid of biochemical tests. Antibiogram of the isolates were examined following the CLSI guidelines. MIC of some selected antibiotics was evaluated against some multi-resistant isolates.
Results: The prevalence was 98.33 % and 71.67 % for *S.aureus* and *Salmonella* respectively. The isolates of *S.aureus* recorded higher level of resistance to the penicillins, erythromycin and tetracycline but had low resistance to gentamicin. Almost all the *Salmonella* isolates recorded a high level of resistance to all the antibiotics. Similarly all the isolates of the two bacteria had multiple antibiotic resistance indices of above 20 %. The MIC results showed a corresponding resistance of the two bacterial isolates to Augmentin, moderate resistance to gentamicin and erythromycin with low resistance(high sensitive) to chloramphenicol. The bacterial load present on the meats reflects the poor hygienic conditions in which they are prepared, stored, transported and sold. In addition to medical misuse, inappropriate use of antibiotics in the agricultural setting is a major contributor to the emergence of antibiotic - resistant bacteria.
Conclusions: The study confirmed high prevalence of *Salmonella and S. aureus* in raw meat products.These data revealed also that the *Salmonella* and *S. aureus* isolates recovered from the retail raw meats were resistant to multiple antimicrobials, and can be transmitted to humans through food products. Thus, it is essential for Nigerian government to implement food handlers training on food safety, conduct periodic medical check-up and continuous monitoring of personal hygiene.

EMP26

Metaproteogenomic analysis revealed a member of the genus *Pelotomaculum* completely oxidizing benzene to carbon dioxide with direct reduction of sulfate

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The enrichment culture BPL dominated by *Pelotomaculum* is able to degrade benzene with sulfate as electron acceptor. Members of the *Pelotomaculum* are known to usually undergo syntrophy with anaerobic respiring microorganisms or methanogens. We hypothesize that the anaerobic benzene degradation is performed by a novel member of the genus *Pelotomaculum* which is able to reduce sulfate as electron acceptor. By using a metagenomics approach, we reconstructed a high-quality genome for *Pelotomaculum* candidate BPL, with 99 % completeness based on the lineage-specific single-copy marker gene analysis. Combing with proteomic data, we were able to reveal the metabolic pathways of *Pelotomaculum* candidate BPL for benzene degradation and sulfate reduction. The predicted pathway of benzene degradation is via carboxylation to benzoate by the *Pelotomaculum* candidate BPL. The central benzoyl-CoA pathway involved reductive dearomatization by a class II benzoyl-CoA reductase followed by hydrolytic ring cleavage and modified β-oxidation. *Pelotomaculum* candidate BPL utilize oxidative acetyl-CoA pathway for complete oxidation to CO_2. Heterodisulfide reductase related proteins might connect the pool of reducing equivalents from the acetyl-CoA pathway to sulfate reduction. *Pelotomaculum* candidate BPL used a similar electron transfer mechanism for dissimilatory sulfate reduction like other Gram-positive sulfate-reducing bacteria. To summarize, *Pelotomaculum* candidate BPL is the first member of the genus that can perform sulfate reduction besides its potential to degrade benzene.

EMP27

Global distribution patterns of methanogenic archaea in natural environments

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Question: Methane (CH_4) is a major greenhouse gas and the second largest contributor to global warming. Biogenic methane is primarily if not exclusively generated by methanogenic archaea. Although our knowledge of methanogenic ecology is expanding, spatial distribution patterns of methanogenic archaea in natural environments remain inadequately understood. The major objective of this work is to determine/identify global patterns of methanogenic community structures in marine and terrestrial environments as well as the corresponding major abiotic factors shaping them.
Methods: Methyl coenzyme-M reductase (*mcrA*) nucleotide sequences from six different global natural environments were retrieved from the NCBI database. Geographical coordinates and environmental conditions of all the research sites were collected from the corresponding publications. Raw sequence data were processed on the MOTHUR platform. Valid sequences were clustered into OTUs at a cutoff of 84 % identity of the *mcrA* gene (Yang *et al.* 2014). Beta diversity was compared and statistically analyzed by different *R* packages.
Results: PCoA ordination based on the semi-quantitative Jaccard distance matrix suggested clear methanogenic patterns related to salinity. Samples from non-saline soils and lake sediments could be further clustered according to pH value. Moreover, samples from the same latitude areas were observed to group closer. Amongst all different habitats, estuaries appear to harbor a higher diversity and more even incidence frequencies than the other habitats. Additionally, soils and lake sediments are inhabited by a diverse methanogenic community with large variations in the taxonomic composition. *Methanoregula* and *Methanocella* are the most frequently identified genera in natural soils while they are absent in marine sediments.
Conclusions: Based on our analysis, salinity is the major environmental factor shaping methanogenic community on a global scale. In addition, pH and latitude have a strong influence on methanogenic β-diversity in natural soils and lake sediments. The hydrogenotrophic methanogens especially *Methanoregula* and *Methanocella* are most common in the studied soils.

Yang S, Liebner S, Alawi M, Ebenhöh O, Wagner D (2014). Taxonomic database and cut-off value for processing *mcrA* gene 454 pyrosequencing data by MOTHUR. *Journal of Microbiological Methods*, 103: 3-5.

EMP28

Algicidal mechanism of *Kordia algicida*

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Question: Phytoplankton is the major primary producer and basis of the food web in marine ecosystems. These microalgae underlie strong modulation by marine bacteria, which even outnumber phytoplankton cells. Studying important bacterial key players may unravel new aspects in marine ecosystems. One organism that greatly influences phytoplankton dynamics is *Kordia algicida* an algicidal bacterium that can terminate entire algal blooms. However, the algicidal mechanism is not yet fully understood. So far studies have shown that algicidal activity is regulated in a quorum sensing-like way.

Furthermore, the activity is not dependent on direct contact but mediated via extracellular compounds. Therefore, we aim to identify the algicidal agent(s).
Methods: We utilize exo-proteomic and exo-metabolomic approaches with customized sample preparation and data analysis to specifically address the problem of highly diluted active substances in a very complex metabolic background.
Results: The combination of these approaches reveals a detailed picture of the regulation of *K. algicida* algicidal activity and of the tools employed by the bacterium to obtain nutrients from lysed phytoplankton cells. We were able to identify potential candidates for bioactive compounds, which can be further tested in bioassays. One class of compounds comprises proteases which are currently discussed as toxic agents.
Conclusions: Overcoming matrix effects and high dilution in omics-analyses of aquatic ecosystems has the potential to unravel new candidates for phycotoxins as well as their secretion and regulation mechanisms.

EMP29

Temperate phages from terrestrial habitats: An induction approach

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Phages are viruses infecting specific host bacteria, thus influencing the mortality and structure of the bacterial community. Even though they are the most abundant biological entities on earth, research has focused mainly on marine ecosystems neglecting terrestrial habitats.
Phages show several life cycles: Temperate phages are characterized by lysogeny which seems to be the most common in the heterogeneous soil environment. During lysogeny the phage genome is temporarily integrated into the host genome as a prophage. This prophage is then replicated within the host until the production of new phage particles is induced by environmental signals causing phylogenetic stress to the bacterial host. In this study, we aimed to isolate and characterize temperate phages from soil and groundwater bacteria.
Bacteria were isolated on solid agar from soil and groundwater samples from the Hainich National Park (Thuringia, Germany) and identified by 16S rRNA-gene sequencing. In a high-throughput approach all isolates were screened for inducible phages using Mitomycin C, which causes mutagenic stress and triggers the release of the phages. The supposedly prophage carrying isolates were grown and induced in a larger volume for subsequent purification of the phages, and characterization via transmission electron microscopy and genome sequencing.
In total we isolated 149 bacteria. Most of the 82 isolates from soil belonged to the class Actinobacteria (35 isolates). In contrast, most of the 67 groundwater isolates affiliated to the Gamma-, Beta and Alphaproteobacteria (20, 19 and 15 respectively). Thirty-four percent of all isolates showed the typical growth curve of bacteria carrying inducible prophages, which is characterized by an initial increase and a following decrease in the optical density (600 nm). In addition, 39 % showed a non-typical growth curve, which may be indicative for the presence of defective prophages, while only twelve percent were not affected by the exposure to Mitomycin C. We will present transmission electron microscopy pictures, which allowed a distinction of the phages into distinct families. First results of the genome sequencing will be also discussed.

EMP30

Diversity and distribution of anammox bacteria and denitrifiers in pristine limestone aquifers

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Despite the increasing problem of groundwater pollution with nitrate, microbial groups involved in processes such as denitrification and anaerobic ammonia oxidation (anammox) leading to nitrogen loss have rarely been studied in aquifers. We investigated the abundance and community composition of denitrifiers and anammox bacteria in two superimposed limestone aquifers in the Hainich region (Thuringia, Germany). We aimed (i) to evaluate the genetic potential for anammox and denitrification in the groundwater of the two aquifers and (ii) to identify key environmental parameters driving anammox bacteria and denitrifier abundance and community composition.
Groundwater samples were taken from eight groundwater wells with sampling depths ranging from 12 to 88 m in monthly intervals over a 18-months-period. Oxygen saturation differed strongly between the two aquifers and ranged from 0 - 20 % in the upper, suboxic aquifer and from 50 - 80 % in the lower, oxic aquifer. Abundances of nitrite reductase genes (*nirK*, *nirS*) as determined by quantitative PCR ranged from 1.9×10^3 to 6.4×10^5 genes L^{-1} for *nirK* and from 2.6×10^3 to 8.2×10^7 genes L^{-1} for *nirS* with about 10 times higher abundances of *nirK* and *nirS* in the upper, anoxic aquifer. MiSeq Illumina sequencing of *nirS* genes pointed to large fractions of autotrophic sulfide or thiosulfate oxidizers such as *Sulfuricella* and *Sulfuritalea* in the denitrifier communities. Abundances of hydrazine synthase genes (*hzsA*) as a molecular marker of anammox bacteria ranged from 6.9×10^2 to 2.1×10^7 genes L^{-1} groundwater with maximum abundances in anoxic groundwater where nitrate and ammonium co-occurred at concentrations of 4 to 225 µmol L^{-1} and 25 to 89 µmol L^{-1}, respectively. qPCR-based results were confirmed by Illumina MiSeq sequencing of 16S rRNA genes, showing that reads affiliated with *Brocadiales* accounted for up to 5 % of the total sequence reads at these sites. Based on both 16S rRNA and *hzsA* gene analysis, the majority of groundwater anammox bacteria was related to *Candidatus Brocadia fulgida*.
Across sites, *hzsA* and *nirS* gene abundances were positively correlated to each other, and *hzsA* gene abundances were positively correlated to concentrations of ammonium. Our results have clearly shown that autotrophic denitrification coupled to the oxidation of reduced sulfur compounds and anammox could play an important role in nitrogen loss in this aquifer system, and that oxygen and ammonium availability appear to be key drivers of denitrifier and anammox bacteria distribution patterns.

EMP31

Acetogenesis and other anaerobic processes in the gut of the methane-emitting earthworm *Eudrilus eugeniae*

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The earthworm gut is saccharide rich and anoxic, and some earthworms such as *Eudrilus eugeniae* emit H_2 and methane, indicating that strictly anaerobic processes such as methanogenesis are ongoing in the earthworm gut. Based on the occurrence of strictly anaerobic processes, we hypothesized that acetogens might also be active in the earthworm gut of *E. eugeniae*.
This hypothesis was addressed by RNA-based stable isotope probing with [^{13}C]-glucose as a model saccharide in anoxic gut contents, the analysis of bacterial 16S rRNA and *fhs* (encoding formyl-H_4F synthetase), and cultivation-dependent methods.
Acetate, CO_2, and methane were enriched in ^{13}C during the degradation of [^{13}C]glucose in anoxic gut content microcosms. Supplemental H_2 as a co-substrate was consumed and drove the production of acetate. An enrichment was obtained from gut contents that converted H_2 to acetate in a ratio of 4:1, a ratio indicative of acetogenesis. *Lachnospiraceae*, *Ruminococcaceae*, and *Enterobacteriaceae* were labeled by glucose-derived carbon, indicating that taxa within those families were involved in the anaerobic metabolism of glucose. *Fhs* sequences closely related to the acetogens *Clostridium glycolicum*, *Blautia producta*, and *Acetobacterium carbinolicum* were detected in microcosms with gut contents, suggesting that these taxa were participants in the methanogenic wood web in the gut of *E. eugeniae*.

EMP32
Survival of pathogens under different storing conditions in different substrates
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Question: As some biotechnological processes are not capable to inactivate pathogens in a proper way there is always need for alternative ways to do so. Storing the material could be an economic way. But are the influences on pathogens all the same no matter what pathogen is present or what substrate is used? To find out more about this storage experiments were performed. Experiment 1 dealt with sewage sludge, biogas fermentation residues and slurry, experiment 2 with biogas fermentation residues of different origin. Here biogas fermentation residues from five different biogas plants with different input materials were chosen. In experiment 1 enteroaggregative hemorrhagic *Escherichia coli* O:104 H:4 (EAHEC) was used as test organism and in experiment 2 *Salmonella* Typhimurium was used.
Methods: In both experiments for each substrate two samples were spiked with the pathogens and stored in climate chambers. In experiment 1 the yearly average temperature in Germany was chosen (10 °C) and in experiment 2 samples were stored at monthly mean temperatures. Samples were taken monthly and were analyzed quantitatively following the MPN method.
Results: Experiment 1 showed that depending on the substrate the reduction rates differed quite wide. After four weeks the bacterial counts varied between 10^6 cfu/ml in sewage sludge, 10^4 cfu/ml in slurry and 10^7 cfu/ml in biogas fermentation residues. And after 24 weeks the bacterial counts were 10^4 cfu/ml in sewage sludge, 10^1 cfu/ml in slurry and 10^5 cfu/ml in fermentation residues.
Experiment 2 showed that the source of the substrate has an influence on the survival of pathogens as well as the season the storage starts. There were differences of up to four log10 steps between the bacterial counts depending on the substrate and the starting temperature.
Conclusion: As the results of both experiments demonstrated that there are differences in the influence on pathogens referring to the composition of the substrate and the temperature during the storing period. We conclude that storing the material may be an economic but not a save way to reduce pathogens in products from biotechnological processes. Therefore, optimization of the biotechnological processes and/or adding additional treatment steps (e.g. composting) are required to obtain safe final products.

EMP33
Towards stable isotope labeling-assisted metatranscriptomics of hydrocarbon degrading aquifer microbes
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Question: Stable isotope probing (SIP) of rRNA is a well-established method in molecular ecology, allowing researchers to distinguish microbes in complex cultures by their ability to metabolize a labeled substrate. However, rRNA can give only taxonomic information, while mRNA is necessary to see actively transcribed functional genes. The only published instance of mRNA-SIP involving next-generation sequencing [1] used pre-centrifugation rRNA depletion, thus potentially limiting the quantitative information on rRNA taxonomy in gradient fractions. Also, metatranscriptomic sequencing is especially valuable for RNA studies, compared to probe-directed methods such as RT-qPCR, as it can provide the entire suite of actively transcribed genes with no requirement for prior sequence knowledge or risk of bias from primer design.
Here, we aim to develop a workflow of Illumina-sequencing-based total RNA-Seq SIP, which provides both taxonomic and functional information on targeted microbiome constitutents. As proof of concept, we apply this method to RNA extracted from ^{13}C-toluene exposed hydrocarbon contaminated aquifer samples. From the sequencing datasets, we search for the transcripts of particular genes of interest (for example, oxygen-dependent vs. anaerobic catabolic pathways in hypoxic systems).
Methods: Method development is ongoing. The small yield of RNA after SIP fractionation and tiny proportion of mRNA in total RNA pose challenges for which linear RNA amplification may be a suitable solution. The risk of mRNA degradation requires careful sample handling and optimized laboratory procedures. Bioinformatic pipelines for total RNA analysis are discussed in literature (ex. [2]), and the continual development of new software offers opportunities for improvement of workflows.
Conclusion: The RNA-Seq SIP approach has significant potential as a process-targeted route in environmental 'omics for researchers in diverse fields of environmental microbiology.

[1] M.G. Dumont, B. Pommerenke, P. Casper. Using stable isotope probing to obtain a targeted metatranscriptome of aerobic methanotrophs in lake sediment. *Environmental microbiology reports* 2013, *5*, 757-764.
[2] A. Tveit, T. Urich, M. Svenning. Metatranscriptomic analysis of Arctic peat soil microbiota. *Applied and environmental microbiology* 2014, *80(18)*, 5761-72.

EMP34
Growth phase-dependent dynamics of nitrogen utilization in *Phaeobacter inhibens* DSM 17395
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Question: In the marine system, available nitrogen sources (e.g. ammonium, nitrate) are commonly present only in low amounts, thus often limiting the growth of heterotrophic microorganisms in the ocean. Due to collapsing algal blooms, input from deep sea water masses in upwelling regions (e.g. off the Namibian coast) or estuary inflow in shelf seas, however, concentrations of dissolved nitrogen species in the waterbody may increase substantially. Although changing nitrogen availability is commonly encountered by members of the marine bacterioplankton, their physiological and molecular adaptations are only poorly understood. Therefore, we studied the physiological, proteomic and metabolic dynamics during heterotrophic growth of *Phaeobacter inhibens* DSM 17395 under nitrogen limiting conditions. *P. inhibens* DSM 17395 belongs to the widely distributed marine Roseobacter group.
Methods: Growth of *P. inhibens* DSM 17395 in seawater medium containing 11 mM glucose and 1 mM ammonium was monitored and depletion profiles of glucose and ammonium were determined. Samples for proteomic and metabolomic analyses were taken throughout the growth curve beginning 15 h after inoculation (early linear growth phase) until late stationary growth phase (100 h).
Results: Complete ammonium depletion was already accomplished during the earliest stages of growth (25 h after inoculation, <1/3 ODmax). This rapid ammonium depletion coincided with the buildup of a high cellular N:C ratio (~ 0.25). The latter continuously decreased by more than a two-fold (<0.1) which was reached at the transition into stationary growth phase, agreeing with growth limitation by nitrogen. The growth-dependent profile of the N:C ratio correlated with an increased intracellular concentration of nitrogen-containing metabolites, mainly branched-chain amino acids (e.g. leucine) or amino acid derivatives (e.g. glycylglycine). Concomitantly, proteins involved in nitrogen metabolism revealed changing abundances, e.g. glutamine synthetase (ranging from -3.0-fold at 15 h to 3.5-fold at 90 h). In addition, regulatory proteins predicted to be responsive to nitrogen limitation were detected, including the key players NtrC and the regulatory protein P_{II} (GlnB1).
Conclusion: The dynamic response of *P. inhibens* DSM 17395 reflects its capability to readily utilize pulses of nitrogen as well as to exhibit a classical nitrogen limitation response.

EMP35
An elevated temperature and heat shock alter lipid composition of inner and outer membranes isolated from *Yersinia pseudotuberculosis*
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Question: In spite of important value of membrane lipids in functions of different bacteria, the information on lipid distribution between inner (IM) and outer membranes (OM) of Gram-negative bacteria is mainly limited to *Escherichia coli* and others mesophilic bacteria. Present work was aimed to study the influence of an elevated growth temperature and heat shock

on phospholipid (PL) and fatty acid (FA) composition of IM and OM from psychrotrophic bacteria *Yersinia pseudotuberculosis.*
Methods: *Y. pseudotuberculosis* was grown at 8 °C and 37 °C or exposed to heat shock by the sharp shifting of temperature from 8 °C to 45 °C. IM and OM were separated by equilibrium sucrose density gradient centrifugation in the accordance with Osborn's method modified by Park *et al.* Total lipids were extracted by method of Folch *et al.* PL and FA composition was analyzed by thin-layer chromatography and gas-liquid chromatography, respectively.
Results: Elevated temperature and heat shock caused the increasing content of lysophosphatidylethanolamine in OM preferably. These changes were accompanied by the decrease of phosphatidylethanolamine (PE) content and drastic increase (up to 3 times) of phosphatidylglycerol (PG) level in OM that causes the increase of the net negative charge of cell envelope. The levels of predominant saturated palmitic (16:0) and cyclopropane FAs were about 1.5 times and 7.5 times higher, respectively, but the content of predominant unsaturated palmitoleic (16:1n-7) and *cis*-vaccenic (18:1n-7) FAs was about 10-30 times lower in both membranes isolated from cells grown at elevated temperature. Due to these changes reflecting process of "homeoviscous adaptation", the ratio between unsaturated and saturated FAs decreased, but still remained higher in IM than that in OM. Simultaneously no essential changes were observed in FA composition of cells subjected to heat shock demonstrating a difference between responses of heat-shocked and heat-adapted *Y. pseudotuberculosis*.
Conclusions: The quantitative differences in the phospholipid composition were found between inner and outer membranes of heat-adapted and heat-shocked psychrotrophic *Y. pseudotuberculosis* cells which change their phospholipids within envelope either reciprocally (PE and PG) or differently (LPE) in comparison with mesophilic *E. coli*.

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EMP36
Differential engagement of diverse sugar driven fermentations and associated communities in the earthworm gut
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The earthworm gut is an anoxic, saccharide-rich microzone in otherwise oxic soils. The concentrations of diverse mucus-derived saccharides decrease during gut passage of *Lumbricus terrestris* whereas concentrations of organic acids increase, indicating that fermentation is an ongoing process in the earthworm gut. Thus far, little is known about fermentation of different gut-associated saccharides and affiliated taxa. The hypothesis of this work was that different gut-associated saccharides stimulate different fermentations and different microorganisms in the gut of *L. terrestris*. This hypothesis was addressed by anoxic microcosm experiments with gut content that was supplemented with gut-associated saccharides and a molecular analysis of bacterial and archaeal 16S rRNA. Galactose, glucose, maltose, mannose, arabinose, fucose, rhamnose, and xylose stimulated the production of fermentation products with gut content but considerably less with pre-ingested soil. H_2, CO_2, acetate, lactate, ethanol, propionate, formate, and succinate were the most abundant fermentation products. The quantity of fermentation end products varied depending of the supplemental saccharide. Approximately 1,750,000 16S rRNA sequences were analyzed and assigned to 37 families. This molecular analysis indicated that a subset of ingested microbes were stimulated by the respective sugars. *Aeromonas hydrophila* was the most abundant species in all sugar-supplemented microcosms. *Rosenbergiella nectarea*, *Enterobacter aerogenes*, and *Citrobacter gillenii* were stimulated by some but not all sugars. Collectively, these results reinforce the likelihood that (a) different mucus-derived sugars stimulate different fermentations, and (b) taxa related to *Enterobacteriaceae* and *Aeromonadaceae* drive these processes in the gut of *L. terrestris*.

EMP37
Impact of biochar application on soil microbial community composition in a laboratory biodegradation test
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Biochar (BC) is widely studied for its potential to improve soil quality and carbon sequestration and reduced emissions of greenhouse gases such as N_2O. Carbon sequestration depends mainly on BC stability, which in turn depends on the type of BC and soil properties including soil associated microbial communities. A biodegradation experiment was performed with three different particle size fractions (< 2, 2-5 and > 5 mm) of two contrasting BCs, a BC generated by low temperature/short-term hydrothermal carbonization of corn silage (htcBC) and high temperature pyrolysis of *Miscanthus* feedstock (pyrBC). Both were mixed with two different soil types (a sandy and sandy loamy soil). Carbon degradation of the BCs was monitored by the measurement of $^{13}CO_2$ effluxes over 120 to 180 days of incubation. A much higher biodegradation was obtained for the htcBC (18.5-31.2 %) than the pyrBC (0.0-1.1 %). The biodegradation of htcBC increased significantly with particle size fractions and was larger in sand than in loamy sand. The effect of BC on soil microbial communities was investigated after total DNA extraction from soil-BC mixtures by PCR amplification of bacterial 16S rRNA gene fragments and subsequent denaturing gradient gel electrophoreses (DGGE) and Illumina amplicon sequencing. Bacterial communities of the two soils showed significant differences among each other but both bacterial community structures were not significantly affected by the BC amendment. However, specific bacterial communities were associated with BC particles (> 5 mm). The relative abundance of the *Candidatus* phylum TM7 significantly increased at htcBC particles from 4 to 11 % in the sandy and from Planctomycetes as well as *Burkholderia* and *Pseudomonas* of the *Beta-* and *Gammaprotebacteria* increased in relative abundance at the htcBC particles. In contrast to htcBC the abundance of the phylum *Nitrospira* strongly increased at pyrBCs in the sandy loamy soil (from 0.5 to 11 %) indicating an increased nitrite oxidation activity at those particles which may be due to the absorption of ammonia by pyrBC. Realtime PCR quantification of the ammonium monooxygenase coding gene (*amoA*) of ammonium oxidizing *Bacteria* (AOB) showed also an increase of AOBs in the sand-loamy soils amended with pyrBC and at the pyrBC particles in both soils. Our data gave a first insight into the activity of BC associated microbes, which may affect carbon and nitrogen cycles in BC amended soils. However, the overall structure of soil bacterial communities was not directly affected.

EMP38
Toxicity of differently sized silver nanoparticles to Gram-positive and Gram-negative bacteria
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Question: Silver nanoparticles (AgNPs) with efficient antibacterial properties are currently the most widely manufactured nanomaterials, which can be found in various consumer products, such as textiles, room sprays, cosmetics and medical devices. Along with the rapid production and application of AgNPs, their release into natural ecosystems inevitably rose accordingly. Therefore, the ecosystems health may bear the risk of gradual accumulation of AgNPs, which might have negative consequences for impairing critical bacteria-driven nutrient cycles or biodegradation of organic matter. Over the last few years, rapid toxicity research in human health has been made, but there is still a critical lack of understanding in toxicity studies towards prokaryotes. In our study, we focused on investigating the toxicity of 10 nm and 30 nm AgNPs to Gram-positive bacteria *Mycobacterium frederiksbergense*, and Gram-negative bacteria *Pseudomonas putida* and *Escherichia coli*, under different physiological states.
Methods: Flow cytometry was used to analyze bacteria on single cell level, combined with fluorescent dyes propidium iodide (PI) and bis-(1,3-dibarbituric acid)-trimethine oxanol ($DiBAC_4(3)$) for indicating the bacterial viability.
Results: Our data showed that with the increasing concentration of AgNPs, bacterial growth was delayed, with Gram-positive *M. frederiksbergense* being the most sensitive species, followed by Gram-negative *P. putida* and *E. coli*. In addition, exponentially growing cells of all strains were much more vulnerable than stationary phase cells. Finally, we demonstrated that the toxicity of AgNPs was size-dependent with smaller sizes AgNPs causing higher toxicity.
Conclusion: AgNP have a negative impact on both Gram-positive and Gram-negative bacteria with smaller AgNP exhibiting highest toxicity to highly active bacteria, i.e. cells in the exponential growth phase.

EMP39

Fermenters in the earthworm gut are affected by the feeding guild

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The earthworm alimentary canal is an anoxic microzone in aerated soils, and cultivable numbers of microorganisms capable of anaerobiosis are up to 1000-fold higher in the gut compared to preingested soil. The feeding habits of *Lumbricus terrestris*, which feeds on leaf litter and soil (anecic), and *Aporrectodea caliginosa*, which feeds only on soil (endogeic), are distinct. Due to these contrasting ecotypes, we hypothesised that fermenters along the two alimentary canals are dissimilar and would yield contrasting processes.
Fermentation products of the crop/gizzard, fore-, mid-, and hindgut sections of *L. terrestris* and *A. caliginosa* (both Lumbricidae) were analysed by GC and HPLC. 16S rRNA and 16S rRNA gene sequences were obtained by Illumina sequencing.
L. terrestris and *A. caliginosa* specimens emitted approximately 11 and 4 nmol H_2 per g fresh weight after 6 h, respectively. The foregut of *L. terrestris* and *A. caliginosa* had high concentrations of glucose (11 mM and 17 mM, respectively) and succinate (5 mM and 8 mM, respectively). Acetate concentrations were similarly high in the crop/gizzard and foregut (5 mM and 2 mM for *L. terrestris* and *A. caliginosa*, respectively). All detected organic molecules decreased towards the hindgut.
16S rRNA sequences within alimentary canal sections of *A. caliginosa* were similar in relative abundance, with quantitative differences between gene and transcript sequences. In marked contrast, dynamic changes in relative gene abundance occurred along the alimentary canal of *L. terrestris* and was even more pronounced at the transcript level. Proteobacteria and Actinobacteria were the most dominant phyla detected.
These results indicate that mixed acid fermentation leads to the production of fatty acids that could be subsequently assimilated by the earthworm. The detected taxa are capable of facilitating mixed acid fermentations, and the phylogenetic dissimilarity of the taxa in *L. terrestris* and *A. caliginosa* likely reflects the different feeding guilds of these earthworm species. H_2, a product of mixed acid fermentation in the gut, is emitted *in vivo* by the earthworm and could act as a source of energy and electrons in aerated soils.

EMP40

The marine Bacteroidetes strains *Formosa sp.* Hel3_A1_48 and Hel1_33_131 are key players in polysaccharide utilization during North Sea phytoplankton blooms

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Question: We could recently show that primary production by algae during a North Sea spring bloom in 2009 triggered a consecutive bloom of distinct clades of bacteria (Teeling *et al.* 2012, Science 336(6081):608-11). Combined metaproteome and metagenome analyses suggested a resource partitioning among abundant clades of *Flavobacteriia* and *Gammaproteobacteria*, in which members of the genus *Formosa* might mediate the decomposition of easily accessible algal polysaccharides during early bloom stages. In the diatom-dominated 2010 spring phytoplankton bloom, two novel *Formosa* species could be isolated from North Sea surface water (Hahnke *et al.* 2015, Environ Microbiol. 17(10):3515-26). The remarkably high abundance of these *Formosa* ssp. isolates during diatom blooms in the North Sea raises questions about the specific niches of these bacteria, in particular with respect to the possible usage of algal polysaccharides.
Methods: The genomes of the isolates *Formosa* sp. Hel1_33_131 and *Formosa* sp. Hel3_A1_48 were fully sequenced and manually annotated in order to characterize their potential to degrade algal polysaccharides. *In vitro* cultivations with defined substrates and comparative proteome analyses were performed to identify proteins induced by specific sugar conditions and to identify the specificity of detectable genetic polysaccharide utilization loci (PULs).
Results: Genome annotation of both *Formosa* strains identified PULs for possible utilization of the diatom storage polysaccharide laminarin (a beta-1,3-linked glucose polymer) and putative clusters for the degradation of mannan and chitin. Semiquantitative analyses of the intracellular, membrane-associated and extracellular proteome fractions revealed a substrate-specific response of *Formosa* sp. Hel1_33_131. For example, laminarinases were only be detected when laminarin was available, but also other proteins involved in the recognition, uptake or degradation of algae polysaccharides were expressed on a significantly higher level with laminarin instead of glucose as a substrate.
Conclusion: Our analyses indicate that while the overall niches of the North Sea *Formosa* species Hel1_33_131 and Hel3_A1_48 are similar, thy differ with respect to polysaccharide utilization, which allows them to coexist during algae blooms.

EMP41

Microbial analyses of fungal N_2O-production in permanent grassland under elevated CO_2

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Nitrous oxide (N_2O) is both a potent greenhouse gas and destructive to the stratospheric ozone layer. During a long-term Free Air Carbon dioxide Enrichment experiment (GiFACE) on a permanent grassland site near Gießen, Germany, a significant positive feedback on N_2O emissions under elevated atmospheric carbon dioxide (eCO_2; +20 %) was observed [1]. The responsible microorganisms and mechanisms of the elevated N_2O emissions are still unknown. Previous studies on this grassland site indicated that fungi might contribute to N_2O production [2].
The aim of this study was to investigate the effects of increased C supply in soil through eCO_2 on fungal diversity and fungal denitrification activity in soils under permanent grassland. The outcome of this research will reveal a better understanding of microorganisms involved in N_2O emissions under eCO_2.
Soil samples were taken from the GiFACE (ambient and eCO_2 rings) and different soil compartments were extracted: rhizospheric soil, bulk soil and soil below the root zone**.** Fungal functional and phylogenetic diversity were investigated by molecular analyses of the fungal nitrite reductases (*nirK*) and ITS2 rDNA as a phylogenetic marker, applying quantitative real-time PCR (qPCR) and Ion Torrent high throughput sequencing.
Results presented give first insights into the fungal diversity and abundance of fungal functional genes involved in denitrification of different soil compartments of permanent grassland soil that might explain higher N_2O emissions under eCO_2.

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EMP42

Diversity of bacterial microbiota associated with the flower pollen

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All plants host a complex microbiome that provides important ecological services, thus having a drastic impact on the plant fitness. Diverse microorganisms colonize the different plant-microhabitats, such as rhizosphere, phyllosphere and endosphere. Some plant-microhabitats are poorly investigated and the role of the associated microbes is still unravelled. Among them, flower pollen was studied so far only with respect to the fungal community, while little is known about the ecology of the associated bacteria. Considering that pollen is involved in the reproduction, we expect that the associated bacteria could be specifically selected by the plant to be transmitted to the next generation.

The aim of this research is to investigate the ecology of the bacterial microbiota associated with different flower pollen through cultivation-dependent and molecular methods. The results of both methods will shed light onto the bacterial community structure, composition, dynamics as well as specificity with respect to various types of pollen.
Flower pollen samples were collected from birch (*Betula pendula*), rape (*Brassica napus*), rye (*Secale cereal*) and autumn crocus (*Colchicum autumnale*) from three different locations. Bacteria were isolated on various media including pollen extract-enriched medium, and were identified by 16S rRNA gene sequencing and phylogenetic analysis. Ion Torrent next-generation sequencing of pollen metagenomics DNA was used to assess the structure and diversity of the bacterial microbiota associated with pollen of the different plants. Fluorescent *In Situ* Hybridization (FISH) with rRNA targeting probes and confocal laser scanning microscopy (CLSM) allowed for the visualization of bacteria within the flower pollen habitat.
Cultivated bacterial isolates from flower pollens belonged to the Phyla *Actinobacteria*, *Firmicutes* and *Proteobacteria*, with notable differences between flower pollen of different plants. Typical plant-associated genera such as *Pseudomonas* and *Bacillus* were retrieved with quite low frequency. Other genera were more recurrent, including *Clavibacter*, *Exiguobacterium* and *Rhodococcus*. The result of FISH-CLSM confirmed bacterial colonization of flower pollen and gave information about the colonization pattern.

EMP43
Identification of naphthalene carboxylase subunits of the sulfate-reducing culture N47
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Non-substituted polycyclic aromatic hydrocarbons are very difficult to degrade in the absence of molecular oxygen and the underlying anaerobic biochemical reactions are only poorly understood. Metabolite analyses have indicated that for the model compound naphthalene, carboxylation was the first reaction activating such a chemically stable molecule. Moreover, Mouttaki *et al.* recently brought biochemical evidence confirming the carboxylation reaction in the sulfate-reducing culture N47. Naphthalene carboxylase converts naphthalene and ^{13}C-labelled bicarbonate to 2-[carboxyl-^{13}C]naphthoic acid at a rate of 0.12 nmol min^{-1} mg^{-1} of protein in crude cell extracts of N47. Additionally, it exhibits an isotopic exchange capability indicating the formation of free reversible intermediates. Previous proteogenomic studies of N47 and the marine naphthalene-degrading strain NaphS2 allowed identification of a gene cluster which products were suggested to be carboxylase-like subunits potentially involved in the initial reaction of naphthalene degradation. So far, the native purification of this enzyme using N47 raw extract was unsuccessful. Therefore, we developed alternative strategies to identify the native naphthalene carboxylase subunits. A differential protein induction analysis on blue native PAGEs led to the identification of potential subunits of the naphthalene carboxylase of N47. Moreover, the identified subunits are encoded in an operon structure within the previously mentioned naphthalene carboxylase gene cluster. Here, we were able to show the interaction of the gene products of this cluster for the first time. Additionally, these findings were supported by a pull-down approach revealing *in vitro* interaction partners of a heterologously produced GST-tagged naphthalene carboxylase subunit. Based on these lines of evidence, we propose the naphthalene carboxylase to be a complex of at least 750 kDa.

EMP44
Grinding in the gizzard – how earthworms feed their feeders
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Earthworms represent the dominant macro fauna in various soils and are considered to be important soil engineers that contribute to soil fertility. They take up soil or decaying plant material, and ingest associated microbiota. Some of the ingested microbial cells (especially larger cells like fungal cells or pseudomonads) are thought to be disrupted by the grinding activity in the gizzard. This disruption of cells theoretically make easily degradable polymers (proteins and nucleic acids among others) accessible for ingested fermenters that can be activated in the anoxic gut. The resulting fermentation products might constitute important nutrients for the earthworm. Despite the potential importance of fermenters that might feed on disrupted microbial cells, nothing is known about their identity or fermentation profiles.
In this study, cell lysates of the yeast *Saccharomyces cerevisiae* was added to gut contents of the earthworm *Lumbricus terrestris* to simulate the release of organic material due to the disruption of microbial cells in the gizzard. Fermentation profiles of anoxic gut content microcosms were analyzed by gas chromatography and high pressure liquid chromatography over a 30 h incubation that simulated gut passage. Ilumina-based 16S rRNA and 16S rRNA gene sequencing was used to analyze gut taxa.
Cell lysates greatly stimulated the production of CO_2, H_2, acetate, and succinate in gut content microcosms. Obligate anaerobes of the *Firmicutes* (*Peptostreptococcaceae*, *Clostridiaceae*, *Lachnospiraceae*) and facultative aerobes of the *Gammaproteoacteria* (*Enterobacteriaceae* and *Aeromonadaceae*) had higher relative abundancies in RNA samples from treatments with cell lysate compared to unsupplemented controls or fresh gut content, suggesting a cell lysate-dependent stimulation of these taxa. Differences in relative abundancies at the RNA level were much more pronounced than at the DNA level, indicating that microbes were differentially activated in the short timeframe of the experiment. Most of the taxa that responded to cell lysates have a fermentative metabolism. However at least one of the dominant species (within the *Peptostreptococcaceae*) was closely related to *Clostridium glycolicum*, for which acetogenic strains are known. This result and the high ratio of acetate compared to other fermentation products indicates that acetogenesis in addition to fermentation might be an ongoing process in the gut.
The collective results demonstrated that organic carbon from lysed cells stimulated obligate anaerobes and facultative aerobes in gut contents. The high amount of fermentation products formed by these microbes likely constitute nutrients for the earthworm.

EMP45
Microorganisms from tailing dams – newly developed community
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Remining is an actual development in mining industry and focuses on mining residues as valuable resource for mining. There are at different points for such approach. It is not necessary digging up new ores for metal production, metals of today's awareness were useless in former times, and third reason it minimize environmental impact of the tailings. The tailings stored in landscapes up to hundred years. During this time a microbial population could be developed in tailing body. These microbial community is a close metabolic network, where some of the organisms actively contribute to the carbon, nitrogen, sulfur, and phosphorus cycle and other microorganisms interact with minerals, metals, and pollutants. On the one hand such a microbial community is well adapted to environment and on other hand it is influenced by the geochemical parameters. Investigation of microbial community of tailings and reference material give a glance inside the development of microbial communities.
Tools describing anthropogenic and natural microbial community are genomic, biochemical, and microbial methods. The most sensitive describing tools are culture independent genomic methods. Metagenomic data will be matched with already existing genomic databases. Genetic methods like PCR fingerprinting techniques and biochemical tests used to identify and quantify microorganisms. Finally different culture techniques will be used to isolated microbial strains from the samples. As sample material from zinc production and lateritic soil will be used.
16 S rDNA analyses were done from the samples. The group of *Pseudomonas* was the strongest. Moreover different cultures were enriched, but some mixed culture and few pure cultures. Biochemical tests (Api 50CH) show similar results as *Burkholderia cepacia*. *Pseudomonas* isolate don't metabolize adonitol, trehalose, and 2-keto-gluconate. Further analyses of *rec*A gene are planed to characterize the *Burkholderia* strain. Enzymatic activities will be analyzed to describe the isolates.

EMP46
Cable bacteria in 1-methyl-naphthalene degrading enrichment culture
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Question: Filamentous *Desulfobulbaceae*, so-called cable bacteria, couple spatially separated sulfur oxidation and oxygen reduction by a long-distance electron transfer over centimeter distances in marine sediments [1], freshwater streams [2], and contaminated aquifers. However, the function of closely related *Desulfobulbaceae* in habitats without molecular oxygen or nitrate as terminal electron acceptors is presently unknown. Within this study

we observed the presence of cable bacteria within an anoxic, iron-reducing, 1-methylnaphtalene-degrading enrichment culture (1Mn) and aimed to determine their role in the culture.
Methods: We grew the enrichment culture 1Mn in the presence of 0.07 mM 1-methyl-naphtalene with 5 mM $Fe(OH)_3$ as electron acceptor in strictly anaerobic groundwater medium which was initially reduced with 0.5 to 2 mM sulfide. During growth we monitored not only Fe(II) and 1-methyl-naphtalene, but also sulfate and sulfide concentrations in regular time intervals and calculated electron balances. The microbial key players were identified by T-RFLP, 454 pyrosequencing and FISH.
Results: After one month of incubation, ferrous iron production and microscopy indicated active growth of culture 1Mn. The microbial community was composed of 64 % Clostridia and 29 % *Desulfobulbaceae*. The later showed 98 % similarity of the 16S rRNA gene sequence to the one of groundwater cable bacteria. Fluorescence *in situ* hybridization identified up to 200 µm long filamentous bacteria as members of *Desulfobulbaceae*.
Conclusion: Groundwater cable bacteria are present in the 1Mn-degrading culture where they might promote solid $Fe(OH)_3$ reduction with electrons from sulfur via a cryptic sulfur cycling.

1. Pfeffer, C., *et al.*, Nature, 2012.
2 Risgaard-Petersen, N., *et al.*, AEM, 2015

EMP47
Relation of conversion rates and temperature dependence of microbial sulphate reduction in acid mine drainage (AMD)

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Question: Acid mine drainage (AMD) is a major environmental problem that affects aquatic ecosystems around the world. AMD results from the oxidation of metal sulphides from abandoned mines. It is characterized by low pH-values and high concentrations of heavy metals and sulphate. Using microbiological processes for AMD treatment sulphate-reducing bacteria (SRB) can be deployed for sulphate reduction and heavy metal removal [1].
Methods: To increase the prosperity of the AMD biotreatment, attempts were made to sustain the anaerobic reduction of sulphate using inexpensive carbon sources. In order to find a proper source SRB were incubated anaerobically with different carbon substrates. In addition the influence of temperature and bacterial concentration was examined to obtain more information about the substrate conversion rate of sulphate.
Results: Testing various organic carbon sources the reduction of sulphate by SRB is most effective with straw. Furthermore the prosperity of the microbiological treatment in AMD can be increased by raising the pH-value. In addition the relation of sulphate, iron and biomass development is shown and the influence of different temperatures on sulphate consumption is presented.
Conclusion: Altering microbial growth conditions such as pH-value, temperature or organic carbon sources during cultivation allows efficient bioremediation with SRB. The knowledge of relations of conversion rates is helpful in order to manage microbial biotreatment of AMD efficiently.

[1] Costa, M. C.; Duarte, J. C. (2005), Water, Air, Soil Pollut. 165 (1-4), S. 325-345. DOI: 10.1007/s11270-005-6914-7.

EMP48
Awakening of the Undead: Regeneration of chlorotic *Synechocystis sp.* PCC 6803 cells

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Question/Introduction: Chlorosis is a process that describes the depigmentation of cyanobacterial cells triggered by different environmental influences. Boresch (1910) was the first to describe the chlorosis as a change in the colour of the cyanobacterial culture. This state ensures long-term survival due to low-level photosynthesis (Görl et al 1998, Sauer et al 2001). Chlorosis is not a dead end for cyanobacteria; actually they are able to regenerate within 48 hours after the addition of a nitrogen source and start to divide again. What is the role of storage compounds in chlorosis and recovery and what are the molecular pathways of recovery?
Objectives: To gain deeper insight in this process, we examined the physiological, morphological and transcriptomic changes during long-term nitrogen starvation and regeneration in the model organism *Synechocystis sp.* PCC 6803.
Methods: Spectral analysis, pulse-amplitude modulation and oxygen consumption/evolution measurements were used to describe the physiological regeneration taking place during the first 24 hours after the addition of nitrogen. Transmission electron microscopy was performed to describe the morphological changes during nitrogen starvation and the regeneration. Besides we performed microarray analyses describing the transcriptional changes during nitrogen starvation and the recovery.
Results: *Synechocystis* produces a wide range of reserve polymers like glycogen and polyhydroxybutyrate (PHB) during starvation conditions, which could possibly be related to the regeneration. We were able to exclude PHB as the storage compound fueling regeneration but confirm glycogen as the driving force for this process.
Conclusion: Based on the performed analyses the regeneration process can be defined in three phases: the first phase, which describes the regeneration of the metabolic apparatus, the second phase, which includes regeneration of cellular structures such as thylakoid membranes as well as the increase of DNA and the third phase, in which the cells start to divide again and enter exponential growth.

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EMP49
Comparative genome analysis of plant associated heavy-metal resistant *Pseudomonas* spp. isolates

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Plant-associated microbial communities can actively enhance plant-growth by various direct or indirect mechanisms such as improved nutrient availability, protection against pathogens or synthesis of phyto-hormones. Microorganisms were also shown to greatly enhance phytoremediation of trace elements contaminated soils. Yet, inferring complex phenotype, such as heavy-metal resistance or plant-microbe interaction, from genomic data is far from trivial. We therefore seek in this study to use comparative genomics to identify genes involved in such traits and describe their genomic context.
To address these questions we focused on the genus *Pseudomonas* often found associated with plants and contaminated environments. We recovered isolates from rhizospheric soil and plant tissues of two biomass producing species: *Miscanthus x giganteus* and *Sida hermaphrodita* in Bytom, Poland. This site has a long history of trace elements contamination typical of calamine soils (Zn, Cd and Pb). All isolates were tested *in-vitro* for production of siderophores, indole-3-acetic acid or hydrogen cyanide, phosphorous solubilization (i.e. plant growth promotion traits) and heavy-metal resistance to Zn, Cd and Pb. Ten strains identified as members of the *Pseudomonas* genus on based on 16S rRNA gene similarity were sequenced using Illumina MiSeq platform. Genomes were assembled and compared with all publicly available *Pseudomonas* spp. genomes with verified phenotypes of plant-association and/or heavy metal resistance.
Using this information, we computed genome-wide phylogenies using sets of proteins markers. The tree based on the full genomes was only partially congruent with 16S rRNA tree, emphasizing the need of large sets of markers for phylogenetic delineation of closely related isolates. When coupling ecological data and genomic results, we identified a cluster within the *Pseudomonas putida* species complex showing elevated resistance to heavy metals tested and endophytic lifestyle, closely related to the poplar endophyte strain *P. putida* W619. A set of shared genes found only in endophyte genomes was identified. The genomic contexts of predicted heavy-metal resistance genes show that many are localized on genomic islands and shows associations with mobile genetic elements. This suggests lateral acquisition of resistance genes allowing endophytes to withstand higher concentration of metals found in tissue of plants accumulating metals.

EMP50

FISH- and qPCR-based quantification of methanotrophic bacteria in a rapid sand filter of a groundwater treatment plant

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Question: Although representing a widespread constituent of reduced groundwater, methane is often neglected in drinking water production from groundwater resources. After oxygenation methane can affect the treatment negatively since it enables the growth of aerobic methane oxidizing bacteria (MOB). Especially in rapid sand filters, biomass and metabolic activities of MOB can cause diverse problems including incomplete manganese and ammonium removal, formation of slimy biofilms, hygienic problems and declined filter hydraulics. A reliable quantification of MOB by molecular methods is necessary for the identification of MOB as one cause among others for treatment difficulties. The objective of this study was to clarify whether quantitative real-time PCR (qPCR) represents a more reliable and faster method for MOB quantification than Fluorescence *in situ* Hybridisation (FISH).
Methods: A rapid sand filter loaded with reduced groundwater containing 50 to 190 µg/L methane and operated with non-optimal backwashing conditions exhibited slime accumulation, channel formation and an increase in filter grain size. Samples from filter material were taken at different depths and analyzed for MOB. Biomass-containing sludge was detached from the surface of the filter material and used for FISH and qPCR. FISH was performed with DOPE-FISH probes targeting type I and type II MOB (Mγ84+Mγ705, Mγ669, Creno445, Mα450). MOB-specific FISH analyses were complemented by quantifying α-, b- and γ-Proteobacteria (ALF1B, BET42α, GAM42α) as well as Eubacteria (EUB338+EUB338II+EUB338III). The qPCR-based quantification was performed with a SYBR® Green qPCR assay targeting the 16S rRNA gene of type I MOB (primers U785F, MethT1bR).
Results: While type II MOB were not detected at all, absolute cell numbers of type I MOB quantified by FISH varied from 7×10^7 to 3×10^8 cells per gram of dry weight of sludge. Corresponding relative cell numbers of MOB ranged from 7 to 12 % of total cell count. Type I MOB gene copy numbers detected by qPCR were about one order of magnitude higher than MOB cell numbers detected by FISH (8×10^8 to 1×10^9 gene copies $g_{dry\ weight}^{-1}$).
Conclusion: At an elevated raw water methane concentration and non-optimal operation conditions of the rapid sand filter a high abundance of MOB was detected. This hints towards a contribution of MOB to the observed treatment problems. The discrepancy between FISH results and qPCR results indicate 1) an overestimation of cell numbers by qPCR due to multiple 16S rRNA gene copy numbers per cell and detection of inactive/dead cells and/or 2) an underestimation of cell numbers by FISH since cells with low ribosome content are not detected. The qPCR method is more precise, faster and enables the handling of higher sample numbers.

EMP51

Active bacterial community composition in the horizontal salinity gradient of the Baltic Sea

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Microorganisms react sensitively and rapidly to any environmental changes. Many studies have shown that salinity has a major impact on distribution patterns of pelagic bacteria in transition between marine and freshwater habitats. The Baltic Sea provides such a natural salinity gradient with highest salinities around 30 PSU at the Skagerak/Kattegat and lowest salinities (below 4 PSU) at the Bothnian Bay. Based on 16S rRNA gene analyses Herlemann *et al.* (2011) could show that the bacterial community of the Baltic Sea was separated into three distinct communities: marine-brackish, brackish and brackish-freshwater communities. Furthermore, they could show that *Alpha-* and *Gammaproteobacteria* as well as *Bacteroidetes* increased in relative abundances with increasing salinity and that *Actinobacteria* and *Betaproteobacteria* had highest abundances at low salinities.
We were interested if the activity of the organisms, represented by 16S RNA as activity indicator, would follow the succession of bacterial assemblages accordingly. For this, surface seawater samples were obtained during the research cruise AL439 on the RV ALKOR in June 2014. During this cruise samples were taken for DNA and RNA analysis, phytoplankton community analysis, inorganic nutrients, oxygen, and chlorophyll a as well as dissolved organic carbon. The phytoplankton community revealed a distinct pattern with mainly *Diatoms* present in the Skagerak/Kattegat and the Belt Sea. *Dinoflagellates* were exclusively present in the Bothnian Bay and filamentous *Cyanobacteria* were found mainly in the Baltic Proper. The bacterial community composition was determined by 16S rRNA gene/16S rRNA taq sequencing from selected stations within the salinity gradient covering the most biogeographical regions of the Baltic Sea; and the results supported the bacterial structures described by Herlemann *et al.* (2011): *Actinobacteria* increased with abundances up to 15 % at low salinities (below 6 PSU), whereas *Betaproteobacteria* had their highest abundances at medium salinities. On the other hand *Bacteroidetes* were the only phylum which was significantly positive correlated with salinity, representing more than 1/3 of the total community in the Kattegat. Cluster-analysis showed that the microbial community was clearly separated by salinity into marine-brackish and brackish communities. However, first data on 16S rRNA level revealed a different community and cluster structure on the activity level. Because the 16S rRNA usually better describes the active portion of the community, our data could reflect environmental short-term influences on the 16S rRNA diversity, a pre-requisite for the use of 16S rRNA blueprints as indicators of environmental stressors.

Herlemann, D. P., Labrenz, M., Jürgens, K., Bertilsson, S., Waniek, J. J., Andersson, A. F. (2011): Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. ISME J. 1571-9.

EMP52

Poking around in microbial dark matter

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Introduction: The majority of existing microorganisms still remains uncultured, obscuring our knowledge of microbial diversity, metabolic potential and evolutionary histories. The constant improvement of next generation sequencing techniques as well as evolving assembly and analysis pipelines make meta-genomics a powerful tool to probe biological "dark matter"[1]. However, Computational resources and bioinformatic background knowledge are often limiting factors for microbiologists. Choosing the most appropriate tools from the available range of choices can be a challenging task for non-bioinformaticians.
Objectives: In order to aid the microbial scientific community, we evaluated the currently available metagenomics assembly tools in terms of ease of use, computational requirements, available documentation and their effect on the interpretation of phylogenetic and functional diversity, using highly diversity Marburg forest soil and low diverse marine Kelp biofilm samples as examples.
Methods: DNA extracts from Marburg forest soil and Kelp Biofilm samples were sequenced on Illumina® HiSeq and MiSeq platforms. A range of publicly available tools were used to conduct assemblies, and different binning methods were employed to attempt partial genome reconstruction of single community members. The employed tools were then evaluated based on assembly statistics, specificity and captured diversity as well as user friendliness, and computational requirements.
Results:
We show that the choice of assembly pipeline and analysis tools greatly affects the observed phylogenetic and functional composition of metagenome datasets.
Conclusion: Metagenomic data can give valuable insights into uncultured bacterial populations. However, the applied assembly and analyses pipelines should be fine-tuned not only for the available computational resources but also for the specific research goal.

1. Rinke *et al.* Nature 499 (2013), p. 431-437

EMP53

Transducing phages provide information on bacterial hosts and the potential transfer of functional genes involved in BTEX degradation

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Bacteriophages represent one of the major factors regulating bacterial abundance and diversity by infecting and lysing their host cells. Additionally, many dsDNA phages are able to transfer genetic information via transduction which is caused by miss-packing bacterial DNA. These generally transducing phages are thus especially interesting because they carry signatures of the corresponding host bacteria.
In this project we aimed to analyse transducing phages in pilot scale constructed wetlands receiving BTEX contaminated groundwater (up to 15 mg/l). The constructed wetlands were set up at a former refinery site near Leuna/Germany to investigate efficient low-cost and near-natural

remediation strategies for BTEX contaminated groundwater. One precondition for phage propagation is an active host, e.g. an actively BTEX degrading bacterium. Analysis of general transducing phages may thus point to the BTEX degrading part of the bacterial community in the pilot plants; moreover this analysis could provide evidence for the potential spread of functional genes involved in BTEX degradation
We will show that 16S rRNA genes in particular from the Proteobacteria and the Firmicutes could be detected in the phage DNA, indicating that these taxa might be involved in transduction-mediated gene transfer. These taxa may thus also harbour potentially active bacteria. We further screened the phage DNA for the presence of mono- and dioxygenase genes relevant in BTEX degradation and compared these sequences with the respective genes present in the total community. Phage samples from different wetland systems indeed harboured some of these genes demonstrating the potential for horizontal gene transfer processes between phages and their hosts.

EMP54
Investigating long-term preservation of RNA for qualitative surveys of aquatic microbial metatranscriptomes
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Microbial communities are the main catalysts of global biogeochemical cycling for multiple elements essential for life. The rapidity of their response to stressors and abrupt environmental changes implies that even fast and infrequent events can affect local transformations of organic matter and nutrients. Studying dynamics in microbial functionality at these subtle temporal- and spatial-scales is complicated by the rapidity of gene transcript turnover in cells, especially with respect to the inevitable delay between the sampling of seawater and the extraction of its RNA. This general obstacle underscores the need for an instrument that will allow the reliable sampling of microbial metatranscriptomes at frequent pre-established or event-triggered intervals, for refined temporal- and spatial-resolution. To advance the development of such a sampling tool, we examined the suitability of phenol fixation for long-term preservation of transcripts.
An artificial bacterial community was aliquoted to be either fixed with 10 % v/v Stop Solution (5 % phenol, 95 % ethanol) or left untreated, and filtered at different time intervals over one month. Following fixation, cell numbers remained constant (~ 10^7 cells mL^{-1}) in contrast to increased cell counts in the unfixed aliquots. Both fixed and unfixed communities lost half their total RNA content after 48h but RNA Quality Number (RQN) revealed more extensive degradation in the fixed communities, at time of fixation and during the storage period. Interpreting this as an indication that 10 % v/v Stop Solution is too aggressive on RNA molecules, we tested alternative fixation procedures on an *in situ* community in a second experiment. Fixation with 1 % v/v Stop Solution provided higher RQN values for long-term storage. RNA-Seq was conducted on a selection of samples from the two experiments to ascertain that fixation efficiently conserves gene expression profiles in both artificial and environmental bacterial communities.

EMP55
Partial purification and characterization of a low molecular weight and industrially important chitinase enzyme from *Streptomyces chilikensis* RC1830, a novel strain isolated from Chilika Lake, India
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Chilika Lake is the largest coastal estuarine brackish water lagoon in Asia situated on the east coast of India and is a designated Ramsar site. In the current study, several chitinolytic microorganisms were isolated and screened by appearance of clearance zone on 0.5 % colloidal chitin agar plate. A strain designated as RC 1830 displayed maximum colloidal chitin degradation by release of 112 µmol/ml/min of N-acetyl D-glucosamine (GlcNAc) in 48h. The strain was taxonomically identified by polyphasic approach based on a range of phenotypic and genotypic properties and was found to be a novel species named *Streptomyces chilikensis* RC1830. The organism was halophilic (12 % NaCl w/v), alkalophilic (pH10) and was capable of hydrolyzing chitin, starch, cellulose, gelatin, casein, tributyrin and tween 80. The partial purification of chitinase enzymes from RC1830 was performed by DEAE Sephacel anion exchange chromatography which revealed the presence of a very low molecular weight chitinase(10.5kD) which may be a probable chitobiosidase enzyme. The study reports the presence of a low MW chitinase (10.5kD) and a chitin decaetylase from a novel *Streptomyces* strain RC1830 isolated from Chilika Lake. Previously chitinases less than 20.5kD have not been reported from any other *Streptomyces* species. The enzymes was characterized with respect to optimum pH, temperature, and substrate specificity and temperature stability

EMP56
Cell surface responses of *Cupriavidus metallidurans* CH34 and *Pseudomonas putida* mt2 to cadmium stress
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Cupriavidus metallidurans CH34 and *Pseudomonas putida* mt2 were used as cadmium (Cd) resistant and sensitive bacteria respectively, to study the effect of Cd on physicochemical surface properties (including surface charge, hydrophobicity) and membrane fluidity. For studying the physicochemical surface properties, first EC_{50} (effective concentration 50) was calculated by the method of Pepi *et al.*, (2008). Surface charge was measured by zeta potential and checked by modifications in the methods of Loosdrecht (1987) and Neumann *et al.*, (2006). Whole cell hydrolyzate method of Hartig *et al.*, (2005) was followed to study the membrane fluidity. EC_{50} of *C. metallidurans* CH34 was found to be 2.5mM while 0.25mM for *P. putida* mt2. Zeta potential analysis revealed that *P. puitda* mt2 cells were slightly more stable than *C. metallidurans* CH34 cells. Cell hydrophobicity analysis showed that *P. putida* mt2 cells behaved as an intermediate hydrophilic whereas *C. metallidurans* CH34 as hydrophobic at their respective EC_{50}. Although belonging to the same Gram-negative group, both bacteria behaved differently in terms of changes in membrane fluidity. Expression of *trans* fatty acids was observed in *P. putida* mt2 strain (0.45 %) but not in *C. metallidurans* CH34 strain (0 %). Similarly, cyclopropane fatty acids were observed more in *P. putida* mt2 strain (0.06-0.14 %) but less in *C. metallidurans* CH34 strain (0.01-0.02 %). Degree of saturation of fatty acids decreased in *P. putida* mt2 (36.8-33.75 %) while increased in *C. metallidurans* CH34 (35.6-39.3 %). Homoviscous adaptation is a suvival strategy in harsh environments which includes expression of *trans* fatty acids and cyclo fatty acids in addition to altered degree of saturation. Different bacteria showed different approaches to homeoviscous adaptations.

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EMP57
Diurnal changes of microbial processes in a toluene-degrading constructed wetland model
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Question: Constructed wetlands are economical and efficient options for the treatment of contaminated waters. In these systems, organic pollutants are primarily degraded in the rhizosphere by microbial processes. Plants provide oxygen and organic exudates to the root zone, thereby stimulating microbial activity. This plant root exudation depends on photosynthetic activity, and thus shows day-night fluctuations. While diurnal changes in CW effluent composition have been observed, the question remains whether these are caused by respective fluctuations of microbial processes.
Methods: Diurnal changes of microbial processes were investigated in a toluene-degrading wetland model[1]. Quantitative real-time PCR was applied to assess diurnal expression patterns of genes involved in aerobic and anaerobic toluene degradation. Additionally, proteomic analyses of day and night samples were carried out to investigate toluene metabolism as well as other microbial processes.

Results: Toluene was steadily degraded aerobically throughout day and night. Expression of the respective genes and proteins showed no diurnal rhythm. Although transcripts encoding for the anaerobic toluene degradation enzyme benzylsuccinate synthase (*bssA*) were highly abundant, no respective proteins could be detected. Instead, proteomic analyses revealed diurnal protein patterns of other metabolic functions such as polyhydroxyalkanoate (PHA) metabolism, transport processes and amino acid metabolism.
Conclusion: Microbial processes such as PHA synthesis and transport seemed to be mainly influenced by diurnal patterns of plant root exudation rather than fluctuating oxygen release. Aerobic toluene degradation was stable throughout the diurnal cycles while anaerobic toluene degradation did not occur.

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EMP58

Hot spots or cold spots? Modelling biodegradation dynamics in response to disturbances

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Microbial degradation of soil pollutants is an important ecosystem function. As soil systems are constantly exposed to disturbances of different spatial appearences and frequencies, their ability to recover the biodegradation function is crucial. However, the effect of disturbance regimes to the long-time biodegradation dynamic is not yet been examined.
We applied a numerical simulation model considering growth, degradation and bacterial dispersal to analyze the spatiotemporal dynamics of biodegradation in response to disturbance regimes. To investigate the influence of bacterial distribution, we simulated different spatial configurations of the disturbances with various degrees of fragmentation. Moreover, we considered bacterial dispersal networks to simulate bacterial movement along fungal networks in soil.
We found that the biodegradation performance decreases in response to periodic disturbances but tends to saturate around a mean. Level of the mean degradation performance increases in presence of dispersal networks and with increasing degree of fragmentation of the disturbance. Spatiotemporal observation of degradation dynamics reveal partitioning of the system in active hot spots and inactive cold spots. In hot spots the function is maintained on a certain level of saturation, whereas in cold spots degradation is completely down. However, size and distribution of hot/cold spots depend on spatial configuration of the disturbance. We identify a critical degree of fragmentation of the disturbance under which the degradation activity concentrates on hot spots. Is the disturbance higher fragmented the whole system is homogeneously active. This effect is due to variation in mean distance between disturbed and undisturbed area. The more distant a disturbed habitat from the next undisturbed the longer the lag-phase of biodegradation recovery and thus the system could absorb higher fragmented disturbances better. However, if dispersal networks are applied this critical degree is shifted as the networks are increasing bacterial dispersal.
Our results show that ecological interaction in reaction to the disturbance pattern is responsible for maintaining the biodegradation performance under disturbance regimes. The degree of fragmentation of the disturbance configuration influences the dynamics which indicates the relevance of spatial processes for functional stability. Besides, fungal networks may increase activity by enhancing bacterial dispersal. However, the influence of fungal networks to the degradation dynamics also regarding other functionalities like substrate/water transport should be analyzed further.

EMP59

Initiating a bacterial revival – mycelia-supplied water and nutrients activate dormant cells trapped in hostile conditions

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Question: Bacteria in soil have evolved strategies to overcome unfavorable environmental conditions such as water and nutrient deprivation by entering less profligate dormant stages. From an ecosystem perspective, however, dormancy is undesirable because important functions and services driven by bacteria are almost completely inhibited. In contrast to bacteria, fungi can maintain growth and activity in water and nutrient depleted soil regions, because they are able to efficiently transport water and nutrients in their mycelium. Here we tested the hypothesis, that mycelia redistribute water and nutrients from a remote area, provide both to dormant bacteria and thus initiate a bacterial revival in a dry and oligotrophic environment.
Methods: Using microscopy and cultivation-dependent techniques we followed the germination of *Bacillus subtilis* endospores in presence and absence of *Pythium ultimum* hyphae, which have access to a source of water and nutrients apart from the dry and nutrient-free spore region.
Results: Microscopy revealed the development of vegetative cells only if mycelia were present in the direct surrounding of the spores. Moreover, we found an increase in total CFU numbers and a decrease of the relative proportion of spores in the presence of mycelia elucidating the diminished adverse conditions for bacteria located in the vicinity of hyphae. The examination of 18-O, 13-C and 15-N incorporation into the bacterial biomass via NanoSIMS can provide direct evidence for the transfer of water and nutrients from the mycelium to the spores.
Conclusion: We propose that water and nutrients supplied by mycelia may trigger bacterial activity in soil, which promotes functional ecosystem resilience.

EMP60

Determination of the most effective inoculation strategy for anaerobic digestion of lignocellulosic biomass and effects on microbial populations

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Animal manure has a great energy potential due to the high organic matter content mostly in the form of lignocellulose from an animal feed and a possible bedding used in the animal shelter. However, during anaerobic digestion process, hydrolysis rate of cellulose is so low and it is stated a rate limiting step in overall digestion. Because of its high cellulolytic activity, rumen liquid is used as a primary inoculum in these systems and using the enrichment of the existing cellulose-degrading bacteria can greatly support the hydrolysis step and improve the acidification rate. In this study, it was aimed to determine the most effective inoculum for enhancing the biogas production of cow manure digestion and establish the effects on the microbial community structure. In this scope, three different inoculums (rumen fluid, digester sludge taken from full-scale plant and the enriched microorganisms responsible for the degradation of cellulosic material in the cow rumen) and their combinations were tested in the batch anaerobic digesters treating cow manure. The quantification of total bacteria, total archaea, and three important fibrolytic bacteria (*Ruminococcus flavefaciens*, *Fibrobacter succinogenes*, and *Ruminococcus albus*) were carried out by StepOnePlusTM platform (Applied Biosystems, Life Technologies). The highest biogas production was measured in the digester seeded with enriched culture and seed sludge. The abundance of total bacteria was not significantly changed for all digester conditions during the operation period. However, at the first day of operation, the quantity of total archaea was measured higher in digesters inoculated with the rumen fluid, then started to decrease. The number of *Ruminococcus flavefaciens, Fibrobacter succinogenes* and *Ruminococcus albus* were the highest in the digesters inoculated with

enriched culture, during the operation the quantities were decrease in all digesters. The results revealed that, addition of lignocellulotic bacteria to the anaerobic digesters enhance biogas production. Inoculum application strategies should concentrate on providing the appropriate conditions for the responsible microorganism and improve the competitiveness with local community.

EMP61
Marine epibiontic filamentous sulfur-oxidising bacteria
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Question: Hydrogen sulfide can be transported by diffusion or by advection to microbes. Gliding *Beggiatoa* live in the transisiton zone of diffusive systems, whereas attached-living *Thiothrix* are well known from advective freshwater systems (1). On a field trip, large white biofilms were discovered growing as epibionts on seagrass with an advective hydrogensulfide source. Which microorganisms formed the white biofilm?
Methods: Morphological characterization by imaging techniques as well as isolation of filaments and partial 16S rRNA gene sequencing were applied.
Results: In a hydrogensulfide-free flow system, the biofilm became colorless. This and the microscopic images suggested the presence of sulfur in the cells. The dominant population of filaments in the biofilm were 1000 to 1500 µm long and had a diameter of 4 µm. The presence of a sheath and the formation of small cells which were interpreted as gonidia indicated the morphology of *Thiothrix* spp.. Many filaments served as attachment substrate for small sulfur containing cells, as previously described by Larkin (2). The community contained also filamentous sulfur-oxidising bacteria with the morphology of *Beggiatoa* as well as large, vacuolated, ensheathed cells in filaments with sizes up to 2000 µm long and 15 µm in diameter.
Conclusion: Unique environmental conditions have transiently formed optimal conditions for a bloom of sulfur-oxidising bacteria. The ongoing characterization of the biofilms may yield novel insights into the diversity of sulfur-oxidizing bacteria.

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EMP62
YocM – a small heat shock protein involved in the stress response of *Bacillus subtilis*
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Small heat shock proteins (sHsp) occur in all domains of life and fulfil their protective role in preventing the irreversible aggregation of proteins. These sHsp are intricately interacting with cellular protein aggregates. This ATP-independent protein aggregate binding activity is often linked to facilitate the activity of molecular chaperones and proteases of the cellular stress response and protein quality control network to support their ability to disaggregate and refold cellular protein aggregates. Until now, no small heat shock protein has been characterised in *B. subtilis* yet.
Our recent experiments investigating thermotolerance development in *B. subtilis* suggested a role of a potential sHsp YocM in the stress response of *B. subtilis*. We observed that YocM-GFP interacts with protein aggregates *in vivo* and is therefore a possible tool to analyse the amount of protein aggregates, indicating the state of stress and the functionality of the stress response system in the cell. Interestingly, sensitivity of *B. subtilis* towards high concentrations of salt increased in a *yocM* deletion mutant strain. However, a strain that overproduced YocM showed an enhanced cellular survival after salt shock. Western blotting experiments revealed the salt stress induced synthesis and localization of YocM to protein aggregates after salt shock. The exact molecular function and role of YocM within the stress response machinery of *B. subtilis* remains to be elucidated in future experiments.

EMP63
Detecting and enriching oxygenic denitrifiers from contaminated groundwater and urban water systems
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The microbial nitrogen cycle has been extensively investigated and was thought to be fully understood. Nonetheless, the recent discovery of a new nitrogen transformation pathway, nitric oxide (NO) dismutation into oxygen and nitrogen gas catalyzed by a putative NO dismutase (Nod), has changed this perspective. This so-called oxygenic denitrification enables microbes to utilize aerobic catabolism in anoxic habitats. Oxygenic denitrification coupled to aerobic methane- and alkane-oxidation has been reported for the denitrifiers *Candidatus* Methylomirabilis oxyfera and the Gammaproteobacterium HdN1, respectively. However, it is not at all clear how widespread oxygenic denitrification could be in natural habitats, and the diversity of potentially coupled oxygen-dependent catabolic pathways is unknown.
Therefore, we have developed the first specific primer sets capable of detecting putative *nod* genes, the key functional marker for oxygenic denitrification, in diverse environmental samples. *nod* sequences were successfully retrieved from anoxic sediments of a BTEX-contaminated aquifer, and from different nitrogen-removing wastewater treatment systems. These genes showed only 70-90 % identity to the *nod* genes of *M. oxyfera* and HdN1. The *nod* genes could also be detected in RNA extracts from the aquifer sediment, suggesting that they were actively transcribed *in situ*. In order to better understand the ecophysiology of these oxygenic microbes and determine whether aromatic hydrocarbon can serve as electron donor for oxygenic denitrification, enrichments were set up using *nod*-rich aquifer sediments as inocula under nitrate- and nitrite-reducing conditions. Enrichment of *nod*-possessing microbes is monitored by quantifying *nod*-gene copy numbers in these cultures. Our results suggest that oxygenic denitrification is much more widespread and abundant in natural and man-made environments than previously perceived, and could be an overlooked nitrogen transforming pathway of great appeal for groundwater remediation and in the urban water cycle.

EMP64 – withdrwan

EMP65
Salt-resistant bacteria isolated from mangroves promote growth of barley in greenhouse under salt stress conditions
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Soil salinity is an increasing problem affecting agriculture of many countries. An environmental-friendly approach to reduce the effect of salinity on crops is based on the application of plant growth promoting bacteria (PGPB) that can support the growth of the plants by improving their resistance to salt stress.
Our aim was to isolate endophytic bacteria from the propagules (reproductive units) of *Avicennia marina* accounted among the most salt tolerant mangrove tree species; to select potential PGPB; to test the best candidates on barley (*Hordeum vulgare* L.) in both gnotobiotic (germination pouches) and greenhouse conditions (non-sterile soil).
Endophytic bacteria were isolated on different media. Representative isolates were characterized for their PGP-traits and resistance to salinity in pure culture. Barley seeds were surface sterilized and inoculated with 12 candidate PGPB encompassing a broad taxonomic diversity. Barley was grown in germination pouches for two weeks and the root colonization pattern was analyzed by fluorescence *in situ* hybridization and confocal laser scanning microscopy (FISH-CLSM). The greenhouse trial was performed with organic-rich substrate (Einheitserde ED-73); barley plants were grown for 8 weeks until production of seeds occurred.
One of the 12 potential PGPB tested, named isolate M40, was able to significantly increase ear dry weight compared to both uninoculated plants and plants inoculated with *E. coli*. The root colonization patterns were very different among various inoculated bacteria and isolate M40 showed an extremely effective rhizoplane colonization. FISH-CLSM analysis

revealed the occurrence of additional bacteria (native seed endophytes) which built up mixed colonies with the inoculated strains.
This is the first study showing mangrove's propagules as a promising source of potential salt-resistant PGPB. Our results using barley as model species, suggest that mangrove endophytes might play a major role in supporting plant growth under saline conditions.

EMP66
Fate of antibiotic resistant bacteria (ARB) and of antibiotic resistance genes (ARG) during sewage treatment and in receiving rivers - culture-based versus qPCR data
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Introduction: Although the number of studies analyzing bacterial communities and abundances of antibiotic resistance genes in soil or aqueous environment by molecular genetic approaches (qPCR, metagenomics) increased, the European guidelines for monitoring the hygienic quality of drinking water (EU drinking water directive, 2011) or bathing water (EU bathing water directive, 2006) are still relying on culture-based approaches.
Objectives: Microbial and molecular-genetic characterization of sewage and river water samples by culture-based and by qPCR assays, comparison of obtained results and discussion whether the thresholds defined e.g. in the EU bathing water directive for culture-based assays could be adopted to quantitative molecular genetic assays.
Material & Methods: 44 sewage samples (raw and treated) and 40 samples taken from the respective receiving river water were analyzed for their inventory of total bacteria, *Escherichia coli*, enterococci and staphylococci as well as for the presence of clinically relevant antibiotic resistance genes.
Results: Whereas plating and qPCR data for all bacteria correlated well in raw sewage, qPCR data of treated sewage and of river water indicated higher cell numbers for *E. coli*. It is unknown if these cells are "only" not growing under standard conditions or if they are dead. Corresponding to the amount of non-culturable cells, the "breakpoints" for monitoring water quality should be adapted. The abundances of clinically relevant antibiotic resistance genes in river water were in the same order of magnitude or even higher as in treated sewage.
Conclusion: For estimation of the resultant health risk, it is necessary to know which species the respective antibiotic resistance genes carry and if they are disseminated via horizontal or lateral gene transfer.

EMP67– withdrwan

EMP68
A multidisciplinary approach towards the evolutionary history of the large sulfur bacteria *Achromatiaceae*, *Beggiatoaceae*, and *Chromatiaceae*
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The large sulfide-oxidizing members of the *Gammaproteobacteria* comprise a heterogeneous group of organisms, not only in terms of size, morphology, physiology, and habitat range, but also in the diversity of storage compounds and compartmentalization. The *Beggiatoaceae* and *Achromatiaceae* are chemolithotrophs while the *Chromatiaceae* are photolithotrophs. All members store elemental sulfur intracellular as electron donors, and they thrive in gradient systems at the interfaces of oxygen and sulfide. While the *Beggiatoaceae* contain a large central vacuole with only a thin peripheral cytoplasmic layer, the *Chromatiaceae* and *Achromatiaceae* contain a continuous network of thin cytoplasmic strings stretching around the inclusions across the entire cell. In line with this shared cell architecture but to the contrary of their physiology, *Achromatiaceae* are closer related to *Chromatiaceae* than to *Beggiatoaceae*. A feature that makes the *Achromatiaceae* unique compared to all currently known *Bacteria* and *Archaea* is their ability to store massive amounts of intracellular calcite. The ecophysiology of large sulfur bacteria is mainly known from *in situ* measurements, and enrichments. Culturing has so far only been successful for a few members of the *Chromatiaceae* and *Beggiatoaceae*, and insights into the genomic repertoire of the three families are still in their infancy. Marine *Achromaticeae* have only recently been rediscovered, and the diversity as well as genetic information of the family is slowly becoming available. Here we present a multidisciplinary investigation of the *Achromatiaceae*, combining traditional and modern staining methods with high-resolution microscopy to elucidate the distribution and localization of intracellular compounds such as calcite and free calcium, elemental sulfur, DNA, and polyphosphate. With the application of different specific membrane dyes we investigate the compartmentalization of the cell interior, such as the formation of cytoplasmic membrane invaginations, intracellular vesicles and vacuoles. Our findings are directly compared to known structures in the *Beggiatoaceae* and *Chromatiaceae*. Tackling their physiological capabilities, we are testing the storage and usage of nitrate by *Achromatium*, which is in line with capabilities of their close relatives, the *Beggiatoaceae*. The major difference being that *Beggiatoaceae* contain a large aqueous vacuole for nitrate storage while the location for nitrate storage in *Achromatiaceae* remains to be revealed. Finally we integrate our morphological and physiological results with a genomic comparison between the sulfur bacteria families. We were able to generate high-quality draft genomes from marine and freshwater *Achromatium*, which allows an unprecedented insight into the phylogenetic history and evolution of this exciting group of large sulfur bacteria.

EMP69
Temperate phages of potentially pathogenic *Vibrio* species from North Sea
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Question: Temperate phages play a major role in bacterial genomic diversity, by transferring mobile genetic elements. Phages may contribute to emergence of disease-causing strains from environmental *Vibrio* species. Thus, potentially pathogenic *Vibrio* species from the North Sea were subjected to phage induction to gather information on the vibriophage related genepool.
Methods: Screening for lysogenic phages from potential pathogenic *Vibrio* strains isolated from North Sea in 2014 was performed. *V. vulnificus*, *V. parahaemolyticus* and *V. cholera* strains were induced using Mitomycin C. After phage induction, each sample was checked for phage production using epifluorescence microscopy. Positive phage induced samples were used for a host screening assay using 154 potentially pathogenic Vibrios of the respective species
Results: During the research cruise 39 potentially pathogenic *Vibrio* strains from the coastal waters were isolated from stations near the coastline. Temperate phages were successfully induced from 18 potentially pathogenic *Vibrio* isolates, in detail 14 correspond to *V. parahaemolyticus*, 3 to *V. vulnificus* and 1 to *V. cholerae*. All inducible phages were found in *Vibrio* strains from coastal stations of Germany and the Netherlands. The induced phage samples presented inhibitory activity against half of the tested *Vibrio* strains, mostly environmental isolates from North and Baltic Sea.
Conclusion: About 46 % of *Vibrio* isolates possess inducible temperate phages, all of them isolated from coastal waters. These phages might play a role in transference of pathogenic genes into the environment. This will be subject of further studies. The present work shows the first insights of temperate phages presence on potential pathogenic *Vibrio* strains from Northern European seawaters.

EMP70
Plasmid spreading during conjugative DNA transfer in *Streptomyces*
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Conjugative DNA-transfer in filamentous bacteria of the genus *Streptomyces* is a unique process requiring a single plasmid-encoded transfer protein (Tra/TraB). After the initial transfer from donor to recipient, *Streptomyces* plasmids colonize the recipient mycelium very efficiently. It was speculated that plasmid-encoded Spread (Spd)-proteins promote migration of the plasmid within recipient hyphae including the passage through cross-walls[1]. This study[2] addressed the following questions: i) Which parts of the hyphae are involved in plasmid transfer ii) Can plasmids spread within recipient hyphae? iii) Which plasmid-encoded proteins are involved in plasmid spreading?
We constructed an eGFP-encoding reporter plasmid based on the *Streptomyces lividans* plasmid pIJ101, leading to a uniform green fluorescence in plasmid-carrying cells. To clearly distinguish donor and

recipient in matings, we used a recipient strain that constitutively expressed mCherry. Matings of these differentially labeled donor and recipient strains were analyzed by fluorescence microscopy. Additionally genetic crosses using *S. lividans* strains resistant to different antibiotics and replica plating were used to determine the extent of plasmid spreading.
Conjugative DNA-transfer was previously proposed to occur at the hyphal tips[3]. Surprisingly, fluorescence microscopic images of matings with the reporter plasmid suggest that plasmid transfer happens at the lateral walls of touching donor and recipient hyphae. The mating junction did not involve complete fusion of donor and recipient compartment, as mCherry was never detected in the donor. In the recipient, the eGFP signal was observed far away from the contact site to the donor, demonstrating that the plasmid has spread within the recipient hyphae through cross-walls. Intramycelial spreading was significantly impaired when the putative *spd* (spread) genes were inactivated showing that effective plasmid spreading depends on the presence of the Spd-proteins. In genetic crosses efficient spreading of a *tra* deletion mutant plasmid was only detected if *tra* was provided in *trans* in the donor and in the recipient, demonstrating that Tra is not only required for the primary transfer, but also for intramycelial plasmid spreading. Moreover, interaction of Spd-proteins and Tra in bacterial two hybrid studies suggest that Tra and Spd-proteins work together to distribute the plasmid in the recipient mycelium.
In adaptation to the filamentous lifestyle of their host, *Streptomyces* plasmids developed a two-step conjugation mechanism. After the initial plasmid transfer at the lateral wall, plasmids invade neighbouring compartments dependent on Tra- and Spd-proteins thereby rapidly colonizing the recipient mycelium.

1. Kieser *et al.*, *Mol Gen Genet* 1982. 185:223-228.
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3. Reuther *et al.*, *Mol Microbiol* 2006. 61:436-446.

FBP01
A bioinformatics analysis of alternative splicing in human-pathogenic fungi
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Question: The process of alternative splicing (AS) increases the number of proteins and thus the functional complexity of an organism. This mechanism is common in all eukaryotes but still little is known about its regulation and effect in fungi [Grützmann *et al.*, 2014]. The presented work is aimed at a better understanding of the role of AS in human-pathogenic fungi.
Methods: Our analyses are based on RNA-Seq data of various human-pathogenic fungi. RNA-Seq is a powerful technique that can be used to distinguish and quantify the expressionof different transcript isoforms. The data were analyzed using five different AS detection tools. Additional filters were applied to exclude artefacts and false positive results. The identied ASevents for all species were compared for a better understanding of the role of AS, especially in the context of host infection.
Results: The observed fungal species use AS as regulatory mechanism. It was possible to determine a number of AS events under various conditions, including pH and stress adaption in *Candida glabrata*. In this fungus, some detected differential spliced genes are present under multiple conditions. A higher number of AS events was identied for the filamentous fungus *Candida albicans*.
Conclusion: AS is an important regulatory mechanism in fungi. Genes detected as AS under multiple conditions in *C. glabrata* may have an important regulatory function. AS is involved in the formation of hyphae and host infection processes in *C. albicans*. This shows that AS takes place in the infection process of human-pathogenic fungi.

K. Grützmann, K. Szafranski, M. Pohl, K. Voigt, A. Petzold, and S. Schuster. Fungal alternative splicing is associated with multicellular complexity and virulence: A genome-wide multispecies study. DNA Res., 21:2739, 2014.

FBP02
Spatio-temporal shift in fungal and bacterial community structure and composition in preserved and non-preserved wood
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Copper-based preservatives are used for wood and plant protection, which leads to an enrichment of copper tolerant microbial communities in respective soil environments. Such communities are overall able to decompose copper-based preserved wood and thus leading to major damages in wooden stakes. To investigate the effect of wood preservatives on fungal and bacterial community structure and composition, three compartments (attached to wood, 1 mm and 7 mm inside of wood) from two sites were evaluated after 17 and 36 weeks of field incubation. Wooden specimens were impregnated with water (A as non-preserved wood) or different biocide-based preservation treatments (containing triazoles and benzalkonium chloride, encapsulated; containing triazoles and benzalkonium chloride, non-encapsulated; and containing copper). Moreover, spatial resolution of attached soil to the wood specimens (2×2×36 cm) and two wood specimen depths were studied. For the assessment of the fungal and bacterial soil community, next-generation sequencing and quantitative PCR by ITS and 16S rRNA gene region were performed, respectively. Both, the fungal and bacterial gene copy numbers decreased significantly over wood depth profile in both soil environments. While the fungal and bacterial copy numbers in attached soil increased over time the respective copy numbers in wood remained unaffected. For all dominant taxa, the structure of fungal and bacterial communities were significantly depth specific and remained less affected by the wood preservative treatment. However, the fungal community structure of both environments differed in treatment A to other wood preservative treatments. In addition, bacterial community structure was less affected by wood preservatives. In conclusion, a decreasing fungal community composition over time indicates that few fungi with accompanying bacterial diversity can cope wood preservative treatments and perform the main wood decay process.

FBP03
Toxic effects of different substances from pyrolysis oil on growth and production of malic acid by *Aspergillus oryzae*
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Question: As part of the "food or fuel" debate a biotechnological malic acid production based on alternative carbon sources which do not compete with food or feed would be desirable. It could be shown, that *A. oryzae* is able to convert several alternative carbon sources, like xylose, which is also a part of ligoncellulosic material (1) to malic acid. Therefore, alternative carbon sources based on lignocellulose, such as pyrolysis oil, as substrates for malate production by *Aspergilli* would be conceivable. Beside usable carbon sources, a lot of possibly toxic compounds are formed during the pyrolysis process. Some of the substances are also well known environmental pollutants. In this study, the toxic effects of different compounds, which represent different chemical groups from pyrolysis, were tested for their influence on growth and malic acid production of the model organism *A. oryzae*.
Methods: *A. oryzae* was cultivated in preculture medium in shaking flasks for 24 hours. Due to nitrogen limitation and an excess of glucose in the production medium the fungus started to produce malic acid. In this phase, main culture medium was mixed with eleven different substances from pyrolysis oil in different concentrations to determine the level where a total malic acid production happened. The same concentrations where used for determine growth limitation concentrations on agar plates.
Results: Toxicity tests showed that *A. oryzae* tolerates pyrolysis oil content between 1 % and 2 % depending on stationary production phase or growth phase. To investigate the reason for this low tolerance level, eleven representative substances from pyrolysis oil, e.g. aldehydes, organic acids, phenols and other compounds were chosen and the limiting concentration for growth and malic acid production determined. Some of the analyzed substances are very critical for both, growth and acid production, some only for one of these phases. On the other hand some of them showed no inhibition in the tested concentration range. One substance could be found, to be responsible for the toleration of 2 % pyrolysis oil in the growth phase.
Conclusions: *A. oryzae* DSM1863 tolerates a pyrolysis oil content between 1 % and 2 % depending on stationary production phase or growth

phase. The inhibitory concentrations of several substances from pyrolysis oil could be determined and possible problematic compounds were identified.

1. Ochsenreither K, Fischer C, Neumann A, Syldatk C. 2014. Process characterization and influence of alternative carbon sources and carbon-to-nitrogen ratio on organic acid production by *Aspergillus oryzae* DSM1863. Appl Microbiol Biotechnol 98:5449-60.

FBP04

p-Hydroxybenzoic acid, a phenolic acid, produced by the *ΔareA* mutant of the wheat pathogenic fungus *Zymoseptoria tritici* during *in vitro* growth experiments

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Question: Do phytotoxins contribute to the infection of wheat by *Zymoseptoria tritici* and if so, do these compounds have any impact on host resistance /susceptibility by inducing the host defense response?
Methods: Fungal growth of the *Z. tritici ΔareA* mutant was performed in minimal medium (MM) with 10 % wheat leaf extract in a 20 L fermenter. The culture filtrate was extracted with ethyl acetate, methanol and dichloromethane (3:2:1). The crude extract was tested on wheat leaves evaluating the phytotoxic potential and was then fractionated by normal phase chromatography. Each active fraction was further purified by preparative HPLC, allowing us to collect the purified compounds for further analysis. The structure of one of the active substances was elucidated by NMR. To investigate if the characterized compound is involved in the induction of the defense response in wheat, real-time quantitative PCR (RT-qPCR) analysis was performed. Therefore 10 days old wheat leaves were placed into Petri dishes containing 300 µM p-hydroxybenzoic acid, 300 µM methyl jasmonate as positive control or water as negative control for 24 hours. RNA was isolated and used as template for RT-qPCR analysis, measuring the changes in the transcript levels of pathogenesis related proteins (PR proteins).
Results: One of the phytotoxic compounds we isolated from culture filtrates of the *Z. tritici ΔareA* mutant was identified as p-hydroxybenzoic acid (p-HBA), a phenolic acid. p-HBA and its derivates are known as common allelopathic agents and were previously reported to be produced by plants in order to inhibit germination, growth and development of neighboring plants. In fermentation experiments performed in MM complemented with 100 µM benzoic acid we were able to show that in *Z. tritici* p-HBA is synthesized by hydroxylation of benzoic acid. The results of RT-qPCR analysis indicate that p-HBA is not involved in the induction of PR proteins in wheat.
Conclusions: Since to date there have been no reports of phytotoxic metabolites isolated from *Z. tritici* but their potential existence is expected to play a crucial role within the pathogenic development, this work is likely to make the first contribution to this field of research. The loss of AreA, the major regulator of nitrogen metabolism leads to a defect in nitrate assimilation and appears to be coinciding with a modification of *Z. tritici* secondary metabolism, as no p-HBA production was observed upon fermentation of the wildtype strain under the same cultivation conditions. We propose that during infection *Z. tritici* is able to metabolize benzoic acid from wheat into p-HBA to facilitate the infection process by the phytotoxic activity of p-HBA. We suppose that toxicity does not occur via induction of the immunity response and the subsequent programmed cell death in wheat, since no changes in the transcript level of PR proteins were detectable by RT-qPCR analysis.

FBP05

Conditional gene expression in *Aspergillus niger* – perspectives and limitations of inducible expression systems

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Synthetic biology tools allowing precise regulation of gene expression are powerful tools to study gene functions *in vivo* and to streamline metabolic pathways. Inducible, tuneable and metabolism-independent gene switches are of special interest as they can be easily controlled and shut on or off at any stage during the life cycle of the organism of interest. Recently, we established a tetracyclin-dependent expression system for the industrial platform organism *Aspergillus niger* that can either be used to switch the expression of a certain gene on (the Tet-On system) or off (the Tet-off system) upon addition of the inducer tetracycline or its derivative doxycyclin.[1,2]
However, if more genes have to be controlled independently, this system reaches its limits. We therefore aimed to design additional inducible expression systems functioning in *A. niger* and other filamentous fungi. We tested the performance and efficacy of two conditional expression systems responding to the antibiotics inducer erythromycin or to the hormone analog diethylstilbestrol. We used luciferase as a reporter gene and applied a MTP-based assay to evaluate and compare the systems with the established Tet-On and Tet-off systems. Corresponding results will be shown.

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[2] F. Wanka, T. Cairns, S. Boecker, C. Berens, A. Happel, X. Zheng, J. Sun, S. Krappmann, V. Meyer, *Fungal Genet. Biol.* 2015, Epub ahead of print

FBP06

Phagocytic escape and survival mechanisms of *Aspergilli* during interaction with the fungivorous amoeba *Protostelium mycophaga*

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The Aspergilli are ubiquitous saprophytic filamentous fungi along with several species cause fatal infections in immunocompromised individuals. Of these, the highest incidences of mortality rates are reported for *Aspergillus fumigatus.* The absence of any highly specific virulence factors suggest that a number of general fungal virulence determinants could have emerged long before the appearance of innate immune systems of infected vertebrates. Amoeba represent one of the largest and most diverse groups among the protozoa, but all share a predatory life-style. Only a handful of bacterivorous species have been studied in more detail, of which the social amoeba *Dictyostelium discoideum* has become a model organism for phagocytosis. Comparably little is known on the evolutionary impact of any fungivorous species. Here, we have studied the antagonistic interactions of the filamentous fungus *Aspergillus fumigatus* with the model amoeba *D. discoideum* and its fungivorous relative *Protostelium mycophaga.*
During *in vitro* confrontation with *D. discoideum*, fungal conidia covered with the green DHN-melanin layer were phagocytized much less effective than white conidia of an *A. fumigatus pksP*-mutant, unable to synthesize the surface-coating pigment. Similar results for conidia have been obtained with innate immune cells, supporting the idea of a universal role to avoid recognition by phagocytes. We are currently testing this hypothesis by extending our model to the fungivorous amoeba *P. mycophaga*. A natural isolate of *P. mycophaga* was found to feed on a wide range of fungi and lives in tight association with a *Pseudomonas* strain which role is currently unclear but was able to prevent invasive growth of hyphae. When exposed to *P. mycophaga*, rodlet and DHN melanin layers efficiently protected the conidia from phagocytosis. Following conidial germination the rodlet and melanin disappeared thereby exposing cell wall polysaccharides which triggered an enhanced amoeba's assault on the fungal hyphae.
These first experiments confirmed that the surface composition of the fungus plays a major role during recognition by *P. mycophaga* support the idea that escape from phagocytosis is major virulence determinants of *A. fumigatus* which origin could have been stimulated by environmental predation.

FBP07

Transcription factor PRO1 is a "Master Regulator" of multicellular development in *Sordaria macrospora*

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Question: Since the late 1950s, the homothallic ascomycete *Sordaria macrospora* is used as a model organism to study fungal sexual development. In recent years, our laboratory has investigated several sterile mutants that generate only immature (protoperithecia) but never mature fruiting bodies (perithecia). One of these mutants is pro1, which carries a gene deletion of a transcriptional regulator, that was thus named PRO1[1]. The protein is characterized by a GAL4-like $Zn(II)_2Cys_6$ binuclear cluster, a DNA binding

motif at the N-terminus, and a putative nuclear localization signal (NLS). We were interested in identifying target genes that are controlled by transcription factor PRO1.
Methods: We used chromatin immunoprecipitation coupled with high throughput sequencing (ChIP-seq) to identify the genome-wide distribution of PRO1 DNA-binding sites. This analysis was performed with a GFP-tagged version of PRO1 either under control of the constitutive *gpd*- promoter or under control of the endogenous *pro1* promoter. *In vitro* protein-DNA binding studies and gene expression analyses were used to validate the results.
Results: We identified putative PRO1 target genes that belong to different signaling pathways of *S. macrospora* and regulate sexual development. Among these target genes are for example genes encoding different MAP kinases and a scaffold protein of the cell wall integrity pathway. Furthermore we predicted a conserved PRO1 DNA-binding motif that is enriched in promoter regions of some target genes.
Conclusion: In summary, our results show that PRO1 seems to control sexual differentiation of *S. macrospora* by regulating different signal transduction pathways. PRO1 can therefore be considered as a "master regulator" of fungal multicellular development.

1 Masloff *et al.* Genetics 152: 191-199 (1999)

FBP08
Sexual development affects volatile production of *Schizophyllum commune*
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Understanding signal transduction pathways by heterotrimeric guanine-nucleotide binding protein (G-protein) signaling is critical for pheromone response in the basidomycete *Schizophyllum commune*. Regulators of G-protein signaling (RGS) are involved in the modulation of heterotrimetric G-protein signaling cascades and control mycelia growth, hydrophobicity and sexual development. G-proteins might be also an important control point for activation of fungal secondary metabolism. In *S. commune* a spontaneous occurring mutation of the RGS gene *thn1* is caused by transposon insertions. These mutants show a partial defect in mating, abnormal clamp formation and an absence of fruiting body development. The aerial mycelia formation is reduced and mutants show an easily wettable phenotype, which indicate that Thn1 regulates surface hydrophobicity. Deletion of *thn1* has a similar effect on vegetative growth, but the Δ*thn1* strain was found to mate unilateral, suggesting the regulation of pheromone signaling by *thn1*. This is reflected in the volatilome. The chemical composition of volatiles was investigated using solid phase microextraction coupled with GC-MS. The wild-type was found to produce mainly esters, whereas transposon mutants and *thn1* deletion strain emit a mixture of different sesquiterpenes, including β-bisabolol as the main component. These findings reveal that synthesis of volatile organic compounds is controlled by Thn1. Sesquiterpenes have diverse biological functions, e.g. as autoinducers, in attraction of pollinators or as defense compounds. In bioassays, volatiles of sesquiterpene producing *S. commune* strains inhibit the growth of various tester fungi. It could be shown that the sesquiterpenes α-bisabolol and bisabolene contribute to the observed growth inhibition. In the genome of *S. commune* we identified 3 genes encoding terpene synthase-like enzymes. They are supposed to be organized in gene clusters with transporters and substrate modifying enzymes. Also transcriptome analysis indicates a regulation of genes involved in the synthesis of sesquiterpenes and suggests a genetic connection between pheromone signaling and secondary metabolism.

FBP09
Analysis of the antimycotic effect of yeast killer toxin zygocin
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The increase in local and systemic fungal infections and also in antifungal drug resistance is one of the major concerns in clinical medicine. Unlike bacteria, eukaryotic yeast and fungal cells are closely related to mammalian cells and, therefore, the treatment of mycosis is often accompanied by many adverse side-effects. Furthermore, most of the common antimycotics either target fungal ergosterol synthesis, which reflects - to a big extent - mammalian cholesterol biogenesis, or interfere with yeast or fungal cell wall components. However, none of these drugs efficiently kills a broad spectrum of pathogenic yeasts and fungi. In addition the molecular mechanisms of yeast cells' adaption processes leading to antimycotic insensibility are poorly characterized. A promising candidate as potential antifungal is the killer toxin Zygocin secreted by the spoilage yeast *Zygosaccharomyces bailii*. This monomeric toxin possesses an unusual wide killing spectrum against various human as well as plant pathogenic yeasts and fungi, including *Candida albicans* and *Candida glabrata*. In this study the biochemical and structural properties of Zygocin and its effects on mammalian cell lines will be further characterized.

FBP10
Genome-wide ChIP-seq analysis of PcVelA identifies methyltransferase PcLlmA as a regulator of development and morphogenesis in *Penicillium chrysogenum*
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P. chrysogenum is the sole industrial producer of the β-lactam antibiotic penicillin, the most commonly used drug in the treatment of bacterial infections. In *P. chrysogenum*, secondary metabolism and morphogenesis were shown to be controlled via velvet, a highly conserved multi-subunit protein complex. However, until today, the output mechanisms of genome-wide velvet protein-mediated regulatory functions on a molecular level remained enigmatic.
We performed chromatin immunoprecipitation combined with next-generation sequencing (ChIP-seq) analysis of PcVelA, one of the core components of the velvet complex. Follow-on analysis included verification of selected PcVelA target genes, DNA-binding studies, as well as functional characterization of a new PcVelA downstream factor.
We present a genome-wide DNA-binding profile and DNA-binding motif of PcVelA, providing experimental evidence for PcVelA acting as a transcriptional regulator on DNA level. Besides a remarkable number of direct PcVelA target genes related to known velvet regulatory functions, e.g. in terms of conidiation, we also identified at least seven PcVelA target genes coding for putative methyltransferases. One of the corresponding proteins, PcLlmA, was submitted to further functional characterization, revealing direct interaction with PcVelA on protein level as well as an involvement in regulation of conidiosporogenesis, pellet formation, and hyphal morphology.
Our work provides deep insight into PcVelA regulatory functions on a genome-wide scale and introduces PcLlmA as a new regulator of development and morphogenesis in *P. chrysogenum*. Most importantly, it sheds light on the whole extent of PcVelA's ambiguous nature as a transcriptional regulator on the one hand and as one of the core components of the multi-subunit velvet complex on the other hand.

FBP11
UV-mutants of the homothallic zygomycete *Zygorhynchus moelleri* affected in sexual morphogenesis
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Developmental mutants are invaluable for the elucidation of morphogenetic processes. In mucorales fundamental problems like the understanding of the hormone-induced sexual development and homothallism may be solved using morphogenetic mutants. Induced by UV-mutagenesis, we have isolated a set of developmental mutants that are impaired at defined, morphologically characterized stages. Heterothallic mucoralean fungi need two complementary mating types, designed (+) and (-), in order to undergo the typical sexual developmental programme from the induction of zygophores via fusion of progametangia to the mature zygospore. The homothallic species *Zygorhynchus moelleri* expresses both mating type information in the same individual. Starting from a mycelial branching, both hyphae develop complementary mating behaviour that finally leads to zygospore differentiation. Here, we describe seven mutants, defective in various stages of zygosporogenesis, which seem particularly suitable to gain access to the molecular characteristics of zygospore development. Two of the mutants, UV139 and UV542, blocked in early stages of the sexual development accumulate a yellowish-orange pigment in a light dependent or independent way, furthermore. In UV44 and UV48 the formation of the gametangial wall is inhibited. Mutant

UV554 seems to be defective in formation of the zygospore, while in UV112 zygosporangial wall formation is repressed. Another mutant, UV127, was isolated as a mating defective mutant, originally. During mutant characterization it was striking that this mutant is a methionine auxotroph. Several experiments showed that the developmental defects of this mutant can be bridged by addition of enhanced methionine concentration and other intermediates of the methionine biosynthesis pathway. This fact reveals the importance of methionine in the developmental processes of *Z. moelleri*. This set of mutants provides an excellent experimental system for the identification of molecular and genetic mechanisms involved in the sexual morphogenesis of *Z. moelleri*.

FBP12

Complete genomes of the mucoralean fusion parasite *Parasitella parasitica* and its host *Absidia glauca* substantiate the frequency of gene transfer between host and parasite

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The mucoralean fungus *Parasitella parasitica* is a facultative biotrophic parasite of many zygomycetes. Fusion parasitism is linked with the transport of organelles from the parasite to the host and gives rise to frequent formation of genetic recombinants between host and parasite. Transfer and expression of genes residing in nuclei have been verified in the host *Absidia glauca* for several genes involved in amino acids biosynthesis. Elucidating the genomes of *P. parasitica* (plus mating type) and *A. glauca* (minus mating type) provides the chance to study a highly efficient laboratory model of horizontal gene transfer among eukaryotes.
The two genomes were obtained by Illumina and 454 sequencing (Eurofins Genomics, Ebersberg, Germany). Prediction of genes was based on AUGUSTUS v3.0.2, using *Rhizopus oryzae* as reference organism. Genes were functionally annotated using BLASTp and InterProScan.
The total genome size of *P. parasitica* amounts to 45 Mbp. The GC content of the *P. parasitica* assembly is 39.0 %. [1]4,507 protein coding genes were identified. The complete mtDNA sequence of *P. parasitica* (1) has a GC content of 30 % and a total length of 83 kbp. A special feature is the remarkably high number of 27 homing endonucleases. The total genome size of *A. glauca* amounts to 49 Mbp. The GC content of the *A. glauca* assembly is 44.5 %. 15,594 protein coding genes were identified. The complete mtDNA sequence of *A. glauca* has a GC content of 28 % and a total length of 63 kbp. The number of homing endonucleases is lower in this fungus. The mtDNA harbours only ten endonucleases. Compared with others, *P. parasitica* ranges among those fungi with larger mtDNA, whereas *A. glauca* shows a more typical chondriome size. For other zygomycetes, lengths differ between 54 kbp in *Rhizopus oryzae* and 62 kbp in *Phycomyces blakesleeanus*. The data support the idea that introns, rendered mobile by acquisition of homing nuclease genes, develop an evolutionary tendency for enrichment in an organism acting as gene donor. Apart from being spread through different populations of the same species after sexual fusion, *P. parasitica*'s mobile introns seem to have found a way for spreading over the host range of the parasite. The larger genome size and the higher number of genes in *A. glauca* indicate the acquisition of genetic material from the parasite by the host.
DNA analysis will facilitate understanding this efficient, naturally occurring horizontal gene transfer system. Further more, comparison of both genomes will support the identification of plus mating type specific enzymes needed for cooperative trisporic acid biosynthesis and new regulatory mechanisms in the complex communication system of zygomycetous fungi.

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FBP13

Differential gene expression and oxidative stress tolerance in the rock-inhabiting fungus *Knufia petricola* A95

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The ability to survive almost absolute dehydration through air-drying is a remarkable feature of a number of prokaryotes and very few eukaryotes. Microcolonial fungi (MCF), which colonise rock surfaces in hot and cold deserts as well as sub-aerial material surfaces in other climatic zones are able to survive temperature fluctuations, UV-radiation and absolute desiccation (also followed by subsequent re-hydration).
MCF are a taxonomically diverse group of melanised ascomycetes that possess a compact colonial structure. This simplified meristematic morphology harbours an impressive range of stress resistance mechanisms. It has been shown that an ancient clade of rock-inhabiting MCF is ancestral to both symbiotic (e.g., lichenized fungi) and pathogenic ascomycetes, which makes them an attractive model to study establishment of symbiotic interactions and evolution of fungal pathogenesis.
This study is focusing on responses to oxidative stress - one of the most significant environmental challenges encountered by MCF - using the rock fungus *Knufia petricola* (Chaetothyriales) strain A95. In comparison to the wildtype strain, treatment with the oxidative agent H_2O_2 (up to 30 mM) shows no dose-dependent reduction of growth rate in the ΔPKS mutant. Knock-out of the single type I polyketide synthase (PKS) in *K. petricola* leads to a complete loss of melanin. Comparative gene expression analyses of a wild type and a ΔPKS mutant are used to identify genes which are especially regulated under oxidative stress conditions to help elucidate mechanisms of cell wall maturation and oxidative stress defence strategies.

FBP14

Transcription factor SomA is required for adhesion, development and virulence of the human pathogen *Aspergillus fumigatus*

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Adherence to host cells represents a key step for pathogenesis of bacterial or fungal microorganisms. Several gene families have been shown to be responsible for adherence in different organisms, among them the *FLO* adhesins (flocculins) of *Saccharomyces cerevisiae* [1, 2]. The transcription factor Flo8/Som1 controls filamentous growth in *S. cerevisiae* and virulence in the plant pathogen *Magnaporthe oryzae* and is activated by the cAMP dependent protein kinase A (PKA) signaling pathway [3]. Consequently we investigated the heterologous transcription factor SomA of the human pathogen *Aspergillus fumigatus*. Via cross-species complementation we found out that *A. fumigatus* SomA rescued in yeast *flo8* mutant strains several phenotypes including adhesion or flocculation in haploids and pseudohyphal growth in diploids, respectively. A ß-galactosidase assay and subsequent analysis of a set of 14 reporter constructs showed that *A. fumigatus* SomA acts similarly to yeast Flo8 on the promoter of *FLO11* encoding a major adhesin in *S. cerevisiae*. In a GFP-trap experiment followed by LC/MS analysis we found that SomA physically interacts with PtaB, which is related to yeast Mfg1 which is part of an activator complex for *FLO11* expression in yeast. Loss of the *somA* gene in *A. fumigatus* resulted in a slow growth phenotype and a block in asexual development leading to aerial hyphae without further differentiation. The deletion phenotype was complemented by a conditional expression of *somA* using the inducible Tet-on system. A biofilm formation assay with the conditional *somA* expression strain indicated that SomA is required for adhesion as well. A *ptaB* deletion strain showed a similar phenotype supporting that the SomA/PtaB complex controls *A. fumigatus* adhesion. Transcriptional analysis showed that SomA regulates expression of genes for several transcription factors which control conidiation or adhesion of *A. fumigatus*. Infection assays with fertilized chicken eggs as well as with mice revealed that SomA is required for pathogenicity. Our data corroborate a complex control function of SomA acting as a central factor of the transcriptional network, which connects adhesion, spore formation and virulence in the opportunistic human pathogen *A. fumigatus*.

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FBP15
CSN5/CsnE is the last subunit incorporated into pre-CSN in *Aspergillus nidulans*
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The COP9 signalosome (CSN) is conserved from filamentous fungi to humans and functions at the interface between cellular signaling and protein half-life control. CSN shows high structural similarity to the 26S proteasomal LID that leads to ongoing comparisons between those two complexes [1]. Both consist of six PCI and two MPN domain-containing subunits. The MPN+ domain (JAMM motif) in CSN5/CsnE confers isopeptidase activity to CSN [2]. Only the fully assembled CSN complex is able to deneddylate cullin-RING ligases (CRLs) [3]. The order of complex assembly for CSN is not known so far. Consequently, we investigated the integration of CSN5/CsnE into CSN in *Aspergillus nidulans.* We showed, that *A. nidulans* strains deleted for a single *csn* subunit gene have a severe phenotype but, in contrast to higher eukaryotes, are viable. All *A. nidulans* single deletion strains show similar defects in deneddylation activity, coordination of development and secondary metabolism. *In vivo* deneddylation assays with each *csn* deletion showed no activity towards CRLs. Those strains cannot develop mature sexual fruiting bodies (cleistothecia) as they stop development at the stage of primordia. The influence of single *csn* deletions on secondary metabolism was observed through presence of orcellinic acid derivatives in hyphae and surrounding media. Applying GFP-Traps with subsequent LC/MS analysis and *in vivo* deneddylation assays, we found a stable seven-subunit pre-complex lacking the catalytically active subunit CSN5/CsnE. CSN isopeptidase activity was only reconstituted by the addition of recombinant CsnE to the pre-complex [4]. A stable seven subunit pre-CSN intermediate is present in *A. nidulans* and its deneddylase activity is controlled by CSN5/CsnE integration as final assembly step. These findings demonstrate a new level of enzymatic activity regulation based on the order of macromolecular complex assembly.

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FBP16
Inhibition of cellulose perception through manipulation of endogenous signaling pathways in *Neurospora crassa*
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Fungi are the major recyclers of lignocellulosic biomass in nature, but at the same time are constant threats to the service life of construction wood. One aim of our group is to contribute to a reduction of wood decay through a better understanding of the fungal responses to lignocellulose. The model filamentous Ascomycete *Neurospora crassa* is useful in this respect since it colonizes woody plant material and shows robust growth on many lignocellulosic substrates. Taking advantage of the functional genomics tools available for *N. crassa* we are deciphering the mechanisms of plant cell wall perception and deconstruction by defining the transcriptional regulatory network underlying the response to different nutritional conditions. For instance, studying the cellulose and mannan regulatory networks and utilization pathways in *N. crassa*, we found that both signaling pathways are interconnected and compete with each other - both at the level of inducer uptake and intracellularly. The further elucidation of this interconnection on a molecular level will aid in the identification of key targets for a rational inhibition of cellulose perception. Ultimately, this knowledge will allow us to develop novel wood protection techniques and more environmentally friendly wood preservation methods.

FBP17
Establishment of translating RNA imaging by coat protein kick-off in *Ustilago maydis*
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The transport of mRNAs ensures a defined localization of encoded proteins as well as a spatiotemporal regulation of expression. In the plant pathogen *Ustilago maydis,* different mRNAs, for example septin mRNAs, are transported in large messenger ribonucleoprotein (mRNP) particles. These contain the key RNA-binding protein Rrm4, the poly(A)-binding protein Pab1 and additional transport promoting factors[1]. mRNPs are co-transported on early endosomes along the microtubule cytoskeleton in a bidirectional manner. It is hypothesized that mRNA transport is coupled with local translation of encoded proteins due to co-localization of the mRNAs and the encoded proteins[2]. In order to verify local translation of mRNAs on endosomes, we want to establish the method Translating RNA Imaging by Coat protein Kick-off (TRICK) which is based on a RNA live imaging technique. In TRICK, the mRNA contains two distinct heterologous RNA binding sites introduced into the open reading frame (ORF) and the 3´UTR, respectively[3]. By virtue of these RNA aptamers different RNA binding proteins (RBP) fused to either green or red fluorescence proteins are recruited to the RNA. Upon the first round of translation, the RBP bound to its cognate RNA hairpins in the ORF is released from the transcript by the ribosome. Hence, a shift in the detectable fluorescence from yellow to red indicates translation of the labeled mRNA. Here we present promising results for establishing TRICK in the filamentous fungus *U. maydis*.

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FBP18
Proteomics of inositol phosphate signaling in the basidiomycete *S. commune*
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The basidiomycete *Schizophyllum commune* has been studied for tetrapolar mating since the early 1900s. Intracellular signal transduction after recognition of mating pheromones involves MAPK, cAMP and Ras signaling. In addition, inositol monophosphatase (IMPase) in inositol signaling is a second messenger which is specifically inhibited by lithium. In *S. commune*, aberrant morphology, growth inhibition, down-regulation of *imp* gene expression, and lower enzyme activity has been observed under lithium presence. Furthermore, *imp* gene expression is down-regulated in a Ras dependent manner, indicating there is a crosstalk between Ras and inositol phosphate signaling cascades. A two dimensional gel-based proteomic approach under LiCl effect was performed in both wild type and constitutively active Ras mutant strains. In the presence of LiCl, inositol phosphatase/fructose-1,6-bisphosphatase (FBPase), Ran BP1, and actin are down regulated in Ras dependent manner strain. FBPase is known to shares similar sequence motifs (Asp-Pro-Ile/Leu-Asp-Gly/Ser-Thr/Ser) with IMPase and known to be inhibited by lithium, Ran BP1 is an important precursor in G-protein Ran signaling cascade which is essential for the translocation of RNA and proteins through the nuclear pore complex. Actin plays a control role in cell polarity, tip growth and long-distance intracellular transport, its down-regulation seems to be linked to growth reduction and hyphal morphology alteration.

FBP19

Modeling the host-pathogen interactions of the human immune system and *C. albicans* using game theory and dynamic optimization

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To understand the complex host-pathogen interactions of the human immune system with *Candida albicans*, computational systems biology approaches are very useful. *C. albicans* is one of the most important human pathogenic fungi. Alterations in the host environment can render the commensal factors of the fungus into virulence attributes once the conditions favor pathogenicity. *C. albicans* then causes infections ranging from superficial mucosal diseases and thrush in immunocompetent hosts to severe, life-threatening systemic infections in immunocompromised individuals. Those systemic infections are associated with a severe morbidity, an unacceptably high mortality and high healthcare costs. With the innate immune system as the primary line of defense against systemic fungal infections the host defense relies mainly on phagocytes, especially neutrophils and macrophages. Using mathematical modeling, particularly game theory and dynamic optimization we gain insights into the interactions of *C. albicans* and macrophages. We start by setting up a differential equation model to simulate the complex dynamics of the host-pathogen interactions and perform dynamic optimization to predict optimal regimes. We then determine pure and mixed Nash equilibria to explain why macrophages sometimes release phagocytosed *Candida* cells instead of killing them, a process known as nonlytic expulsion.

FBP20

Metal homeostasis in *Candida albicans*

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Question: Metals serve as both structural and catalytic co-factors for many enzymes and are required in a multitude of biological processes. Iron and zinc availability is recognized as a central factor in bacterial and fungal infections and mammals restrict access to essential metals in order to prevent infection in a process known as 'nutritional immunity'. However, pathogens can counter this defense through the expression of high affinity transporters and metal binding proteins in order to capture limited metals from the host environment.
The aim of this project is to investigate metal homeostasis in *C. albicans* and to determine the mechanisms fungus exploit in order to cope with metal restriction.
Methods: Microarray and qRT-PCR analyses were performed in response to iron and zinc limitation. Genes which were differentially regulated were selected and the corresponding knock-out mutants were created. These mutants were phenotypically analyzed using metal limitation growth assays.
Results: The transcription factor Hap43 was shown to be essential for adaptation to iron limitation in fungal pathogens [1-3]. The ortholog of Hap43 - HapX - consists of cysteine-rich domains, which regulate the repression of iron utilization and activate iron uptake in *Aspergillus fumigatus* [4]. These domains are also present in the *C. albicans* Hap43 protein. Phenotypic analyses of mutants, lacking each domain, did not show their requirement for proper protein function under low iron levels.
Transcriptome analyses showed that many biological processes are directly affected by zinc limitation. As expected, the 'zincophore' zinc acquisition system, consisting of the secreted Pra1 and the zinc transporter Zrt1, was up-regulated during our *in vitro* zinc starvation system. Moreover, up-regulation of genes of the vacuolar zinc homeostasis systems (*ZRT3* and *ZRC1*) reflected external zinc limitation.
The *zrt2*Δ, lacking the central zinc importer Zrt2, exhibited a severe growth defect under zinc limitation. *Zrt2*Δ is largely unable to obtain free zinc, but we found that it is still able to utilize zinc citrate.
Conclusion: *C. albicans* is able to adapt to metal restriction. Hap43 is required under low iron levels in *C. albicans*, although the conserved domains, described for other fungi, are not essential for proper protein function. The zinc up-take and storage machineries are up-regulated under low zinc content and are critical for *C. albicans* growth. The ability of the zrt2Δ to grow better in the presence of zinc citrate indicates that the fungus may use zinc citrate as an alternative source of zinc.

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FBP21

Growth impairment of the plant pathogenic fungus *Verticillium* by fluorescent pseudomonads requires only phenazine in a redundant glucose medium but a gacA/gacS controlled combination of secondary metabolites in a plant pectin environment

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Verticillium dahliae und *Verticillium longisporum* are plant pathogenic fungi. Whereas *V. dahliae* can infect a variety of different host plants, *V. longisporum* infects *Brassica napus*. To antagonize fungal crop infection, the biocontrol potential of fluorescent pseudomonads is utilized. We investigated the relevance of different secondary metabolites like phenazines and gacA/gacS-regulated mycotoxins secreted by fluorescent pseudomonads in co-culture with *Verticillium*. The antagonistic potential of Pseudomonas with and without production of phenazines or gacA/gacS-controlled metabolites against *Verticillium* was quantified in a glucose- or pectin/amino acid-rich environment, by microfluidic *in situ*- and *in planta* co-cultivation. *Verticillium* transcriptome under *Pseudomonas* influence was analyzed to identify genes and related pathways, which might be involved in the fungal response towards the bacterium. Different strains used in our experiments displayed different biocontrol potential. *Pseudomonas fluorescens* derived from *Brassica*-rhizosphere exhibited a similar inhibition potential for the *Brassica napus* pathogen *V. longisporum* as for the tomato pathogen *V. dahliae*. Bacterial strategies to control fungal growth are distinct in different environments. The *Pseudomonas* mycotoxin phenazine specifically reduced *Verticillium* growth in high glucose conditions whereas in plant pectin environment the gacA/gacS-regulation-system of multiple mycotoxin pathways is essential for the biocontrol function. *Pseudomonas* influences *Verticillium* hyphal polarity through phenazines or gacA/gacS-regulated mycotoxins. *P. protegens* caused the strongest polarity defect in *V. longisporum* which coincides with 16 % up-regulated genes and a decrease of about half of the fungal transcripts. Fluorescent pseudomonads can control *Verticillium* infection *in planta* via a gacA-mediated pathway. Taken together our results suggest that fluorescent pseudomonads establish their biocontrol activity against the plant pathogen *Verticillium* depending on the nutritional habitat either via the secretion of phenazines or via a combination of gacA/gacS-controlled mycotoxins.

FBP22

Three alcohol dehydrogenase genes are responsible for ethanol degradation in *Y. lipolytica*

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The non-conventional yeast *Y. lipolytica* is able to utilize a wide range of different substrates like glucose, glycerol, ethanol, acetate, proteins and different hydrophobic molecules [1]. Although most metabolic pathways for the utilization of these substrates have been clarified by now, the responsible genes for ethanol degradation have been neither identified nor characterized. It was still unclear whether ethanol is degraded by alcohol dehydrogenases [2] or by an alternative oxidation system [3].
In order to detect the genes that are required for ethanol degradation in *Y. lipolytica*, eight alcohol dehydrogenase (*ADH*) genes and one alcohol oxidase gene (*FAO1*) have been identified and respective deletion strains were tested for their ability to metabolize ethanol. As a result of this, we found that the availability of *ADH1*, *ADH2* or *ADH3* is required for ethanol utilization in *Y. lipolytica*. A strain with deletions in all three genes is lacking the ability to utilize ethanol as sole carbon source. Adh2p is considered to be the main enzyme for ethanol degradation because it showed by far the highest enzyme activity of the three enzymes.
As *Y. lipolytica* is a non-fermenting yeast, it is neither able to grow under anaerobic conditions nor to produce ethanol [1]. To establish alcoholic fermentation, the respective key genes of *S. cerevisiae*, *ScADH1* and *ScPDC1*, were overexpressed in an *ADH* deletion strain of *Y. lipolytica*. But instead of producing ethanol, the respective strain regained the ability to use ethanol as single carbon source and was still not able to grow under anaerobic conditions.

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FBP23

New interpretation of *Circinella simplex* based on molecular phylogenetic and morphological analysis

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The genus *Circinella* (*Mucorales, Mucoromycotina*) is morphologically similar to *Mucor* differing only in the production of circinate supporting hyphae of the sporangia. The last revision of the genus originates from 1955 and resulted in the acceptance of eight species. A recent molecular phylogenetic study on biodiversity of the *Mucorales* showed that *Circinella* is a polyphyletic group, with *C. simplex* and *C. rigida* being nested in the genus *Mucor*. Therefore, the authors transferred *C. rigida* to *Mucor* (*M. durus*), but they maintained *C. simplex* due to the fact that no type material of this species was available and only a single strain had been studied. During a study on the diversity of *Mucorales* in soil of the Atlantic Rainforest in Brazil, some strains that fit into the current morphological concept of *C. simplex* where isolated. Our primary intention was to use these strains in order to perform a proper neotypification of *Circinella simplex*. Therefore we performed molecular phylogenetic analyses based on the three different DNA regions (ITS, LSU and mcm7), mating experiments, morphological studies as well as a research of the original literature. The phylogenetic analyses clearly show that the studied strains represent a monophyletic linage belonging to the genus *Mucor*. However, all strains studied had irregularly shaped sporangiospores, were isolated from soil or plant material and originated from South America, Africa and Oceania, while the strain of the original description of Van Tieghem (1875) had ellipsoid spores and was isolated from dog dung in France. In our opinion the irregular shape of the sporangiospores is a striking character that could not be overlooked by Van Tieghem. Based on the differences in the shape of the spores together with the deviating ecology and distribution we interpret the studied strains as a discrete undescribed species for which we propose the name *Mucor circinatus* sp. nov.

FBP24

***Mucor laxorrhizus* and *M. indicus* (Mucoromycotina, Mucorales) where isolated for the first time in South America**

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Mucor Fresen. comprises species characterized by producing simple or branched sporangiophores that emerge directly from the substrate bearing non-apophysate sporangia. Some species can present rhizoids and stolons not are observed. This genus includes about 80 cosmopolitan species and is the most representative and studied among Mucorales. Taxa of the *Mucor* can be isolated from a wide variety of substrates, such as soil, stored grains, fruits, vegetables rotting material and excrement of herbivores. During a survey on Mucorales in semiarid areas in the northeastern Brazil two specimens of *Mucor* were isolated from dung and soil samples collected in the cities of Areia and Sertânia, located in Paraiba and Pernambuco State of Brazil, respectively. A morphological description was performed in malt extract agar and in potato dextrose agar at 15, 20, 25, 30 and 35ºC for 15 days. Phylogenetic analyses of the sequence datasets ITS (ITS1-5.8S-ITS2) and LSU (D1 and D2 domains) of rDNA confirmed both isolates as *M. indicus* Lendn. and *M. laxorrhizus* Y. Ling. *Mucor indicus* strains presented morphological similarities to *M. circinelloides*. However, short and abundant branches observed in *M. circinelloides* are not present in *M. indicus*, and the columellae predominantly globose in the latter are distinct from the obovoid ones checked in *M. circinelloides*. Moreover, thermotolerance inherent to *M. indicus* is not found in *M. circinelloides*. *Mucor laxorrhizus* differs from the other species of *Mucor* in presenting sporangiophores repeatedly branched at right angles showing one to several septa. These taxa are being recorded for the first time in South America, expanding the knowledge of the distribution of Mucorales in semiarid regions.

FBP25

Laboratory evolution of *Candida albicans* in macrophages rewired the hyphae signaling network

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Question: Adaptation to the host is an on-going process in the evolution of pathogenic microbes. We aimed to investigate the ability of the human fungal pathogen, *Candida albicans*, to re-wire its transcriptional networks in order to escape host-imposed stresses (1). We investigated the genetic changes induced by this laboratory evolution to elucidate novel regulators of morphology and pathogenicity in *C. albicans*.
Methods: For the laboratory evolution experiment, a non-filamentous *C. albicans* mutant (*efg1*Δ/*cph1*Δ) was continuously exposed to cell-culture murine macrophages (J774A.1). After regaining its ability to filament, genetic changes in the mutant were determined by Illumina DNA and RNA sequencing. Virulence of the strains was determined by an established systemic murine infection model.
Results: Our long-term incubation of a non-filamentous *C. albicans* mutant with macrophages led to a re-appearance of its ability to filament. These filaments allowed the strain to kill and escape from the macrophages, providing it with the necessary selection advantage. Interestingly, the filamentation was not constitutive, but responded to known triggers of hyphae production in the original wild type. In a systemic mouse infection model, the virulence - nearly abolished in the original mutant - was mostly regained, accompanied by hyphae formation in the organs, which was not observed in pre-evolved mutant strain. Genomic and transcriptome sequencing, combined with transfer of evolved alleles, revealed a single nucleotide exchange to be responsible for regaining the ability to filament. This mutation in a kinase of the mediator complex was then shown to increase the propensity to form hyphae in the wild type even under non-optimal induction conditions.
Conclusion: The re-appearance of hyphae formation in our laboratory evolution experiments shows both, the importance of filamentation in fungus-macrophage interactions and the adaptability of the signalling networks involved in this process. The exchange of a single nucleotide was sufficient to rewire the information flow from external triggers to effector genes of hyphae formation, in effect lowering the threshold for inducing filamentation. This sheds light on the past evolution of the fine-tuned filamentation program of *C. albicans*, and allows us to detect thus far unknown components of the otherwise well-investigated *C. albicans* hyphae formation program.

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FBP26

Iron regulation in pathogenic fungi: Functional domain analysis of the central regulator HapX in *Aspergillus fumigatus*

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Aspergillus fumigatus is a ubiquitous saprophytic mould with clinical relevance, since this fungus is capable to cause life-threatening diseases in immunocompromised patients. During infection, sufficient iron supply is crucial for fungal growth. Iron is a vital nutrient, but can be harmful in excess by triggering the formation of cell damaging reactive oxygen

species. As a result, *A. fumigatus* has evolved fine-tuned mechanisms to maintain iron equilibrium.
Adaptation to iron limitation is mediated by the bZIP transcription factor HapX, which represses iron consuming pathways and activates iron uptake. Additionally, HapX contributes to resistance against iron excess by activation of vacuolar iron storage (Gsaller *et al.,* 2014).
For gene repression during iron starvation and activation of iron detoxification, the physical interaction of HapX with the heterotrimeric CCAAT-binding complex (CBC) is essential. Currently, it is unclear whether cooperation of HapX with the CBC is also required for gene activation under low-iron conditions. In this study, we analyzed the functional role of both HapX CBC-binding domain and DNA-binding domain in gene activation *in vitro* and *in vivo*.
Via surface plasmon resonance interaction analysis using recombinant *A. fumigatus* CBC and HapX proteins that included deletion of the CBC-binding domain or mutations within the DNA-binding domain, we demonstrate here that both the CBC-binding and DNA-binding domain of HapX are mandatory for combinatorial sequence-specific DNA-binding of the CBC and HapX *in vitro*. *In vivo*, lack of either the HapX CBC-binding domain or the HapX DNA-binding domain phenocopied HapX-deficiency. Activation of siderophore biosynthesis was repressed in both mutant strains, whereby deletion of the CBC-binding domain had a greater impact than mutation of the DNA-binding domain.
In summary, these data provide first evidence for combinatorial DNA-binding of HapX with the CBC to activate siderophore biosynthesis during iron starvation.

Gsaller *et al.* (2014) The Janus transcription factor HapX controls fungal adaptation to both iron starvation and iron excess. *EMBO J* 33:2261-76

FBP27

Dicer-dependent small RNA formation in the β-lactam producing fungus *Penicillium chrysogenum*.

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Small non-coding RNAs (sRNAs) with a size of about 22 nt can trigger post-transcriptional regulation of gene expression in a wide range of eukaryotes. In this study, we identified and characterized sRNAs in the industrial β-lactam producer *Penicillium chrysogenum* P2niaD18 [1]. Therefore, sequencing of sRNAs representing different growing conditions and developmental stages were performed. Furthermore, single and double deletion mutants of the two Dicer-like protein encoding genes *dcl2* and *dcl1* were constructed and sRNA sequencing libraries of the recipient strain and the Dicer-deficient-double mutant were constructed and sequenced. To distinguish between Dicer-dependent and -independent sRNA formation, a comparative transcriptomic analysis was performed and 661 loci were identified that produce sRNAs in a Dicer-dependent manner. 368 of these loci generate sRNAs in sense and antisense orientation from the same genomic source, mostly originating from intergenic and exonic regions [2]. Moreover, the amount of sRNAs on both strands of selfish genetic element, like Copia13-like transposable elements, was significantly reduced in the Dicer-deficient double mutant. This observation is consistent to results in other fungi and support the existence of a defense mechanism against selfish regulatory elements in *P. chrysogenum*. Furthermore, *in silico* predictions revealed 34 sRNA loci that share typical characteristics of previously discovered fungal microRNA-like RNAs (milRNAs), like the RNA stem-loop formation, a strong preference for uracil at the 5'-end, and the typical length distribution [3]. Northern blot hybridization of two milRNAs validated the existence of milRNAs and demonstrated that milRNAs are processed from a single stem-loop RNA precursor either in a complete or partial Dicer2-dependent manner.
Our study highly suggests that the diverse classes of sRNAs in *P. chrysogenum* play a so far unconsidered role in post-transcriptional gene regulation in *P. chrysogenum*. This will help us to increase our knowledge of sRNA-dependent gene regulation processes, which is an important prerequisite to develop effective strategies for improving industrial fermentations with *P. chrysogenum*.

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FBP28

Mycoparasites on *Agaricus macrosporus* observed in nature

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White button mushroom (*Agaricus bisporus*) industry is threatened by various mycoparasites such as *Lecanicillium fungicola* causing dry bubble disease and *Cladobotyrum dendroides* causing cobweb disease. The pathogens infect stages in mushroom development. They induce various symptoms on the host, such as bubbles, split stipes and spotty caps and destroy theirs host´s hyphae [1, 2]. Observations on these mycopathogens in nature are rare. Here, we observed some new mycopathogens in nature, isolated them and tested the behavior with different basidiomycetes in the laboratory.
Mushrooms of *Agaricus macrosporus* regularly appear on grounds of the University of Göttingen in August to November as drumstick-like closed young fruiting bodies underneath a *Pseudotsuga menziesii* tree. Within 3 days the mushrooms open by perforating the veil at the edges of the cap, the caps flatten in the next 2 days, to stretch out their pinkish-brownish gills for spore release to subsequently degenerate. In 2015, deformed fruiting bodies of irregular stipe and cap shape appeared. Fluffy mycelium covered the surface of the mis-shaped parts of the fruiting bodies. Microscopic analysis revealed ascomycetous types of conidiophores producing multiple 1-3 celled conidia. The pathogens were isolated from infected *Agaricus macrosporus* mushroom tissues and used to infect in the lab commercially produced *Agaricus bisporus* and *Pleurotus ostreatus* mushrooms. The pathogens were very aggressive towards *A. bisporus* and produced huge amounts of conidiospores on the mushrooms. *P. ostreatus* in contrast was highly resistant and slight infections were observed only in few cases at stipe regions of *P. ostreatus*. The pathogenicity was further tested on vegetative cultures of lab strains *Coprinopsis cinerea* and *Pleurotus ostreatus.* Only *C. cinerea* was attacked by the mycoparasites. ITS sequencing is done to determine the identity of the isolates.

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FBP29

Evaluation of cellulolytic activities for consolidated bioprocessing

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Question: Cellulose could replace fossil oil as renewable feedstock for the production of various compounds. A main economical challenge, thereby, is the hydrolysis of cellulose into soluble sugars, which relies on the effective production of cellulolytic enzymes.
Many efforts have been made to screen different organisms for their cellulolytic potential. However, the hydrolytic activity of their culture-supernatants is often evaluated on different cellulosic model substrates, which do not necessarily represent true plant biomass.
With this approach, mainly cellulase producers that produce high amounts of soluble extracellular cellulases were identified that are especially effective in hydrolyzing model substrates under optimized conditions.
In our search for effective cellulase producers for consolidated bioprocessing we therefore reevaluated cellulase producing organisms under *in situ* fermentation process conditions.
Methods: *Trichoderma reesei RUTC30* is the best publicly available cellulase producer evaluated by classical screening procedures such as filter paper assay. The cellulase activity of this fungus was compared against other fungi with different methods, including the standard filter paper assay and newly developed methods that monitor the total *in situ* cellulase activity including cell and substrate bound cellulase activities. Thereby, the influence of substrate loading on the different methods was investigated.
Results: *T. reesei* shows by far the highest filter paper activity among all tested fungi.
The partitioning between soluble and cell or substrate bound cellulases differs between organisms but also during cultivation of the same organism, suggesting a release of cell bound cellulases over time.
The activity measured by filter paper assay depends strongly on the substrate loading of the sample and cellulases can be simply removed from

the supernatant by adding substrate. In contrast when measuring the total cellulase activity, substrate loading has only minor effect.
Conclusion: Classical supernatant-based cellulase assays are highly biased towards soluble cellulases and therefore inadequate for evaluating the true cellulolytic potential of an organism. We propose instead a method to evaluate the activity based on the *in situ* sugar release rate measured in the fermentation broth under fermentation conditions, while blocking sugar uptake of the organism.

FBP30
New *trp1*$^+$ based marker system for sequential transformation of *Coprinopsis cinerea*
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Question: A mayor bottleneck in DNA-mediated transformation of fungi is the limited number of available selective marker genes, especially when sequential transformations of the same strain are necessary. In the model basidiomycete *Coprinopsis cinerea*, the *trp1*$^+$ gene is commonly used as selection marker for transformation to complement *trp1* auxotrophies. The *trp1*$^+$ encoded tryptophan synthase is a bifunctional enzyme which catalysis the final two reactions in the biosynthetic pathway of tryptophan. Each of the final two reactions is carried out by a separate functional domain. The N-terminal A-domain (encoded by the *trp1*$^+$ α-subunit) is responsible for the conversion of indole-3-glycerol-phosphate into indole, while the C-terminal B-domain (encoded by the *trp1*$^+$ ß-subunit) catalyzes the final step, the subsequent production of tryptophan from serine and indole. The *trp1.1,1.6* mutant allele used in *C. cinerea* hosts for transformation carries a mutation in each domain, which prevents the strain from completing tryptophan biosynthesis. Due to the locations of the mutations, both final catalytic reactions of tryptophan biosynthesis are independently blocked. Taking advantage of this situation, we developed from the *trp1*$^+$ vector pCc1001 a new set of vectors containing either just the A- or the B-domain encoding sequences. Using vector pCc1001 containing the complete wild type *trp1*$^+$ gene in transformation leads to simultaneous complementation of both mutations, whereas the new marker set enables the independent complementation in separate transformations.
Methods: Vectors containing either the single α- or ß-subunit under regulator control of the original *trp1*$^+$ promotor and terminator sequences were created via homologous recombination in yeast using the yeast shuttle vector pRS426. Plasmids pYSK7 harboring the *C. cinerea* laccase gene *lcc1*$^+$ and p004iGM3 containing the *egfp* gene were used in co-transformations of *C. cinerea*. Laccase expressing transformants were detected using a plate assay with 50 µM ABTS and *egfp* expression was observed using a fluorescence microscope.
Results: Using the α-subunit containing vector in co-transformation with pYSK7 in the first transformation enabled positive transformants to grow on indole supplemented selection medium. Positive transformants showing laccase expression (25 % of total colonies) were selected and used in a second co-transformation with the ß-subunit containing vector and p004iGM3. Transformants were tryptophan prototroph and 15-18 % of the transformants show also *egfp* expression.
Conclusion: In this study we successfully created a new set of *trp1*$^+$ based auxotrophic markers to sequentially transform *C. cinerea*, avoiding the need of creating strains with multiple auxotrophies. Furthermore, this is the first report of functional fungal tryptophan synthase domains which are not encoded together by a single ORF.

FBP31
Characterization of some developmental regulators in the mushroom *Coprinopsis cinerea*
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Three putative genes involved in developmental processes in *Coprinopsis cinerea FLU1-II*, *crg1* and *NWD2* are being investigated. In *Aspergillus nidulans,* the homolog of *FLU1-II* (*fluG*) and *crg1* (*flbA*) work jointly to activate the process of conidiation by initiation the expression of the gene *brlA* [1]. Inactivation of these two genes in *A. nidulans* results in fluffy colonies [2]. *A. nidulans* FluG contains an N-terminal amidohydrolase and a C-terminal glutamine synthase I (GSI)-like domain and only the GSI-like domain is essential for function as a regulator to activate the specific developmental pathway [3]. In *C. cinerea,* there are two *FLU1-II* genes coding only for the C-terminal GSI-like domain. *C. cinerea* Crg1 possesses two DEP (Dishevelled, Egl-10, and Pleckstrin) domains which function in subcellular targeting, and a C-terminal regulator of G-protein signaling (RGS) domain. *crg1* homologs have been reported before in several fungi and shown to participate in regulation of processes such as vegetative growth, asexual sporulation, mating, mycotoxin and pigment production and pathogenicity [4]. The *C. cinerea nwd2* gene encodes a signal transduction protein with NACHT-NTPases and has been found to suppress a defect in primary hyphal knot formation (*pkn1*) of *C. cinerea* mutant Proto159. We are investigating the functions of *crg1* and *FLU1-II* in oidiation, mating, vegetative growth of mono- and dikaryons, and fruiting body formation, with the aid of overexpression of *FLU1-II*, *crg1* and *NWD2* and by homologous gene targeting using a Δ*ku70 C. cinerea* monokaryon that is inactivated in the non-homologous end joining pathway and was derived from crosses of a wildtype *A5 B6* monokaryon with the self-compatible *Amut Bmut* Δ*ku70* homokaryon generated by Kamada and colleagues [6].

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FBP32
Two new species of *Mucor* (Mucoromycotina, Mucorales) isolated from the semi-arid region of Brazil
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Mucor Fresen. is characterized by the production of simple or branched sporangiophores that bears non-apophysate, globose and/or subglobose sporangia. Few species produce rhizoids and stolons are not observed. This genera has a worldwide distribution, with most species described as saprobes commonly isolated from soil, stored grains, fruits, vegetables and the excrement of herbivores. In a study of the Mucorales from semi-arid regions in Brazil, two *Mucor* specimens that differ morphologically and genetically from the other species of the genus were isolated from soil and dung samples collected in the cities of Buíque and Arcoverde, located in Pernambuco State of Brazil, and are being described as new to science. A morphological description was performed in malt extract agar and in potato dextrose agar at 15, 20, 25, 30 and 35ºC for 15 days. Phylogenetic analyses of the sequence datasets ITS (ITS1-5.8S-ITS2) and LSU (D1 and D2 domains) of rDNA of the specimens confirmed them as new to science. *Mucor caatinguensis* sp. nov. is distinguished from the other species of the genus as it simultaneously produces numerous chlamydospores in mycelia (sometimes in sporangiophores), unbranched or weakly branched sporangiophores, columellae and sporangiospores that are variable in shape and size. *Mucor merdicola* sp. nov. is morphologically similar to *M. circinelloides* f. *circinelloides.* The former is distinguished from *M. circinelloides* f. *circinelloides* as it produces globose, subglobose and applanate columellae, differing from the obovoid to ellipsoidal columellae of *M. circinelloides* f. *circinelloides.* Additionally, *M. merdicola* sp. nov. presents sporangiospores smooth-walled, mostly ellipsoid to fusiform, but also ellipsoid and subglobose, whereas *M. circinelloides* f. *circinelloides* sporangiospores are only ellipsoidal. This study contributes to the knowledge of the diversity of Mucorales in the semiarid region of Brazil.

FBP33

Raman spectroscopic characterization of *Candida albicans* evading the immune response of neutrophils

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Question: As an opportunistic pathogen, *Candida albicans* (CA) may cause severe infection associated with a high mortality rate in immunocompromised patients. This is due to late recognition and treatment of the pathogen, but it has also been shown that CA can escape immune response in whole blood infection models[1]. 10-15 % of the inoculated fungi remained extracellular, proposing an unknown mechanism that makes them resistant to phagocytosis by neutrophils and monocytes. To elucidate this question, Raman spectroscopy was used which allows an insight into the overall biological state of cells, fungi or bacteria[2]. Combined with chemometric methods, it is possible to characterize even small changes in cells.
Methods: From whole blood of healthy volunteers neutrophils were isolated by negative selection. The untouched cells were then co-incubated with CA for 90 min before the sample was split in half in order to characterize extracellular and phagocytized fungi. In the first aliquot, extracellular fungi were measured using a confocal Raman spectroscope. In the second sample, extracellular CA were fluorescently labelled using specific antibodies prior to cell lysis. Retrieved phagocytized fungi were spectroscopically characterized and compared to non-phagocytized fungi using multivariate statistical methods. For imaging of intracellular CA, neutrophils were fixed and scanned. The recorded Raman maps were analysed using the N-FINDR algorithm.
Results: Significant spectral differences could be identified between intra- and extracellular CA in the fingerprint region of the Raman spectra indicating biochemical differences between the two groups. In the mean spectra of both groups the most significant differences were detected in the bands of phenylalanine and C-C/C-N stretching vibrations representing mainly differences in protein composition. A classification model was built from the Raman data using combined principal component - (PCA) with linear discriminant analysis (LDA). Furthermore, false color Raman images visualizing structural features of phagocytized CA within neutrophils could be obtained just using the spectral information without any further labeling steps.
Conclusions: Although the extracellular fungi were often hyphae, the identified marker bands found were not the same bands which occur if yeast and hyphae are compared. It has been shown that 75 % of the extracellular fungi stay viable in a blood infection model[1] which indicates that the spectral differences we found are not just caused by the killing process, but it is probable that these marker bands point to the molecular mechanism behind the escape process.

1 Hünniger & Lehnert *et al.,* PLoS Comput Biol (2014), 10, e1003479.
2 Rösch *et al.*, J Raman Spectroscopy (2005), 36, 377.
Financial support of DFG via JSMC, BMBF via the CSCC (FKZ 01EO1502) and EU via the project 'HemoSpec' [CN 611682] are highly acknowledged.

FBP34

Cellular division of *Schizosaccharomyces pombe* studied by single molecule localization microscopy

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After DNA replication, each chromosome in the cell consists of two sister chromatids, both of which contain identical genetic information. In order for a cell division to result in two fully functional new cells, these chromatids must be segregated, divided and pulled apart with great precision, so that both daughter cells end up with identical genomes. The microtubules that move the chromatids cannot bind directly to DNA; instead they require a specialized linker. The kinetochore is a multi-protein complex which acts as the linker between DNA and microtubule and as such, regulates chromosome segregation. Since incorrect distribution of genetic material during division usually results in non-viable or heavily deficient cells, the kinetochore plays an absolutely vital role in the cell cycle (Yamagishi *et al.* 2014).
By the use of state-of-the-art quantitative single molecule localization microscopy techniques and heavily supported with localization based algorithms for data analysis and molecular techniques for the design of model organism strains it now becomes possible to target also complex processes like the cellular division of *Schizosaccharomyces pombe* (Lando *et al.* 2012).
Here, we discuss the current strategies for accessing the inner core kinetochore components as well as the freshly replicated DNA by single molecule methods and present our recent results on the individual protein counts and their replenishment in the complex following the cell cycle, their spatial distribution and their co-localization.

Lando, D., *et al.* (2012) Quantitative single-molecule microscopy reveals that CENP-A(Cnp1) deposition occurs during G2 in fission yeast. Open Biol 2(7), 120078.
Yamagishi, Y, Sakuno, T, Goto, Y, Watanabe, Y (2014) Kinetochore composition and its function: lessons from yeasts. *FEMS Microbiology Reviews* 38, 185-200.

FBP35

Studying the regulatory networks governing polysaccharide perception in the filamentous fungus *Neurospora crassa*

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Question: Due to their active role in biomass mineralization, fungi are an indispensable part of the global carbon cycling. In particular filamentous fungi are of great economic importance as sources of industrial enzymes such as for polysaccharide hydrolysis, but also as a cause for food spoilage and soft-rot decay of construction wood. However, both, the rational engineering of filamentous fungi for improved plant cell wall deconstruction, as well as the development of novel wood-protection mechanisms, is hampered by incomplete knowledge of the regulatory and metabolic networks under various nutritional conditions.
Methods: Holistic, systems-level analyses are the best way to achieve a sufficient knowledge base and predictive power for targeted strain bioengineering. The filamentous ascomycete *Neurospora crassa* is ideally suited for this kind of analysis since it is not only a well-known model system for eukaryotic cell biology and genetics, but also shows robust growth on lignocellulosic material. Leveraging the genomic resources for *N. crassa*, we initiated a large-scale study elucidating the transcriptional responses of *N. crassa* to nutritional variations.
Results & Conclusions: Our comparative analysis of fungal carbon perception has already led to the identification of several novel factors taking part in plant cell wall degradation processes as well as the construction of metabolic maps and regulatory networks going well beyond what was possible so far. Moreover, based on these data, gene annotations could be validated on a genome-wide scale.

FBP36

Identification of pathogenicity factors in basal fungi by comparative genomics and pathogenomics approaches

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Mucormycoses are life-threatening infections caused by members of the fungal order Mucorales. Although these infections are uncommon fungal infections they have been increasingly recognized in patients during the last decades. More than twenty different species are associated with mucoralean infections and *Lichtheimia* species represent the second-most common cause of mucormycosis in Europe. To date, only three of the six

described *Lichtheimia* species are known to cause infections in humans. Despite their clinical importance little is known about the pathogenicity and the molecular virulence determinants of these fungi. Moreover, only few genomes of mucoralean fungi are available and information about genome structure and evolution in basal fungi is still lacking.
In order to get insights into genome structure and evolution of mucoralean pathogens the genomes of several strains of *Lichtheimia* species were sequenced and analyzed. Strains were selected based on phylogeny, physiology and virulence potential. Comparative genomics of different clinical and non-clinical *Lichtheimia* species revealed a high similarity in gene content and genome organization between the species. In addition, putative virulence factors were conserved also in non-clinical species. Additional transcriptomic analyses under selected stress conditions gave first insights into the stress-response of these basal fungal pathogens.
The genome sequences of additional strains of the clinical species with reduced virulence and distinct physiological defects were analyzed using comparative genomics in order to identify factors which explain differences in physiology and virulence of the species.
This is the first study comparing various strains from closely-related species of mucoralean fungi and gives first insights into virulence factors of basal fungal pathogens.

FBP37 – withdrawn

FBP38
Humanized yeast – a tool to study Parkinson's disease
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Parkinson's disease is a neurodegenerative movement disorder, associated with the progressive loss of dopamine-producing neurons in the *substantia nigra*. The pathological hallmark of the disease is the accumulation of intracytoplasmic inclusions known as Lewy bodies (LBs) that consist mainly of the presynaptic protein α-synuclein. The budding yeast *Saccharomyces cerevisiae* represents an established model system to study the molecular mechanisms associated with neurodegenerative disorders. Expression of α-synuclein in yeast leads to inclusion formation similar to neurons and significant growth reduction, resembling the pathology of the disease. This eukaryote represents a valuable model system for studying cellular pathways that are involved in the degradation of protein aggregates, associated with neurodegenerative diseases. Various modifications change α-synuclein posttranslationally and alter its inclusion formation, cytotoxicity and the distribution to different clearance pathways. Several of these modification sites are conserved from yeast to human. Phosphorylation is one of the major modifications of α-synuclein in LBs, whereas sumoylation has recently been described. The interplay between α-synuclein phosphorylation and sumoylation is poorly understood. Here, we examined the interplay between these modifications as well as their impact on cell growth and inclusion formation in yeast. We found that α-synuclein is sumoylated *in vivo* at the same sites in yeast as in human cells. Impaired sumoylation resulted in reduced yeast growth and increased number of cells with inclusions suggesting that this modification plays a protective role. Inhibition of sumoylation prevented autophagy-mediated aggregate clearance. Phosphorylation of α-synuclein at serine-129 by expression of human G protein-coupled receptor kinase 5 (GRK5) suppressed the defect, associated with impaired sumoylation and rescued the autophagic aggregate clearance. These findings suggest a complex interplay between sumoylation and phosphorylation of α-synuclein, which may open new opportunities for the development of therapeutic strategies for Parkinson's disease.

FBP39
The biotechnological potential of *Ustilago maydis*: from basic research to applied sciences
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The corn smut fungus *Ustilago maydis* is currently gaining increasing momentum as a eukaryotic model organism. Diverse areas of basic research ranging from homologous recombination, cell biology, plant-pathogen interaction, to RNA biology are studied extensively in this fungus. Furthermore, *U. maydis* and related smuts are also more and more used in applied research. Their biotechnological potential ranges from the production of valuable secondary metabolites and organic acids, promising enzymes with novel properties, biomass degradation by intrinsic hydrolytic enzymes, to the production of proteins via a novel unconventional secretion machinery evading N-glycosylation.
Unconventional secretion has first been observed for the chitinase Cts1 during basic research. This enzyme does not harbor a classical N-terminal secretion signal and thus, circumvents the Endoplasmic Reticulum passed by conventionally secreted eukaryotic proteins. Interestingly, Cts1 can deal as a carrier to export heterologous proteins of interest, thereby avoiding N-glycosylation. Hence, Cts1-mediated unconventional secretion is currently exploited to establish a novel protein production platform. Pharmaceutical proteins are one interesting target of this secretory pathway, because inappropriate N-glycosylation may lead to allergic reactions in humans. The system is currently optimized on different levels including extracellular protease activity, culturing conditions and downstream processing to achieve competitive protein yields in the future.

FBP40
C-terminal tyrosine modifications play a major role in α-synuclein cytotoxicity in yeast model of Parkinson´s disease
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Parkinson´s disease (PD) represents a neurodegenerative disorder affecting six million people worldwide. PD proceeds with selective loss of dopamine-producing neurons in the *substantia nigra* leading to movement disorders. Phenotypic hallmark of PD are α-synuclein (αSyn) aggregates that are part of proteinaceous inclusions called Lewy bodies (LB). One established system for modelling human diseases is *Saccharomyces cerevisiae.* Overexpression of αSyn in *Saccharomyces cerevisiae* results in growth impairment and formation of cytoplasmic inclusions resembling the aggregates observed during pathogenesis of the disease. We use yeast as model tool to elucidate the effect of post-translational modifications of tyrosines on αSyn cytotoxicity. The three C-terminal tyrosine residues 125, 133, 136 (Y125, Y133, Y136) are proposed as putative nitration and phosphorylation targets. Phosphorylation of αSyn at serine 129 (S129) is prominent in PD and influences autophagic clearance of αSyn inclusions. Our study shows that αSyn is nitrated *in vivo* and forms stable αSyn dimers originating from covalent crosslinking of two tyrosine residues. Analysis of the tyrosine residues involved in crosslinking revealed that rather αSyn C-terminus than N-terminus is modified by nitration and di-tyrosine formation. The A30P variant that is not toxic in yeast forms more dimers than the wild-type αSyn. In contrast, the nitration level of wild-type αSyn is higher compared to A30P implicating that tyrosine residues but not di-tyrosine dimers contribute to αSyn cytotoxicity. Notably, modification of Y133 is required for protective phosphorylation of αSyn at S129 and for S129 independent proteasome clearance. Increased cellular nitrative stress by deletion of the yeast flavohemoglobin gene *YHB1* resulted in increased cytotoxicity of A30P and A30P-induced mitochondrial fragmentation. Overexpression of the human homolog of *YHB1* neuroglobin protected against αSyn aggregation in mammalian cells. Our findings reveal that post-translational modification of Y133 plays a major role in αSyn aggregate clearance by promoting phosphorylation of S129. Our data suggest that increased nitration level of C-terminal tyrosines is involved in pathogenicity which can be partially detoxified by αSyn dimerization. This reveals a complex interplay between S129 phosphorylation and C-terminal tyrosine modifications of αSyn.

FBP41
Studying the regulatory networks governing polysaccharide perception in the filamentous fungus *Neurospora crassa*
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Question: Due to their active role in biomass mineralization, fungi are an indispensable part of the global carbon cycling. In particular filamentous fungi are of great economic importance as sources of industrial enzymes such as for polysaccharide hydrolysis, but also as a cause for food spoilage and soft-rot decay of construction wood. However, both, the rational engineering of filamentous fungi for improved plant cell wall deconstruction, as well as the development of novel wood-protection

mechanisms, is hampered by incomplete knowledge of the regulatory and metabolic networks under various nutritional conditions.
Methods: Holistic, systems-level analyses are the best way to achieve a sufficient knowledge base and predictive power for targeted strain bioengineering. The filamentous ascomycete *Neurospora crassa* is ideally suited for this kind of analysis since it is not only a well-known model system for eukaryotic cell biology and genetics, but also shows robust growth on lignocellulosic material. Leveraging the genomic resources for *N. crassa*, we initiated a large-scale study elucidating the transcriptional responses of *N. crassa* to nutritional variations.
Results & Conclusions: Our comparative analysis of fungal carbon perception has already led to the identification of several novel factors taking part in plant cell wall degradation processes as well as the construction of metabolic maps and regulatory networks going well beyond what was possible so far. Moreover, based on these data, gene annotations could be validated on a genome-wide scale.

FBP42 – withdrawn

FBP43
In-depth characterization of the *Aspergillus fumigatus* mating-type system
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Sexual reproduction of the human fungal pathogen *Aspergillus fumigatus* was assumed to be absent or cryptic until fertile crosses among geographically restricted environmental isolates were described in 2008 by O'Gorman *et al.* The existence of cryptic sexuality in this species had been proposed before, based on genomic and genetic analyses revealing the presence of mating-type idiomorphs (*MAT1-1* and *MAT1-2*) and of several putative genes orthologous to recognized determinants of pheromone signalling, mating, karyogamy, meiosis, or fruiting body formation in the fertile species *Aspergillus nidulans*. Furthermore, the products of *A. fumigatus MAT1-1* and *MAT1-2* genes were shown to be functional in *A. nidulans*. We provide evidence for mating, fruiting body development, and ascosporogenesis accompanied by genetic recombination between unrelated clinical isolates of *A. fumigatus*, which reveals the generality and reproducibility of this long-time undisclosed phase in the lifecycle of this heterothallic fungus. Furthermore, we demonstrate that successful mating requires the presence of both mating-type idiomorphs *MAT1-1* and *MAT1-2*, as does expression of genes encoding factors presumably involved in this process. Comprehensive transcriptional profiling studies reveal the depth of the *MAT1*-driven transcriptomes. Functional categorization of genes that are significantly up- or down-regulated in these transcriptomes led us to further investigation of candidate genes and gene clusters that are under control of the bipolar mating-type system, especially those involved in secondary metabolism, which are ideal for validation on the product level. Secondary metabolite profiling of recombinant strains that are deregulated or mis-regulated in sexual development confirms the association. Furthermore, functional analysis of a novel presumed mating-type gene *MAT1-2-4* associated with the *MAT1-2* idiomorph indicates its necessity for fruiting body formation, assigning the corresponding gene product a functional role in the mating process. With the help of yeast two-hybrid screening we were able to identify an interactor with the product of *MAT1-2-4*, and further investigation of this genuine interaction is ongoing.

FBP44
Comparative transcriptomics of the two β-lactam producers *Penicillium chrysogenum* and *Acremonium chrysogenum* elucidate the regulatory effects of conventional strain improvement programs.
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The filamentous fungi *Penicillium chrysogenum* and *Acremonium chrysogenum* are the only industrial used producers of β-lactam antibiotics. While *P. chrysogenum* is used for the production of penicillins, *A. chrysogenum* is a cephalosporin producer. Beside the ability to generate anti-infectives, both species do not have much in common and are taxonomically only distantly related within the Ascomycota. To achieve higher amounts of β-lactam antibiotics in each fermentation run of *P. chrysogenum* and *A. chrysogenum*, the wild-type isolates *P. chrysogenum* NRRL1951 and *A. chrysogenum* ATCC 11550 were randomly mutated during several rounds of conventional mutagenesis. Since the beginning of strain improvement programs, various strains and lineages were generated from the wild-type strains that produce increased amounts of the desired antibiotic. Although, previously research had discovered the complete β-lactam biosynthesis pathway and the enlarged knowledge about regulatory processes involved in the formation of penicillin and cephalosporin C, there is still a lack of knowledge how the increased β-lactam production was achieved during the conventional strain improvement programs.
To elucidate regulatory effects that occurred during strain improvement, we performed RNAseq analysis of the two wild-type strains, an industrial production strain of both species (*P. chrysogenum* P2niaD18 and *A. chrysogenum* A3/2), and a deletion mutant of the regulator of secondary metabolism ΔPcvelA in *P. chrysogenum* and, respectively, ΔAcveA in *A. chrysogenum* [1,2]. Comparative transcriptomics of the dataset including intra- and interspecific analyses gave an insight into the regulatory changes that were caused through conventional strain improvement. Furthermore, comparison of the effects found in the industrial producer strains and the deletion strains ΔPcvelA and ΔAcveA revealed a highly interesting intra- and interspecific correlation of the regulatory control of secondary metabolite gene clusters within these strains. Our results will contribute to develop novel strategies for strain improvements of the β-lactam antibiotic producers *P. chrysogenum* and *A. chrysogenum*.

[1] Kopke K, *et al.* (2013) Eukaryotic Cell 12:299-310.
[2] Dreyer J, *et al.* (2007) Appl. Environ. Microbiol. 73:3412-3422.

FBP45
Inhibition of cellulose perception through manipulation of endogenous signaling pathways in *Neurospora crassa*
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Fungi are the major recyclers of lignocellulosic biomass in nature, but at the same time are constant threats to the service life of construction wood. One aim of our group is to contribute to a reduction of wood decay through a better understanding of the fungal responses to lignocellulose. The model filamentous Ascomycete *Neurospora crassa* is useful in this respect since it colonizes woody plant material and shows robust growth on many lignocellulosic substrates. Taking advantage of the functional genomics tools available for *N. crassa* we are deciphering the mechanisms of plant cell wall perception and deconstruction by defining the transcriptional regulatory network underlying the response to different nutritional conditions. For instance, studying the cellulose and mannan regulatory networks and utilization pathways in *N. crassa*, we found that both signaling pathways are interconnected and compete with each other - both at the level of inducer uptake and intracellularly. The further elucidation of this interconnection on a molecular level will aid in the identification of key targets for a rational inhibition of cellulose perception. Ultimately, this knowledge will allow us to develop novel wood protection techniques and more environmentally friendly wood preservation methods.

FBP46
A transcriptome meta-analysis proposes a novel biological role of the antifungal protein AnAFP in *Aspergillus niger*
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Although *Aspergillus niger* is used since decades in industrial biotechnology for the production of organic acids and proteins, it largely depicts a black box and we are far from understanding how most of the internal cellular processes work on the molecular level. However, the availability of its genome sequence and hundreds of microarray data for this fungus make it now feasible to shed light into this black box. Our interest in AnAFP is due to the fact that the growth-inhibitory effect of the protein and its homologs from other filamentous *Ascomycetes* seems to be restricted to fungi. No detrimental effects have been observed against bacterial, plant and mammalian systems, making this group of proteins interesting for application in red, green and yellow biotechnology.
We have recently established a database that stores 377 high-throughput microarray data for *A. niger*. The database includes 158 different

cultivation conditions related to carbon source and carbon availability, nitrogen metabolism, conditions related to stress, temporal and spatial stages during its asexual life cycle and many more. We have performed a transcriptome-meta analysis of this database, which enabled us to zoom into the gene expression networks and physiological processes under which AnAFP is expressed.
The corresponding transcriptome meta-analysis of *A. niger* suggests a novel prominent biological role of AnAFP. Remarkably, *anafp* gene expression is apparently regulated in a non-defense manner. Instead, upon carbon starvation, *anafp* is strongly upregulated and its expression profile resembles that of genes involved in nutrient mobilization and with a predicted role for autophagy. In addition, *anafp* expression strongly increases when the mycelium becomes committed to asexual development. Its expression is several fold upregulated in both a *ΔflbA* and *ΔbrlA* background. As the *flbA* mutant depicts an autolytic phenotype, we propose AnAFP has a function during the asexual life cycle of *A. niger* and is somehow linked to autophagic processes during normal development.
Our in-house transcriptomic database depicts a valuable tool which enabled us to zoom into the gene expression networks and physiological processes of *A. niger*. Further analysis of this database will definitely help to increase our knowledge of the complex regulation of *A. niger's* gene network.

IBP01
Molecular characterization of *dichelobacter nodosus* serogroup H from footrot of sheep of Andhra Pradesh, India.
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Question: Footrot is a contagious disease causing lameness in sheep and goats with interdigital necrotic or suppurative lesions. The disease is caused by primary pathogen *Dichelobacter nodosus* an anaerobic, Gram-negative, rod shaped bacterium, and is often heavily fimbriated. The fimbriae are highly immunogenic for sheep and are the major host-protective immunogen. The current Australian classification system classified *D. nodosus* in to 10 serogroups (A-I and M) based on K-type agglutination. Despite its worldwide presence, the disease has significant economic impact in those sheep farming countries that have temperate climates such as Australia and New Zealand. In India the disease has become enzootic in throughout the state of Jammu and Kashmir with temperate climate for the last 18 years. Tropical climate of Andhra Pradesh which is considered as unusual niche for the survival of the causative organism *D. nodosus* still recorded incidence of ovine footrot. Prevalence of serogroups A, B, C, E, F and I were reported so far in Andhra Pradesh. Present report deals with the identification of serogroup H for the first time in the region and its characterization.
Method: A total of 331 foot swabs collected from inter digital spaces of sheep with clear cut footrot lesions were screened for 16 rRNA of *D. Nodosus* by PCR. Out of the 331 samples, 129 (38.97 %) were found to be positive. All the positive samples were subjected to multiplex PCR for targeting *fimA* gene for identification of serogroup of *D.nodosus*.
Results: Srogroup B was found to be predominant (41.86 %) followed by serogroup I (25.58 %), A (13.95 %), C (10.85 %), E(3.10 %) and H(4.65 %). The serogroup H was identified for the first time from the Indian subcontinent. The sequence and phylogenetic analysis of the present sequence was done with the available serogroup H sequences of GenBank revealed close association with the serotype H1.
Conclusions: Multiple serogrops of *D. nodosus* are responsible for footrot in sheep in India.
D. nodosus is exclusively present in foot lesoins of sheep of Andhra Pradesh with tropical climate which is the unusual niche for the survival of the organism.
The vaccnie need to incorprate all the avilable strains of *D. nodosus* for effective control of footrot.

IBP02
Structure and specificity of *Helicobacter pylori* aminopeptidase
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Question: The standard *H. pylori* eradication therapy has lost its efficacy, with an eradication rate dropping to as low as 60 % in Western Europe. Aiming to develop an alternative therapy, we have performed initial characterisation of *H. pylori* M17 aminopeptidase (HpM17AP). To address the structural basis of catalysis and inhibition of this enzyme, we have established its specificity towards an N-terminal amino acid of the substrate and determined the crystal structures of HpM17AP and its complex with the inhibitor bestatin.
Methods: We have analysed the diffraction data sets for HpM17AP and its bestatin complex. HpM17AP activity was screened against a fluorogenic substrate library containing both natural and unnatural amino acids.
Results: The position of phenylalanine moiety of the inhibitor with respect to the active-site residues and with respect to other M17 aminopeptidases suggested that it represents the S1 subsite. In contrast to most characterized M17 aminopeptidases, HpM17AP displays preference to L-Arg over L-Leu.
Conclusions: A close similarity between the structures of HpM17AP and its homologues from other bacteria has allowed the structural features that determine differences in their substrate specificity to be analysed. The results have interesting implications for metabolic utilisaton of arginine for the production of primary amines, and cysteine scavenging through degradation of mucosal glutathione.

IBP03
Influence of *Enterococcus faecalis* mobile genetic elements on proteome composition and virulence
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Question: *Enterococcus faecalis* is a commensal Gram-positive bacterium inhabiting the gastrointestinal tract of humans and animals. However, *E. faecalis* is also a major nosocomial pathogen causing a number of diseases, like endocarditis, sepsis or urinary tract infections. The pathogenicity of *E. faecalis* derives from an arsenal of different virulence factors and the treatment of *E. faecalis* infections becomes increasingly difficult due to a variety of antibiotic resistances. *E. faecalis* is highly recombinant and can modify its pathogenic properties thereby representing the possibility of acquisition and transfer of virulence-related genes from and to other pathogens. In the current study we analyzed the proteome composition and phenotypic changes that accompany the transfer of a pathogenicity island (PAI) between two different *E. faecalis* strains.
Methods: To assess the influence of the pathogenicity island the common laboratory strain *E. faecalis* OG1RF and a transconjugant carrying the PAI of strain UW3114 were analyzed with regard to the expression of different virulence factors. Proteins isolated from planktonic and biofilm-grown cells were subjected to a comprehensive, quantitative mass spectrometry-based analysis for a global view on protein expression using GeLC-MS/MS and LC-IMS^E approaches. Phenotypical assays were carried out to complement the results from our proteome analysis. Finally, the virulence of OG1RF and the PAI-carrying transconjugant was tested in a *Galleria mellonella* infection model.
Results: In total, we could analyze the expression of more than 1,100 proteins in the cytosol as well as in the extracellular fraction. Expression of PAI-encoded genes in the transconjugant could be verified by proteomics approaches. The Gls24-like protein, which is probably important for virulence of *E. faecalis*, is one of the most abundant proteins in the transconjugant. Cytolytic acitivity and biofilm formation are enhanced as well. In addition, the *G. mellonella* infection model revealed a higher virulence of *E. faecalis* OG1RF after acquisition of the PAI.
Conclusion: The transfer of the pathogenicity island from the *E. faecalis* isolate UW3114 led to phenotypic changes and enhanced virulence in the laboratory strain OG1RF supporting the idea that mobile genetic elements are important factors in adaptation of *E. faecalis* to clinical environments.

IBP04

Genome sequencing of two novel EHEC/EAEC hybrid strains isolated from human infections

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Introduction: The so far highest number of life-threatening hemolytic uremic syndrome was associated with a food-borne outbreak in 2011 in Germany which was caused by an enterohemorrhagic *Escherichia coli* (EHEC) of the rare serotype O104:H4. Most importantly, the outbreak strain harbored genes characteristic of both EHEC and enteroaggregative *E. coli* (EAEC). To evaluate the importance of EHEC/EAEC hybrid strains in human disease, we analysed the EHEC strain collection of the German National Reference Centre for Salmonella and other Enteric Bacterial Pathogens (NRC). Additionally to molecular methods, we here analysed the strains of interest by means of whole genome sequencing (WGS).
Methods: The search for EHEC/EAEC strains and their subsequent analysis included the following methods: PCR or Southern blotting for the detection of EHEC (such as *stx* and *eaeA*) and EAEC marker genes (such as *aatA*) as well as for aggregative adherence fimbriae genes (AAF), characterization of adherence pattern and cytotoxicity, analysis of antibiotic resistance profile, macrorestriction analysis / pulsed-field gel electrophoresis, multi locus sequence typing, *stx* sequence analysis and PacBio and Illumina MiSeq WGS.
Results: After exclusion of O104:H4 EHEC/EAEC strains, out of about 2400 EHEC strains sent to NRC between 2008 and 2012, two strains exhibited both EHEC and EAEC marker genes, specifically were *stx2* and *aatA* positive. One of the novel EHEC/EAEC, isolated from a patient with bloody diarrhoea in 2010, harboured *stx2*a, was serotyped as O59:H$^-$, belonged to MLST ST1136, and exhibited genes for type IV AAF. The second strain was isolated from a patient with diarrhea in 2012, harboured *stx2*b, was typed as Orough:H$^-$, and belonged to MLST ST26. No AAF genes corresponding to fimbrial types I to V were detected in this strain. WGS revealed a genome size of ~ 5.2 Mb for both strains and 3 to 4 plasmids of 7 to 124 kb carrying important virulence and adherence genes. The WGS data enabled us to confirm and newly detect virulence markers, to perform genoserotyping and to compare the core genome of the stains with stains of the same MLST sequence type, the outbreak strain EHEC/EAEC O104:H4, EHEC O157:H7 EDL933 and EAEC O44:H18 042. For the strain from 2012 we found genes coding for new potential adherence structures on the pAA-plasmid. By screening other AAF I-V negative EAEC we found further stains carrying similar gene structures, indicating that these new potential adherence genes are not that uncommon.
Conclusion: So called mixed *E. coli* pathovars or hybrid strains have been seldom described and show a high virulence potential. We found two novel strains isolated from human disease cases in Germany in 2010 and 2012 belong to MLST sequence types and/or serotypes seldom associated with human disease. In addition to *stx2* these strains harbour EAEC characteristics which further qualify them to cause severe disease.

IBP05

Biological growth and toxin release in silicone breast implants as a cause for adverse events?

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Silicone gel mammary prostheses are composed of an outer shell of a highly cross-linked silicone elastomer and an inner partly cross-linked silicone gel serving as a filler material. The industrial production should follow GMP principals to avoid chemical and biological contaminations. We here report on investigations in explanted silicone gel breast implants indicating the possibility of biological growth like fungi or bacteria inside the gel. We identified a series of approximately 10 % of the total explanted silicone mammary prostheses with intact shells which showed small visible inclusions like snow-flakes in the inside gel resembling organic growth. In turn this would give rise to the assumption of toxin production and release by the micro-organisms which are protected by the silicone shell and gel against the immune system. The prerequisites for biological growth implicate failures of the manufacturing causing biological contaminations of the inside material, failure of sterilization as a final step of production, influx of nutrients and water once being implanted, optional oxygen supply, and convenient growth conditions inside the implant or body respectively. We explain how these conditions may accidently coincide. Toxin release may be a new explanation for the development of complications associated with silicone gel implants including loss of breast tissue by apoptosis and the development of anaplastic large cell lymphoma [ALCL]. We demonstrate in this presentation a series of photographs and micrographs to visualise the phenomenon. The clinical impact of our findings may be underlined by the fact that ALCL as a site specific lymphoma is reported in a still rising number in the literature and seems to be associated with silicone breast implants. Up to now the understanding of this development is poor.

IBP06

Effect of antibiotics treatment on the microbiome of bacterial vaginosis patients

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Bacterial vaginosis (BV) is a disease of the female genital tract which is characterized by a change in bacterial diversity from a uniform flora dominated by Lactobacilli to a flora that is highly diverse. BV has a high rate of recurrence which might be caused by biofilms that survive antibiotic therapy. Therefore a clinical study was conducted to test antibiofilm treatments after standard therapy with the antibiotic metronidazole.
16S rDNA amplicon sequencing showed that all women diagnosed with BV and a biofilm on clue cells had a highly diverse microbial flora with *Atopobium vaginae* being the most abundant species followed by *L. iners, Sneathia sp., Prevotella sp.* and *Gardnerella vaginalis* among many others. All of these taxa were present in every woman with BV. In the majority of cases (n=37, 84 %), metronidazole treatment changed the microbial profile drastically to a healthy flora dominated by Lactobacilli, concurrent with lack of clinical symptoms. Recurrence to a microbial community with high diversity similar to BV was observed in 11 (30 %) of those 37 cured women during the next 4 months. Interestingly, Lactobacilli in women diagnosed with BV were dominated by *L. iners* (69 %) even after successful treatment, whereas healthy women from a control cohort were colonized mainly by *L. crispatus* and *L. gasseri* (80 %) and only to a smaller extent by *L. iners* (16 %). This confirms the ambiguous role of *L. iners* in vaginal health. Most importantly and unexpectedly, these results show the effectiveness of metronidazole in treating acute BV. Further work will focus on the factors leading to recurrence of BV and will investigate why some women did not respond to metronidazole treatment using RNA sequencing.

IBP07

Seroprevalence of *Helicobacter pylor*i among asymptomatic students in Jazan university, Saudi Arabia

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Helicobacter pylori has been associated with peptic ulcer and gastric carcinoma. The present study aimed to find the seroprevalence of *Helicobacter pylori* infection in some male students of Jazan University, Kingdom of Saudi Arabia.
A total of 41 students were enrolled in this study (n=41). Informed consent was obtained from the students. 2 ml of blood was collected intravenously in a vaccutainer evacuated blood collection tube with no additives. The blood was allowed to clot at room temperature. The serum was seperated and tested for *Helicobacter pylori* antibodies immediately using I-tell [tm] Rapid diagnostic test for the invitro diagnosis of *Helicobacter pylori* antibodies.
A total of 23 (56.10 %) students tested positive for *Helicobacter pylori* antibodies.
The seroprevalence of *Helicobacter pylori* was found to be high in some male University students and is a cause of concern regarding their health. The students were counseled and were encouraged to undergo confirmatory test and get medical intervention. Further large scale studies need to be done to plan action against this disease causing organism so as to improve the health of students.

IBP08

Magnitude of gene mutations conferring drug resistant in *Mycobacterium tuberculosis* strains among pulmonary tuberculosis cases in Southwest Ethiopia

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Background: The magnitude of mutations in rifampicin (RIF) and isoniazid (INH) resistant *M. tuberculosis* isolates vary considerably according to the geographic locations. However, information regarding specific mutational patterns in Ethiopia remains limited.
Methods: In this cross-sectional study, sputum smear-/culture-positive TB cases were enrolled from October 2013 to September 2014. Mutations associated with RIF and INH resistance were studied by GenoType MTBDRplus assay in 112 *M. tuberculosis* isolates.
Results: Mutations conferring resistance to INH, RIF and MDR were detected in 36.6 % (41/112), 30.4 % (34/112) and 27.7 % (31/112) of *M. tuberculosis* isolates respectively. Among 34 RIF resistant isolates, 82.4 % (28/34) had *rpoB* gene mutations at S531L, 2.9 % (1/34) at H526D and 14.7 % (5/34) had mutations only at wild type probes. Of 41 INH resistant strains, 87.8 % (36/41) had mutations in the *katG* gene at Ser315Thr1 and 9.8 % (4/41) had mutations in the *inhA* gene at C15T. Mutations in *inhA* promoter region were strongly associated with INH monoresistance.
Conclusion: High rate of drug resistance was commonly observed among failure cases. The most frequent gene mutations associated with the resistance to INH and RIF were observed in the codon 315 of the *katG* gene and codon 531 of the *rpoB* gene respectively.

IBP09

Localization and function of the E-cadherin complex in gastric epithelial cells after infection with *Helicobacter pylori*

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Question: *Helicobacter pylori* (*H. pylori*) is a class I carcinogen that can induce chronic gastritis, gastric ulcerations, MALT (mucosa-associated lymphoid tissue) lymphoma and gastric cancer. Among several virulence factors, *H. pylori* expresses the serine protease and chaperone high-temperature requirement A (HtrA) as a pathogenicity-supporting factor. HtrA cleaves the host cell adhesion protein and tumor suppressor E-cadherin on gastric epithelial host cells to disrupt intercellular adhesions. However, the intracellular domain of E-cadherin forms a complex with α-catenin, ß-catenin and p120, which are important signaling molecules associated with cell migration and cancer development. Therefore, the functional consequences of HtrA-mediated ectodomain shedding are investigated in this project.
Methods: The subcellular localization of the intracellular E-cadherin domain was investigated by immunofluorescence analyses. Distribution of β-catenin, α-catenin and p120 was further analyzed in E-cadherin-negative gastric epithelial cells, which re-expressed either E-cadherin wild type or an E-cadherin mutant that lacked the HtrA-targeted cleavage site. The disruption of the E-cadherin complex was analyzed in co-immunoprecipitation experiments.
Results: Infections of E-cadherin-positive epithelial cells with *H. pylori* resulted in a drastic loss of cell-cell adhesion and led to massive cell elongation. The cleavage of the adhesion protein and tumor suppressor E-cadherin by HtrA resulted in the degradation of the cadherin-catenin complex. Detecting the intracellular E-cadherin domain in *H. pylori*-infected cells, E-cadherin localized in cellular speckles. A similar localization was observed for β-catenin, α-catenin and p120. Thereby translocation of p120 into the nucleus could be observed. Furthermore α-catenin was found to co-localize with *H. pylori.*
Conclusion: These data indicate that cleavage of E-cadherin by the pathogenicity promoting factor HtrA of *H. pylori* resulted in the disintegration of the cadherin-catenin complex in infected epithelial cells. Further studies will reveal the significance of complex destabilization in *H. pylori*-associated disease.

IBP10

Comparative analysis of the immune response against *Aspergillus fumigatus* conidia in immunosuppressed patients versus healthy blood donors

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Question: The opportunistic human pathogen *Aspergillus fumigatus* is the most important cause of fatal fungal infections in immunocompromised individuals, such as chemotherapy patients or solid organ transplant recipients. Generally, infection is established by fungal conidia which are ubiquitiously present in the air. To date, a reliable diagnosis is difficult and treatment options are limited resulting in high mortality rates of infected patients. Phagocytes, i.e. monocytes and neutrophil granulocytes, are in the first line of defense against invading fungi. We aim at the elucidation of potentially different responses of human phagocytes towards *A. fumigatus* from immunosuppressed patients versus healthy controls to promote a better understanding of the mechanism of invasive fungal infection, which is the key towards improved prophylaxis and therapy of patients.
Methods: To this end, leucocytes from healthy blood donors or from immunosuppressed patients were co-incubated with resting or pre-swollen FITC-labelled *A. fumigatus* conidia for 0.5 hours, 2 hours and 4 hours. Leucocytes without conidia served as control. Afterwards, cells were analysed by flow cytometry for phagocytosis as well as monocyte and neutrophil antigen marker expression.
Results: Up to 63 % of leucocytes from healthy donors performed phagocytosis of *A. fumigatus* conidia. This maximum was achieved by neutrophils after 2 hours and by monocytes after 4 hours of co-incubation. Preliminary analysis of patient-derived cells revealed no significant difference in monocyte behaviour but phagocytosis by neutrophils was massively impaired (35 % or lower) at all time points. The known upregulation of neutrophil-specific CD66b upon cell activation was observed in both healthy and immunosuppressed neutrophils. However, in healthy neutrophils, CD11b was upregulated after 0.5 hours of co-incubation compared to conidia-free controls and returned to basal levels within 4 hours. By contrast, CD11b expression in patient-derived neutrophils remained at elevated levels after an initial increase. These effects could not be observed in monocytes. Instead, upon co-incubation monocytes and neutrophils of both healthy and immunosuppressed origin showed a massive down-regulation of CD33 and monocytes also of CD274.
Conclusion: Compared to healthy individuals, we assume a delayed response of neutrophils against *A. fumigatus* conidia in immunosuppressed patients due to a decreased phagocytosis rate as well as the lack of upregulated CD11b returning to a basal level. The relevance of these findings is to be analysed.

IBP11

The role of the pore-forming toxin Ece1 of *Candida albicans* during translocation through the intestinal epithelial barrier

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The opportunistic pathogen *Candida albicans* is a common and mostly harmless inhabitant of the human gastrointestinal tract. However, the fungus can cause life-threatening blood stream infections in immunocompromised patients by translocating from the gut lumen into the blood stream. To date, the molecular mechanisms of this translocation are widely unknown. It is clear, however, that a healthy microbiota is important to restrict the overgrowth and virulence of *C. albicans*. We discovered that the well-known hyphae-associated gene *ECE1* encodes a polypeptide, which is processed into eight peptides via the protease Kex2.

One of the peptides, Ece1-III, acts as a pore-forming toxin. Here we want to elucidate the effect of Ece1-III on probiotic bacteria and its role for the translocation of *C. albicans*.
We created an *ece1*Δ/Δ deletion mutant, a revertant *ece1*Δ/Δ+*ECE1* and a mutant that lacks only the Ece1-III-encoding sequence and assessed their influence on different processes that contribute to translocation. These include barrier function integrity (measurement of electrical resistance, TEER), damage (LDH assay) and a newly established translocation assay. For all analyses we used differentiated Caco-2 C2BBe1 intestinal cell monolayers. We also tested various synthetic Ece1-III variants to investigate the role of specific substitutions on damage or TEER of C2BBe1 cells. The same Ece1-III variants were tested for their ability to kill probiotic bacteria by measuring metabolic activity, membrane integrity or minimal bactericidal concentration.
Data obtained with the *ece1* mutants showed that Ece1-III is dispensable for filamentation, adhesion and invasion, but is essential for epithelial damage, integrity loss and translocation through Caco-2 cells. However, the synthetic Ece1-III variants have almost no effect on Caco-2 cells except for an N-terminal shortened version (Ece1-III$^{68-92}$). All tested bacteria were not killed but agglutinated by Ece1-III and they were effectively inhibited by Ece1-III$^{68-92}$.
Ece1-III of *C. albicans* is an important factor responsible for damage by and translocation of the fungus through intestinal epithelia, which presumably needs hyphal formation for full function. Probiotic bacteria aggregate, but are not killed by native Ece1-III. However, a shorter peptide version of Ece1-III efficiently killed bacteria.

IBP12
Protein S-mycothiolation functions in redox regulation of the glyceraldehyde 3-phosphate dehydrogenase Gap in the pathogen *Corynebacterium diphtheriae*
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Question: *C. diphtheriae* utilizes mycothiol (AcCys-GlcN-Ins, MSH) as thiol-redox buffer. In response to oxidative stress, MSH forms mixed disulfides with proteins, termed as protein S-mycothiolation. Protein S-mycothiolation was identified as important redox modification in Actinomycetes that protects proteins against overoxidation and regulates protein functions. In *C. diphtheria,* we have recently identified 28 S-mycothiolated proteins under hypochlorite stress. The glyceraldehyde-3-phosphate-dehydrogenase GapA (DIP1310) was the most abundant S-mycothiolated in *C. diphtheriae* that is a conserved target for redox-regulation and protein S-thiolation across all domains of life. Thus, we were interested to study the changes in the activity of GapA in response to oxidative stress and the redox-regulation of this enzyme by protein S-mycothiolation and the mycoredoxin and thioredoxin pathways.
Methods: To identify and quantify *S*-mycothiolated GapA in *C. diphtheriae* we applied shotgun-LC-MS/MS and the OxICAT approach. Recombinant GapA was produced as His_6-tagged fusion protein in *E. coli* and used for antibody production. The GapA activity and the electron transfer pathways *in vitro* were monitored spectrophotometric by consumption of NAD^+ and NADPH, respectively.
Results: The results revealed that GapA is S-mycothiolated in *C. diphtheriae* under hypochlorite stress at its active site Cys153. Using enzyme kinetics, GapA's glycolytic activity could be reversibly inhibited *in vitro* by increasing H2O2 concentrations in the presence of MSH due to S-mycothiolation and could be reactivated by DTT. Inactivation of GapA using H2O2 without MSH was irreversible due to overoxidation of the active site Cys since no reactivation was possible with DTT. Further results revealed that both, the Mrx1 and Trx-pathways are able to reduce Gap-SSM to restore its activity.
Conclusion: Our results document the important role of protein S-mycothiolation and the Mrx1 and Trx reducing pathways as mechanisms for GapA redox regulation during oxidative stress conditions. As key enzyme in the glycolysis, Gap provides the energy by regulation of the substrate level phosphorylation step. Thus the reversible redox-regulation and protection of Gap under oxidative stress conditions is essential for bacterial survival.

IBP13
Suppression of the heat sensitive Δ*gpsB* phenotype by mutations affecting the initial step of peptidoglycan biosynthesis in *Listeria monocytogenes*
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Question: DivIVA proteins are involved in various processes like cell division, growth and virulence in most Gram-positive bacteria. Firmicutes possess a DivIVA paralog, named GpsB, which is important for the coordination of septal and lateral cell wall biosynthesis in *Bacillus subtilis* (1) and essential for septal ring closure in *Streptococcus pneumoniae* (2). In the human pathogen *Listeria monocytogenes*, GpsB controls the activity of the bi-functional penicillin binding protein PBP A1, mediating the last two steps in cell wall biosynthesis (3). Deletion of *gpsB* caused morphological aberrations and strong virulence attenuation in *L. monocytogenes* due to defects in cell wall biosynthesis. Remarkably, the Δ*gpsB* mutant is unable to grow at elevated temperatures (42 °C) but can be stably maintained at lower temperatures (30 °C, 3). Besides its effect on PBP A1, the function of GpsB is only poorly understood. Here, we present a suppressor screen for the isolation of *gpsB* suppressors that restore viability at 42 °C.
Methods: A *L. monocytogenes gpsB* mutant was streaked on plate and incubated at 42 °C. Spontaneous suppressors became visible after two days of incubation, were isolated and sequenced using next generation sequencing (MiSeq) to map the mutations leading to the ability to grow at 42 °C. The identified genes and their impact on the heat-sensitive *gpsB* phenotype were studied in genetic experiments.
Results: We isolated two suppressors carrying a mutation in the *clpC* gene, which encodes the ATPase-subunit of the Clp-protease, and three suppressors, carrying mutations in the *murZ* (*lmo2552*) gene. MurZ is one of two UDP-N-acetylglucosamine 1-carboxyvinyltransferases, which are present in *L. monocytogenes*. All five suppressors showed wild-type growth at 42 °C. Deletion of the *clpC* and *murZ* genes in the Δ*gpsB* background also suppresses the Δ*gpsB* phenotype. Western blot analysis showed that MurA, the major UDP-N-acetylglucosamine 1-carboxyvinyltransferases of *L. monocytogenes*, is overexpressed in all suppressor strains as well as in the *clpC* and *murZ* deletion mutants. Mutations in other UDP-N-acetlyglucosamine consuming pathways (decoration of wall teichoic acids by Lmo2550 and GtcA) also suppressed the *gpsB* phenotype. These data represent the first reported genetic link between *gpsB* and initiation of peptidoglycan biosynthesis.
Conclusion: Our results show that increased protein level of MurA can suppress the heat sensitive phenotype of the Δ*gpsB* mutant. MurA is responsible for the first step of peptidoglycan synthesis, therefore giving a further connection between GpsB and cell wall biosynthesis in *L. monocytogenes*.

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IBP14
Candidalysin
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Question: Hyphae formation in *Candida albicans* is a well-known virulence factor, although the molecular mechanisms underlying the damaging potential of this fungal morphology remain to be unraveled. The *ECE1* gene has long been known as a gene highly expressed during - but dispensable for - hyphae formation in *C. albicans*. This characteristic led to the hypothesis that its function might be accessory to hyphae formation. Here we

disassemble the role that Ece1 has in pore-forming mediated damage of human host cells.
Methods: A set of *ece1* mutants have been phenotypically screened for damaging, adhering, and invading potential of different host cell types, including epithelial cells (ECs) and red blood cells (RBCs). Ece1-derived synthetic peptides were tested for damaging potential. LC-MS analysis was adopted to gain insight into the processing of the Ece1 polypeptide. Biophysical analysis was used to test the membrane composition that was most susceptible to peptide intercalation and pore formation.
Results: All *ece1* mutants were indistinguishable from the wild-type in many traits, such as hyphal length, adhesion or invasion ability, but a new clear phenotype emerged: *ece1ΔΔ* was strongly reduced in its damaging potential during infection of all tested cells, including human oral, intestinal and vaginal ECs and RBCs. An *in silico* analysis of the Ece1 amino acidic sequence hinted towards the protein being a polypeptide composed of eight peptides ending (all except the last one) with a common motif, i.e. Lysin-Arginin (KR). This is a recognition site for the endoprotease Kex2. All eight peptides have been synthesized and tested for their cytolytic activity, but only Ece1-III was able to cause damage. This potential was observed in all tested cells. Ece1-III intercalated into host membranes and formed lesions, a feature that was highly enhanced by the presence of phosphatidylserine, tested by Förster resonance energy transfer. LC-MS analyses confirmed the presence of Ece1-III in *C. albicans* hyphal supernatants, together with partial hits of other predicted peptides.
Conclusion: During hyphal formation, Ece1 is processed into smaller peptides and secreted into the surrounding media. The secreted *C. albicans* hypha-associated peptide toxin then intercalates into mammalian cell membranes, forming pores and resulting in host cell lysis. We named this toxin *Candidalysin*. This is the first peptide toxin described in a human fungal pathogen.

IBP15
Identification of new immune evasion proteins of the human pathogen *Staphylococcus aureus*
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S. aureus, similar to many other pathogenic microbes, binds and thereby recruits human complement regulators to its surface in order to evade host complement attack. *S. aureus* and other pathogens e.g. recruit Factor H, a regulator of the alternative complement pathway. Here we identify two novel staphylococcal proteins that bind Factor H and protect the pathogenic bacterium from host complement attack: Complement regulator acquiring surface protein 7 (CRASP7) is a moonlighting protein that binds Factor H and is also involved in purine biosynthesis. CRASP8, the second staphylococcal Factor H binding protein is a member of a family of superantigen-like proteins.
In order to identify novel Factor H binding proteins of *S. aureus* a screening via a protein microarray was performed. The two Factor H binding proteins CRASP7 and CRASP8 were identified, subsequently cloned, recombinantly expressed and purified. Binding was analysed by ELISA and biolayer interferometry. Factor H, when bound to each CRASP protein maintains complement regulatory activity, e.g. acts as a cofactor for the protease Factor I, assisting in cleavage of C3b. This cleavage decreases complement amplification at the level of the C3 convertase.
Although both staphylococcal proteins bind to Factor H, they influence complement in different ways. CRASP8 inhibits the alternative pathway of complement, as demonstrated in a complement activation assay. However a staphylococcal CRASP7 insertion mutant, that does not express CRASP7, shows attenuation effects regarding survival compared to the wildtype in *in vivo* assays in the wax moth larvae *Galleria mellonella* and mice kidney abscess and lung infection models.
Both staphylococcal CRASP7 and CRASP8 also bind C3, the central human complement component. Therefore further studies will investigate if the CRASP variants upon binding to C3 inhibit complement at the level of the C3 convertase via phagocytosis assays and complement specific experiments.
S. aureus evolved multiple complement and immune evasion strategies. As there is an ongoing spread of antibiotic resistant staphylococci research aims to identify and characterize immune evasion proteins and identify their mode of action in order to develop new antistaphylococcal compounds. CRASP8 turns out to be a novel staphylococcal inhibitor of the alternative pathway, likely due to Factor H and C3 binding, protecting *S. aureus* from the complement attack. Mice infection models demonstrate the impact of the moonlighting protein CRASP7 on *S. aureus* virulence. Thus CRASP7 and CRASP8 can provide prospective targets for therapeutic immune interference.

IBP16
B cells and antibodies in protective immunity to *Candida albicans* infection
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Candida albicans colonizes mucosal surfaces of most healthy individuals but may become an invasive pathogen in the immunocompromized host. While in the context of acute bloodstream infection innate immune responses appear to predominate protection, adaptive immunity is required for long term immunological memory and immunization. The present project focuses on the role of antibodies and B cells in the maintenance of benign *C. albicans* colonization, as well as in protection from secondary challenges with *C. albicans* bloodstream infections, to approach the question whether and how a protective antibody response to *C. albicans* can be triggered in humans.
Blood and gastrointestinal samples of C. albicans-colonized mice were analyzed and first results indicate that although blood concentrations of the different antibody isotypes do not change substantially upon colonization, there may be an increase in IgA in the gastrointestinal tract of these mice. Moreover, at day 21 post-infection a significant amount of specific IgG1 can be detected in the blood of colonized mice.
In 2012, Wächtler *et al.* showed that epithelial cell damage caused by *C. albicans* was strongly reduced upon incubation with human serum. Analysis of *C. albicans*-infected epithelial cells showed that the reduced damage is serum concentration-dependent, and that although antibodies appear not to be the main responsible factor, they may contribute to this protection. Further analysis of the effect of human serum antibodies on *C. albicans*-infected epithelial cells will provide more insights into the role of antibodies in protection against cell/tissue damage.
To stimulate antibody production by B cells, fungal products might trigger specific signals in these cells. We are investigating the role of fungal cell wall components in B cell stimulation, differentiation and antibody production.
These three distinct parts of the project attempt to clarify the role of B cell/antibodies in the response mechanisms against *Candida albicans*, since although the importance of adaptive immunity in *Candida albicans* infection has been extensively studied, the contribution of B cells and antibodies is still not clear.

IBP17
Genome-wide screening for plasma-sensitive mutants reveals molecular mechanisms for bacterial inactivation
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Research on cold atmospheric plasmas in the field of biomedicine is of growing interest. Next to enhanced wound healing and improved blood coagulation, disinfection and sterilization are major applications of cold plasmas. Hitherto, little is known about the molecular and genetic mechanisms resulting in comparably short survival times of microorganisms when exposed to plasmas [1, 2].
The genetic features of bacteria supporting the survival of a critical plasma dose were investigated in the model organism *Escherichia coli* using a single-gene knockout library. This strain collection constructed at Keio University includes approximately 4,000 deletion mutants, each missing one non-essential gene [3]. A comprehensive screening of this library

against non-lethal doses of plasma generated by an atmospheric-pressure plasma jet was performed resulting in a set of 87 mutants with increased sensitivity towards plasma. For some of the genes knocked out in theses mutants the involvement in the course of plasma tolerance appears obvious, such as *katE* or *oxyR* coding for an H_2O_2 detoxifying catalase and a transcriptional regulator of the oxidative stress response, respectively. For other genes, their part in plasma tolerance is not as clear, as for instance for *flgB* and *flgG*, both coding for proteins of the flagellar apparatus. In order to identify for each "plasma-tolerance" gene the individual plasma factor it mediates resistance against, the knockout mutants were further treated with different stressors mimicking single components generated by plasma, *e.g.* decreased pH value, paraquat as inducer of superoxide stress, diamide for simulation of disulphide stress, or peroxynitrite as reactive oxygen and nitrogen species. For more than 80 % of the 87 plasma-sensitive mutants at least one stressor was found causing a growth deficiency similar to plasma treated samples.
The overall findings indicate that a set of plasma-tolerance genes increases the ability of bacteria to survive under life-threatening plasma conditions, while many of these genes seem to be involved in cellular stress resistance mechanisms protecting bacteria from various environmental stressors.

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IBP18

Establishment of a commensal gut model to study interactions between *C. albicans* and Lactobacilli

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Question: *Candida albicans* is both, a harmless commensal on mucosal surfaces and the most common cause of life-threatening nosocomial fungal infections. The main reservoir for systemic *C. albicans* infections is the gut. In both stages, as a commensal and as a pathogen, *C. albicans* not only interacts with the host but also with other members of the microbial flora. Furthermore, removal or imbalance of the bacterial microbiota is a significant predisposing factor for disseminated *Candida* infections. Our aim is to investigate the commensal-to-pathogen shift of *C. albicans* and the role of nonpathogenic bacteria during this shift.
Methods: To establish a commensal gut model, two intestinal cell lines, different Lactobacilli strains and different culture conditions were tested upon infection with *C. albicans*. As a read-out of these coincubations, we quantified adhesion- and damage potential and fungal morphology. Next, an antibiotic treatment will be used to induce a shift from the commensal to a pathogenic state of *C. albicans*. Transcriptional profiling of *C. albicans* and intestinal cells will be used to gain detailed information about their gene expression pattern during this transition.
Results: A commensal *in vitro* gut model was established, which was stable for at least 48 h. The incubation of intestinal cells with different *Lactobacillus* strains showed a dose- and time dependent protective effect against damage caused by *C. albicans*. Adhesion of *C. albicans* was not influenced by the presence of Lactobacilli, but rather by differences in the composition of different intestinal cell lines. Further reduction of intestinal cell damage was achieved by incubation at hypoxic shock conditions. More detailed studies will help to elucidate the protective mechanisms behind these current observations.
Conclusion: The established commensal gut model and the obtained results are the first steps to characterize the commensal-to-pathogen shift of *C. albicans*. Lactobacilli seem to be effective nonpathogenic bacteria to protect intestinal cells against *C. albicans* damage and could possibly play a similar role during the commensal state of *C. albicans* in the gut.

IBP19

The influence of divalent cations on *Helicobacter pylori* HtrA's E-Cadherin cleavage activity

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Question: Eukaryotic as well as prokaryotic HtrAs (high temperature requirement A) act as serine proteases and molecular chaperons and therefore play an important role in protein quality control by degrading misfolded proteins. The epithelial adherence glycoprotein and tumour suppressor E-Cadherin has been shown to be actively cleaved on gastric epithelial cells by *Hp*HtrA from the human pathogen and class-I-carcinogen *Helicobacter pylori* (*H. pylori*). E-Cadherin cleavage has drastic consequences for the integrity of the epithelial barrier as it contributes to the loss of cell-cell adhesion, to alterations in cell signalling pathways and finally to carcinogenesis. The stability of E-Cadherin-mediated adherence is determined by calcium binding, which is essential for homophilic interactions of the ectodomains of E-Cadherin. Hence, we investigated the effect of calcium and other divalent cations on E-Cadherin cleavage by *Hp*HtrA in cell culture and *in vitro* cleavage experiments.
Methods: To determine the effects of divalent ions ($CaCl_2$, $ZnCl_2$, $MgCl_2$, $MnCl_2$ and $BaCl_2$) on E-Cadherin cleavage and *Hp*HtrA activity, we performed infection experiments with different gastric epithelial cells and *H. pylori* as well as *in vitro* cleavage assays using recombinant human E-Cadherin as a substrate. The cleavage activity was measured by detection of the soluble ectodomain of E-Cadherin in the supernatant of infected cells or E-Cadherin fragments in *in vitro* cleavage assays. The regulation of the caseinolytic activity of *Hp*HtrA by divalent ions was analysed by zymography.
Results: *In vitro* cleavage experiments revealed that calcium ions strongly inhibited E-Cadherin cleavage, whereas the chelators EDTA and EGTA favored the cleavage activity. This was also observed in infection experiments, but to a lesser extent. At the same time, calcium ions had no effect on the caseinolytic activity of *Hp*HtrA, indicating a direct effect on E-Cadherin structure, but not on *Hp*HtrA activity. Furthermore, we could show that zinc as well as manganese ions were able to inhibit E-Cadherin cleavage and decrease *Hp*HtrA activity *in vitro*.
Conclusion: Calcium ions decrease E-Cadherin cleavage by *Hp*HtrA *in vitro* as well as in infection experiments. We suggest that the inhibitory effect of calcium ions was due to alteration of the dimerization and accessibility of E-Cadherin for HtrA. Additionally, zinc ions and to lower degree manganese ions seemed to inhibit *Hp*HtrA activity, which leads to the conclusion that divalent salts can affect HtrA´s activity as well as the cleavage accessibility of E-Cadherin and therefore might influence *H. pylori* pathogenesis.

IBP20

Pylogenetic overview of ß-lactamases

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Introduction: The worldwide spread and emergence of extended spectrum ß-lactamases (ESBL) and carbapenemases has been recognised as one of most serious threats to public health by international authorities. The main burdens of ESBLs and carbepanemases are the limited treatment options and failures of routine diagnostic, both caused by fast plasmidal transfer between species and co-expression of different variants and other resistance determinants in one isolate. Thus, molecular diagnostics gaining in importance but high sequence diversity of β-lactamases and the different sequence conservation within the groups are highly challenging for this applications. Due to fast growing number of sequenced genomes, identification of new β-lactamase variants is booming. Currently, approximately 1500 different β-lactamases are known, some are highly clinically relevant, others represent a natural environmental reservoir for dissemination in humans. The strongly increased research interest on ß-lactam resistance led to numerous reviews in the last years; those however discussing only specific classed or groups of β-lactamase.
Methods: We present a comprehensive phylogenetic and molecular overview of all known 1504 β-lactamase variants based on amino acid and DNA sequences. Those were retrieved from the NCBI database, MLST database of Pasteur Institute bigsdb.web.pasteur.fr/klebsiella for OXY, OKP and LEN β-lactamases and LacED (www.laced.uni-stuttgart.de) for B2 and B3 metallo-β-lactamases, considering www.lahey.org/Studies. Sequence analyses were performed using CLC Mainworkbench 7.0.2 software, ncbi-blast-2.2.31+ and PERL with BIOPERL package.
Results & Conclusions: A phylogenetic based analysis allows to find conserved and variable pattern that can be used to design more precise and predictive molecular diagnostics. Our results based on similarity based network also indicated that the classification according to Ambler should be expand to further classes and groups as they forms clear own phylogenetic clusters.

IBP21

In vivo imaging reveals foci of _Candida albicans_ colonization in the murine gut

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Question: *Candida albicans* is an opportunistic fungal pathogen, which is found as a commensal on mucosal epithelia, especially the human gut. Under predisposing conditions *C. albicans* can translocate through the intestinal barrier into the bloodstream. Entering blood vessels, the fungus can disseminate into a variety of organs, leading to life-threatening disseminated infections. Both colonization and dissemination are poorly understood to date. Therefore, the aim of this project is to characterize colonization and translocation with regard to the involved anatomical sites, cell populations and immune response.
Methods: In order to determine at which anatomical sites translocation of *C. albicans* occurs and which host cells are involved, a murine *in vivo* colonization/dissemination model (according to Koh *et al.*; 2008) was established. Following antibiosis, mice were infected orally by gavage with a bioluminescent strain of *C. albicans.* In order to characterize the model, fungal burden and morphology were analyzed in feces, intestinal and internal organs. Flow cytometry was employed to determine the host response. *In vivo* or *ex vivo* bioluminescence imaging was performed to visualize sites of colonization.
Results: Animals remained clinically healthy throughout the experiments. All fungal morphologies were found in the content and in the homogenates of the stomach, small intestine, cecum and colon at different time points after infection. While fecal burden remained relatively stable over the course of colonization, *in vivo* imaging revealed a rather dynamic behavior with changing intensity and localization of signals. *Ex vivo* imaging of the gastrointestinal tract revealed distinct foci with bioluminescent signals. By extracting these foci for flow cytometry analysis and comparison to control animals, a distinct immune response of the host was observed upon colonization.
Conclusions: While *C. albicans* was found to persistently colonize different parts of the murine gut in stable numbers during antibiosis, bioluminescence revealed foci with increased signal intensity. Whether these foci indicate sites of increased fungal density and/or focal invasion is currently under investigation.

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IBP22

Bile-mediated resistance of _Candida albicans_ against antifungals

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In previous studies an *in vivo* imaging system was applied to study efficacy of caspofungin and fluconazole in therapy of disseminated candidiasis caused by *Candida albicans*[1]. Imaging revealed that fungal burdens in kidneys were significantly reduced under therapy and complete fungal clearance was observed between 5 and 10 days post infection. Interestingly, around 30 % of mice in the treatment group expressed a bioluminescence signal that derived from the gall bladder. This signal persisted despite successful clearance of infection in other organs. Subsequent *in vitro* analyses confirmed that bile significantly decreased antifungal drug efficacies. However, the reason for this protective effect remained unclear. Bile is a natural detergent produced by the liver and mainly consists of bile acids or bile salts, cholesterol, phospholipids, biliverdin and proteins. Here, we analyzed the protective effect of different bile components for their protective properties. Interestingly, while unconjugated bile salts revealed strong toxic effects on *C. albicans*, taurine or glycine conjugates were well tolerated. Subsequent analyses revealed that conjugated bile salts conferred resistance against some, but not all antifungals tested. In addition, conjugated bile salts protected *C. albicans* from uptake of the fluorescent dye rhodamine 6G, indicating that drug protection occurs outside of the fungal cell. Therefore, the most likely scenarios for the protective effects will be discussed.

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IBP23

Insights into the role of the transcription termination factor Rho of _Staphylococcus aureus_

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Question: We analyzed the transcriptome of *S. aureus* HG001, a derivative of the model strain NCTC 8325, under multiple experimental conditions using strand-specific tiling arrays. These conditions cover a broad spectrum of the bacterium's lifestyles ranging from optimal *in vitro* growth to interaction with host cells. *In silico* data analysis comprised a systematic mapping of transcription units, classification of promoters according to sigma factor dependence, identification of new potential targets for several known transcription factors and annotation of non-coding RNAs including antisense RNAs. This revealed a relatively low abundance of antisense RNAs in the *S. aureus* wild type, where they overlap only 6 % of the coding genes. Previous studies had shown that the transcription termination factor Rho plays a major role in suppressing antisense transcription in *E. coli* [1] and *B. subtilis* [2], and transcriptome analysis of an *S. aureus rho* deletion mutant indeed revealed a remarkable overall increase in antisense transcription in the absence of Rho. In contrast to results reported for *E. coli*, elevated antisense transcription significantly affected sense transcript levels. Based on these observations the goal of the present study was a detailed comparative analysis of *S. aureus* HG001 and its isogenic *rho* mutant.
Methods: *S. aureus* strains were grown in different culture media and exposed to several stress stimuli. For investigation of the proteome, cytoplasmic and secreted proteins of cells grown to exponential and stationary phases were analyzed by mass spectrometry.
Results: The comparison of the growth behavior in the different media and after exposure to stress stimuli revealed only minor differences between the *S. aureus* wild type and the *rho* mutant. However, the proteome analysis showed significant differences in the abundance of several proteins, namely increased amounts of SaeSR-dependent virulence factors like extracellular adherence protein (Eap) and fibronectin-binding proteins (FnbA and FnbB) in the absence of Rho. Elevated expression of the regulon controlled by the SaeSR two-component system perfectly confirmed the mRNA data of the tiling array study. Remarkably, the *rho* mutant exhibited increased virulence in a murine bacteraemia model.
Conclusion: Our data suggest that under conditions of Rho deficiency the SaeSR regulatory system of *S. aureus* is activated by a so far unknown mechanism leading to the observed induction of the SaeSR regulon which was shown to increase virulence *in vivo*. Currently, we use cell culture infection models to further explore the impact of the higher levels of SaeSR-dependent virulence factors and the reduced expression of other genes due to antisense transcription in the *rho* mutant.

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IBP24

Novel synthetic antimicrobial peptides against _Streptococcus pneumoniae_

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Background: According to WHO, 1.6 million deaths are caused by pneumococcal infections every year with 0.7 to 1 million in children younger than 5 years mostly in Asia and Africa (1). Like other Gram-

positive bacteria, *Streptococcus pneumoniae* is increasingly difficult to treat due to the irrational use of antibiotics (2). Antimicrobial peptides (AMPs) represent a possible alternative for current antibiotics against drug resistant pathogens.

Materials & Methods: In this study, thirteen antimicrobial peptides were designed based on two natural peptides indolicidin and ranalexin. The *in vitro* activity of these peptides was investigated using broth microdilution assay, hemolytic activity assay, time killing assay, and toxicity assay against two cell lines WRL-68 and NL-20.

Mechanisms of action of peptides were assessed using transmission electron microscopy (TEM), scanning electron microscopy (SEM), DNA binding assay, and *in silico* molecular docking against three virulent factors.

Results: Our results revealed that four hybrid peptides RN7-IN10, RN7-IN9, RN7-IN8, and RN7-IN6 possess potent antibacterial activity against 30 pneumococcal clinical isolates (MIC 7.81-15.62µg/ml). These four hybrid peptides showed broad spectrum antibacterial activity (7.81µg/ml) against *S. aureus*, methicillin resistant *S. aureus* (MRSA), and *E. coli*. Furthermore, the time killing assay results indicated that the hybrid peptides were able to eliminate *S. pneumoniae* within less than one hour which is faster than the standard drugs erythromycin and ceftriaxone. The cytotoxicity was tested against human erythrocytes, WRL-68 normal liver cell line, and NL-20 normal lung cell line. The results revealed that none of the thirteen peptides have cytotoxic or hemolytic activities at their MICs.

TEM and SEM results showed that these four peptides are killing the bacteria by destroying the integrity of their membranes. DNA binding assay revealed that the hybrid peptides were able to bind to DNA at 62.5 µg/ml preventing it from migration through the agarosre gel.

Conclusion: In conclusion, our results indicated that hybrid peptides RN7-IN10, RN7-IN9, RN7-IN8, RN7-IN7 and RN7-IN6 represent promising first templates for developing a new class of antibacterial agents against *S. pneumonia*. Hence, with the growing resistance to traditional antibiotics, our peptides can offer an alternative to today's antibiotics to protect against resistant bacteria. Currently, *in vivo* study is being carried out to investigate the toxicity and therapeutic efficacy of peptides in animal models.

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IBP25

Translocation and phosphorylation of *Helicobacter pylori* CagA in different epithelial and non-epithelial cells

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Helicobacter pylori (*H. pylori*) is a gram-negative bacterium, which can induce gastric diseases like severe gastritis or even gastric cancer in the human stomach. *H. pylori* expresses cytotoxin-associated gene A (CagA) protein as an important virulence factor, which is translocated into host cells via a type IV secretion system (T4SS). In the cytoplasm, CagA is tyrosine-phosphorylated by Src and c-Abl kinases of the host cell and it deregulates cancer-associated signal transduction pathways. In monocytic cells, it was shown that the phosphorylated full-length CagA protein (135 kD) was cleaved into two fragments with a molecular weight of approximately 40 kD and 100 kD with unknown functions. Aim of this work is the investigation of the CagA translocation and phosphorylation in different established cell lines.

The translocation, phosphorylation and fragmentation of CagA was analysed in the gastric epithelial cell lines AGS, MKN-28, MKN-45 and SNU-1, and the B cell line MEC1. The cell lines have been infected with *H. pylori* wild-type for different time periods and the CagA phosphorylation was determined by Western Blot analysis using anti-phosphotyrosine antibodies and antibodies directed against CagA.

H. pylori-infected gastric AGS and MKN-28 cells showed a strong translocated tyrosine-phosphorylated full-length protein, but no fragmentation of CagA. In contrast to MKN-28 cells, AGS cells drastically elongated leading to the formation of the CagA-dependent scattering or "hummingbird"-phenotype. Infection experiments with MNK-45, SNU-1 and MEC1 cells revealed an efficient translocation and phosphorylation of CagA followed by a specific cleavage of the full-length CagA protein into two distinct fragments. The amount of these cleavage products increased whereas the intensity of the full-length protein decreased in a time-dependent manner. In the *H. pylori*-infected B cell line MEC1, the phosphorylated full-length CagA was shown, but the cleavage was stronger compared to the MKN-45 cells as reflected by the high amount of the 40 kD CagA fragment within one hour after the infection. Differences in the cellular phenotype were not obvious.

These data indicate that CagA is translocated and tyrosine-phosphorylated in different epithelial and non-epithelial cell types. While the function of CagA in AGS cells is well understood, the biological significance of CagA in other cell types needs to be investigated in more detail.

IBP26

Genome analysis of the insect-killing and humanpathogenic fungus *Conidiobolus coronatus* (Entomophthoromycota, Zygomycota)

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Conidiobolus coronatus is a soil inhabiting and insect killing fungus, which is able to become a human pathogen in immunocompetent patients. Little is known about the way of infection and mechanisms of pathogenicity. The infection comprises local necrosis of skin and mucosal tissue. Treatment encompasses surgery and subsequent antimycotic treatment. We analysed the genome, transcriptome and secretome including the hemolytic activities against human erythrocytes in order to gain insights into the pathogenicity mechanisms at the molecular level. Different fluorescence and electron microscopy methods were used to look for morphological differences and variations between the strains JMRC:FSU:04392 and JMRC:FSU:11506. A variety of media were used to simulate different host or environmental conditions. For example, chitin, keratin and collagene were tested for their inductive potential in virulence-related conditions. As a result, we deciphered important keyplayers at the -omics level.

In addition, the infection process were analysed at the Deutsches Elektronen Synchrotron in Hamburg (DESY). We monitored the infection process *in vivo* using the µCT compartment. The aim of this study was the comparison of the colonization by the insect host with other insect killing, but non-human pathogenic, entomophthoralean fungi. This project was funded by the CRC/Transregio 124 FungiNet.

IBP27

Proteome profiling of *Burkholderia pseudomallei* quorum sensing mutants

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Question: *Burkholderia pseudomallei*, the causative agent of melioidosis is a Gram-negative soil bacterium in tropical areas. *B. pseudomallei* employs serveral acyl-homoserine lactone (AHL)-mediated quorum sensing (QS) systems which activate specific sets of genes as a function of cell density [1,2]. The genome of *B. pseudomallei* encodes genes for three QS systems with one *luxI* and one *luxR* homologue, respectively, and additionally three orphan *luxR* homologues [3]. The *luxI* homologues encode AHL synthases, which produce specific AHL, which bind to the respective transcriptional regulator. Thus regulate expression of specific genes involved in virulence like biofilm formation, siderophore biosynthesis or swarming motility [4]. The aim of this study was to clarify the influence of the different QS systems on virulence factor expression.

Methods: We constructed single mutants of the three complete QS systems. Subproteome fractions of the mutant strains were subjected to mass spectrometry analysis to identify targets of the different QS systems. We used the DIA approach IMS^E in combination with the Hi3 approach for quantification of cytosolic proteins and the GeLC MS/MS approach for the analysis of extracellular and surface-associated proteins. Furthermore,

we assessed the biofilm-forming capacity as well as the motility of the different QS mutants.
Results: Our comprehensive proteome analysis of the QS mutants revealed a number of differentially expressed proteins. Among them proteins that are already described as QS-regulated in other Gram-negative opportunistic pathogens, e.g. *Burkholderia cenocepacia* and/or known to be involved in pathogenicity. We also observed a clear involvement of the QS systems in biofilm formation and motility of *B. pseudomallei*.
Conclusions: The *luxI* and *luxR* homoloques influence protein expression by up- and downregulation of AHL-dependent proteins in the QS circuitry of *B. pseudomallei* and thus influence the pathogenicity of this bacterium.

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IBP28

Lichtheimia corymbifera and its interaction and long term survival within alveolar macrophages

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Mucormycosis, an infection caused by mucoralean fungi is one of the devastating fungal infections in humans with the mortality rate of approximately 90 %. *Lichtheimia* species are the 2nd and 3rd most causative agents for the infection in Europe and USA, respectively. The main route of this infection is *via* the respiratory tract where alveolar macrophages (AM) belong to the immune system's first line of defense and yet the interaction between AM and this fungus are not well known. In our study, we used two strains of *L. corymbifera*; JMRC:FSU:09682 (fully virulent) and JMRC:FSU:10164 (attenuated) to the pathogenesis of *L. corymbifera*. First, the phagocytic efficiency of murine alveolar macrophages (MH-S) was measured. Spores were prepared in various conditions such as resting, pre-swollen, and opsonised to mimic *in vivo* situations. Interestingly, spores of both strains differed in uptake especially in opsonised conditions. Quantification of the complement activation product on these spores showed that virulent strain together with other strains of *L. corymbifera* have higher opsonisation on their surfaces than that of the attenuated strain despite of the similarity in spore shape and size. Also we have found difference in surface protein component between two strains which may play a role in recognition and uptake by MH-S. Analysis regarding the survival of *L. corymbifera* spores in macrophages showed that both strains can survive for up to 24 h. In contrast to *Aspergillus fumigatus, L. corymbifera* spores do not escape from macrophages by the formation of hyphae but remain as spores and show long-term persistence in macrophages similar to *A. terreus*. The non-acidified phagolysosome contributes to the long-term survival within the host. Dissecting the interaction between the fungi and immune system will reveal more possibilities to enhance the treatment and management against mucormycosis.

IBP29

Identification of antibiotic targets by chromatographic co-elution

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Question: Due to a wide spread of bacterial resistances, development of novel antibiotics has become urgent. A prerequisite for the application of promising novel compounds is to understand their mode of action and identify the cellular target. This allows e.g. the estimation of the risk of cross-resistances or potential cytotoxicity for the host. Target identification can be time- and ressource-consuming and often requires chemical modification of the compound for immobilization or labeling. Recently, a widely applicable non-denaturating, label-free method for target identification by chromatographic co-elution (TICC) was developed [1]. We set out to adapt this technique for the discovery of antibiotic targets in order to complement our existing platform for mode of action studies based on the proteomic response of model organism *B. subtilis*.
Methods: For TICC, the antibiotic is first incubated *in vitro* e.g. with cytosolic protein extract and subsequently fractionated by native mixed-bed HPLC. Due to drug target binding during incubation, the target-bound antibiotic elutes in different fractions compared to the free compound. These target-containing "shift"-fractions are identified by characterization of the antibiotic elution profile using LC-MS. Potential protein targets are subsequently identified after tryptic digest using LC-MS. Two model systems, composed of known antibiotic-target-pairs, were used to establish TICC for antibiotic research. First, a low-complexity system consisting of purified FabF and the fatty acid biosynthesis inhibitor platensimycin was used [2]. Rifampicin, which inhibits DNA-dependent RNA polymerase by binding to RpoB [3], was used as a proof-of-concept model for identifying antibiotic targets in cytosolic extracts of *B. subtilis*.
Results: TICC was successfully established for antibiotic target identification in cytosolic protein extracts of *B. subtilis*. The retention times of platensimycin and rifampicin shifted during native HPLC and the target proteins were identified in the "shift"-fractions. FabF, which requires substrate binding for interacting with the antibiotic [2], revealed a limitation of TICC. Due to the used protein extraction method, metabolites were removed from the extract. As a consequence, target proteins like FabF might be inactive during drug-target incubation, which eventually prevents drug binding and target identification by chromatographic co-elution.
Conclusion: The identification of known antibiotic targets in cytosolic protein extract of *B. subtilis* was successful. Rifampicin/RpoB was established as positive control for future target identification studies on antibiotics with unknown targets.

[1] Chan *et al.* (2012) Mol Cell Proteomics 11:M111.016642
[2] Wang *et al.* (2006) Nature 441:358-61
[3] Campbell *et al.* (2001) Cell 104:901-12

IBP30

The effect of spore surface modification on the virulence of *Lichtheimia corymbifera*

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Mucoralean fungi, an order of zygomycetes, can cause mucormycosis, a life-threatening disease such as invasive aspergillosis in immunocompromised patients. In our study, we analysed the influence of different spore surface alterations on the phagocytosis of murine alveolar macrophages and on the virulence in an invertebrate infection model using larvae of the wax moth (*Galleria mellonella*). Two strains were used, which were shown to be virulent and attenuated, respectively, in an avian infection model [1], were used in this study. Different physical and chemical treatments of the spore surface were performed using swelling, heat inactivation, glucanases (VinoTaste), proteases (pronase E, trypsin) and detergents (Tween 20). The highest phagocytosis was achieved with the trypsin, pronase E and opsonization treatment. When comparing the two strains, the phagocytosis indeces for resting, swollen and opsonized spore conditions were higher in the virulent strain, whereas protease E and tryptic treatment decreased phagocytosis of the virulent strain. Heat inactivation, Tween 20 and VinoTaste glucanase treatments generally increased phagocytosis of both strains regardless of their virulence behavior in the embryonated hen egg model. Therefore, spores subjected to six different surface treatments were tested in *Galleria mellonella* larvae in order to confirm existing virulence data and to verify the impact of spore treatments in the virulence potential to living hosts. Furthermore, the role of Dectin-1 (b-glucan) and Dectin-2 (a-mannan) receptors was investigated by incubation of the macrophages in laminarin and mannan prior to confrontation with spore stimuli. No participation of known receptors from *Aspergillus fumigatus* (Dectin-1) and *Candida albicans* (Dectin-2) in phagocytosis was observed. The findings presented in this study will shed light into the recognition of *Lichtheimia corymbifera* by phagocytes of the murine innate immune system and invertebrate hosts which raise important measures to mammalian infection models.

[1] Schwartze *et al.*, (2012) *Lichtheimia* species exhibit differences in virulence potential. PLoS One 2012; 7: e40908

IBP31
Selection of protein interaction partners for therapeutic and diagnostic applications
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Protein protein interactions play a central role in nearly all biological processes. Interactions between food-borne pathogens and human host cells lead to invasion of the cell by the bacteria and human body infection. We investigate protein protein interactions for therapeutic and diagnostic applications, e.g. to target *Listeria monocytogenes*. *L. monocytogenes* is a pathogenic bacterium responsible for foodborne infections and listeriosis, a disease with a high mortality rate. In spite of treatment with antibiotics, 20-30 % of clinical infections result in death. Media report frequently about recalls of contaminated meat and cheese products. Hence, it is very important to detect listeria in food and to prevent the entrance in human cells. Using a technique called phage display, we develop peptides which inhibit harmful protein protein interactions, enable diagnosis or immobilize specific proteins to surfaces. Similar molecules are used, inter alia, in many areas of modern medicine and biotechnology.

IBP32
The role of biotin in *Candida glabrata* – macrophage interaction and virulence
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Question: *Candida glabrata* is a human opportunistic pathogen, which can cause superficial but also life-threatening systemic infections. *C. glabrata* has evolved strategies to resist killing by macrophages and can even replicate within these immune cells.
In a screening of a *C. glabrata* mutant collection for attenuated survival in human monocyte-derived macrophages (MDMs) we identified 24 mutants with a reduced intracellular fitness [1]. One of these mutants, *vhr1*Δ, showed strongly attenuated survival in MDMs and in an *in vivo* systemic mouse infection model [2]. Vhr1 is a positive regulator of biotin uptake, and the orthologue in the related yeast *Saccharomyces cerevisiae* is known to regulate the expression of the biotin transporter gene *VHT1*. Thus, the aim is to elucidate the role of Vhr1, Vht1 and biotin for intracellular survival of *C. glabrata* in macrophages.
Methods: We created and analyzed *VHR1* and *VHT1* deletion and revertant strains in MDMs and under biotin-limited conditions. To investigate the Vhr1-dependent transcriptional activation of *VHT1* in *C. glabrata*, we performed quantitative Real-time PCR experiments in the presence of different exogenous biotin concentrations and after phagocytosis by macrophages. To determine whether biotin is important for intracellular fitness, we tested the survival of biotin-starved and biotin-fed wild type cells in MDMs through determination of CFUs. *In silico* and growth analysis in comparison to *C. albicans* and *S. cerevisiae* were performed to study the general biotin metabolism in *C. glabrata*.
Results: We confirmed a Vhr1-dependent expression of the transporter gene *VHT1* in *C. glabrata* under low biotin conditions and demonstrate that *VHT1* is required for survival in macrophages. Thus, both, *VHR1* and *VHT1* have crucial roles during *C. glabrata* - macrophage interaction. Prestarvation of *C. glabrata* for biotin lead to reduced intracellular survival, whereas feeding with high exogenous biotin increased intracellular fitness within macrophages. In general, pathogenic *Candida* spp. are auxotrophic for biotin, but some are able to synthesize biotin in the presence of biotin biosynthesis precursors. However, in contrast to *C. albicans* and *S. cerevisiae*, *C. glabrata* is not able to utilize these precursors due to the loss of biosynthesis genes.
Conclusion: Our data demonstrate that biotin or biotin-dependent processes are required for survival of *C. glabrata* within the phagosomal compartment of macrophages. The Vhr1-dependent transcriptional activation of *VHT1* is crucial for intracellular fitness. However, it remains unclear whether *C. glabrata* can use host biotin or whether the fungus is dependent on its own intracellular biotin pool during interaction with macrophages.

[1] Seider K *et al.*, 2014. Eukaryot Cell. 2014 Jan;13(1):170-83.
[2] Brunke S *et al.*, 2015. Dis Model Mech. 2015 Mar 18. pii: dmm.019901.

IBP33
Establishment of an antibiotic signature library for *Pseudomonas aeruginosa*
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Question: Worldwide spread of antibiotic resistance greatly impairs the treatment of bacterial infections. Therefore, antibacterial agents with new mechanisms of action are urgently needed as well as new innovative and cost-effective techniques for the characterization and validation of new drugs. Gel-based proteomics in combination with quantitative gel-free proteomics have emerged as valuable tools to study the physiology of microbes under antibiotic stress conditions. The presented study aims at the creation of a comprehensive antibiotic signature library for *Pseudomonas aeruginosa*. Protein signatures of *P. aeruginosa* treated with well characterized antibiotics will then be used to identify the cellular targets and the mode of action of new compounds showing anti-microbial activity.
Methods: *P. aeruginosa PAO1* was exposed to various concentrations of different antibiotics with well-defined molecular targets to determine their minimal inhibitory concentrations (MIC): aztreonam 0.5 µg/mL; ceftazidime 8 µg/mL; chloramphenicol 16 µg/mL; ciprofloxacin 0.03 µg/mL; colisitin 2 µg/mL; gentamicin 4 µg/ml; rifaximin 8 µg/mL; tetracycline 2 µg/mL. For the proteomic analyses, multiples of the MICs were tested in growth experiments to identify antibiotic concentrations that reduced the growth rate of the bacteria but did not inhibit growth completely in order to map the specific antibiotic stress response. Different sub-cellular protein fractions of *P. aeruginosa* PAO1, harvested 30 and 120 min after antibiotic treatment, were prepared and proteins identified using a gel-free LC-IMS^E approach in combination with the Hi3 method for absolute protein quantification.
Results: Although each antibiotic showed an individual protein expression profile, signature proteins specific for a common drug target were identified (e.g. cell wall, ribosome, and replication machinery). These subsets of proteins whose expression levels are characteristic for a specific antibiotic treatment have been designated as "proteomic signature".
Conclusions: We established a workflow for a comprehensive antibiotic stress proteome signature library of *P. aeruginosa*. Using this pipeline, new compounds can now be evaluated to gain insight into their *mode-of-action.*

IBP34
Regulation of phospholipases D virulence factors in *Acinetobacter baumannii*
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Question: Hospital acquired (nosocomial) infections with multiresistant bacteria are a rapidly emerging threat worldwide. Especially infections with multiresistant opportunistic pathogen *Acinetobacter baumannii* strains have substantially increased over the last decade. Adaptation of *A. baumannii* to clinical habitats is fostered by its persistence on dry surfaces. Moreover, *A. baumannii* is well adapted to host cells, thriving under iron- and phosphate-limitation at low pH-values and under oxidative stress. However, information with respect to molecular mechanisms of adaptation to clinical environments and the human host is scarce. Detection of three phospholipase D (PLD) genes in the genome of *A. baumannii* ATCC 19606 raised the question whether the PLD's play a role in infection and whether transcription of the *pld* genes is modulated by different physiological conditions found in host cells.
Methods: To analyse the role of phospholipases D in pathogenicity of *A. baumannii* a markerless mutagenesis system was established and used to generate *pld* mutants. These mutants were analysed in infection studies. Transcriptional regulation of the *pld* genes was analysed by qRT-PCR.
Results: All three phospholipases D of *A. baumannii* were found to play a role as virulence factors. Different stress conditions including growth phase and temperature were identified to affect *pld* gene expression.
Conclusions: The three PLD's act in a concerted manner as virulence factors and play a role in host cell invasion. In addition, the three *pld* genes undergo transcriptional regulation.

IBP35

Biochemical and molecular characterization of the parvulin-type PPIase PrsA2 of *Clostridium difficile*

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Introduction: *Clostridium difficile* is the main cause for nosocomial antibiotic associated diarrhea. Despite appropriate antibiotic treatment up to 10 % of the patients die, and in 40 % the disease relapses due to re-colonization by *C. difficile*. Because of this *C. difficile* is becoming a major burden for the health care systems of industrial countries. Two large toxins are the primary virulence factors that exert their functions by glucosylating small GTPases of enterocytes. This results in the destruction of tight junctions, infiltration by immune cells followed by heavy inflammation and development of pseudomembranous colitis.
Objectives: Besides the two toxins, other virulence factors and their contribution to development and progression of disease are only insufficiently studied. Under this aspect peptidyl-proly-*cis/trans*-isomerases (PPIases) constitute an interesting class of proteins, as many bacterial PPIases have been described in the context of virulence. Further on, the parvulin type PPIases PrsA of *Bacillus subtilis* and its homolog in *Listeria monocytogenes*, PrsA2, are membrane associated lipoproteins that are involved in the secretion of proteins including virulence factors. *C. difficile* has two putative parvulins (CD630_15570 and CD630_35000) with predicted extracellular location. Of these, CD630_35000 has the highest sequence homology to PrsA2 of *L. monocytogenes*, and was chosen for detailed analysis.
Materials and Methods: CD630_35000 (CdPrsA2) was cloned into the expression vector pSSBM106. Nine highly conserved amino acids in the catalytic cleft of CdPrsA2 were replaced by alanines using site directed mutagenesis. Wild type CdPrsA2 and its mutants were recombinantly produced in *B. megaterium*. Further on, a PrsA2-deficient mutant was generated in the *C. difficile* 630*Δerm* background using the ClosTron technology.
Results: PrsA2 and its site directed mutants could be produced in high yields and purity, and were correctly folded as assessed by CD-spectroscopy. By this, catalytically active amino acids as well as the substrate specificity of PrsA2 could be identified. The PrsA2-defficient mutant showed differences in its motility and the composition of its secretome as well as surface layer proteins when compared to its isogenic wild type.
Conclusion: Here we present initial results on the molecular cloning and enzymatic characterization of the secretory PPIase PrsA2 of *C. difficile*. Future studies aim at evaluating the influence of CdPrsA2 on virulence in more detail.

IBP36

Biochemical characterization of two distinct phospholipases C of *Acinetobacter baumannii*

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Question: In the past decades *A. baumannii* became an emerging threat in hospital environments. Multidrug resistance paired with an array of different virulence factors have led to the development of epidemic lineages with growing mortality rates. Among the virulence factors of *A. baumannii* are phospholipases C (PLC) and phospholipases D (PLD). Recently, we have identified three PLD of *A. baumannii* being important for cell adhesion and invasion as well as interbacterial competition (1). In addition, one of the two PLC encoded in the genome of *A. baumannii* has already been shown to mediate cytotoxic effects on epithelial cells (2). To get insights into the physiological role of both PLCs we performed biochemical analyses of both PLCs.
Methods: The *A. baumannii plc* genes were heterologously expressed as his-tag fusion proteins in *E. coli* and purified by Ni-NTA affinity chromatography. Biochemical analyses were performed with the purified PLCs.
Results: Both PLCs cleaved the cromogenic substrate *p*-nitrophenol phosphorylcholine giving rise to the products phosphorylcholine and *p*-nitrophenol. Maximum specific activity of both PLCs was detected between 37 °C and 40 °C. However, the maximum specific activity of PLC_2 was 10-fold higher than higher than the one of PLC_1. Furthermore, the activity of both PLCs differed significantly in dependence of the pH. The PLC_1 exhibited maximum specific activity at pH 7 whereas the optimum for PLC_2 activity was pH 9.
Conclusion: The differences of the PLCs in pH optimum and maximum specific activity suggest that the PLCs of *A. baumannii* play distinct roles under different physiological conditions.

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2. Camarena L., Bruno V., Euskirchen G., Poggio S., Snyder M., (2010) *PLoS Pathog* 6:e1000834.

IBP37

Gene-expression profiling of human monocytes after stimulation with pathogens of systemic infections

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In the innate immune response against microbes that cause bloodstream infections, human monocytes are of particular importance because they are able to phagocytose, release cytokines and chemokines and trigger adaptive immune responses. Genome-wide gene expression analysis of monocytes after stimulation with a fungal pathogen, a Gram-negative and a Gram-positive bacterium were used to dissect the response of monocytes towards different pathogen classes and identify pathogen specific patterns.
Monocytes of five healthy male donors were isolated from venous blood via Ficoll density centrifugation and CD14+ separation. The cells were stimulated with *A. fumigatus*, *N. meningitidis* or *S. aureus* for 3 h and 6 h. RNA of monocytes was used for microarray analysis. Differentially expressed genes (FDR < 0.05, Fold Change > 1.5) were determined for each pathogen.
The results showed that transcriptional patterns of monocytes are clustered *Aspergillus* or bacteria specific. Within the stimulated samples the expression pattern of both timepoints can be differenciated. Data revealed a comprehensive pathogen-independent core program of gene expression induced by all three pathogens. Within this group mainly genes encoding chemokines (e.g. *CCL7*, *CXCL5*), pro-inflammatory cytokines (e.g. *IL6*, *IL8*, *TNF*) or surface marker for activation or adhesion (*CD40*, *CD44*) were found. *A. fumigatus* induced a strong specific response on genes corresponding to the MAPK-signaling pathway (e.g. *MAP2K1*, *MAP4K3*, *MRAS*), to protein processing (e.g. *HSPA7*, *HSPA1L*) and to the TNF-receptor superfamily (e.g. *TNFSF14*, *TNFRSF10D*, *TNFRSF12A*). In contrast, the bacterial pathogens shared a large number of differentially expressed genes encoding chemokines (e.g., *CXCL, CXCL13*), cytokines (e.g. *Il19*, *Il16*) or transcription factors of the Jak-STAT signaling pathway (*STAT1*, *STAT2*).
These characteristic transcriptional differences between the pathogens can be used as a fundament for future studies to aid the diagnosis of sepsis and to characterize a pathogen specific immune response.

IBP38

Distribution of specific Glycopeptidolipids (GPLs) within subspecies of *Mycobacterium avium*

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Members of *Mycobacterium avium* (*M. avium*) belong to the direct and indirect human environment; they have been found in soils and waters worldwide. *M. avium* subsp. *hominissuis* (MAH) is an opportunistic pathogen responsible for infections in humans, swine and other mammals. *M. avium* subsp. *avium* (MAA) and *M. avium* subsp. *silvaticum* (MAS) are the causative agents of avian tuberculosis; *M. avium* subsp. *paratuberculosis* (MAP) causes Johne's disease in ruminants. Cell wall glycolipids (GPLs) are discussed to contribute to the virulence of *M. avium* [1] Different genes involved in the synthesis of GPLs would be expected to modify therefore indirectly the interaction of the bacteria with their hosts. The aim of this study was to investigate the distribution of genes involved in the synthesis of GPLs among subspecies of *M. avium* and among individual strains.
M. avium isolates of different origin were screened for the presence of six selected genes of the GPL cluster. All isolates had been characterized

before by MIRU-VNTR, RFLP and MLST genotyping. Altogether 76 *M. avium* isolates belonging to MAA (n=10), MAH (n=25), and MAP (n=40) originating from different hosts and some from the environment were investigated including the reference strains ATCC 25291 (MAA), ATCC 19698 (MAP), DSM 44175 (MAS), and additionally the ATCC 13950 (*M. intracellulare*). The isolates were tested for the presence of selected nonspecific (ns)GPL genes *gtfA, rtfA*, and *mtfC* as well as serotype-specific (ss)GPL genes: *mdhtA*, *merA*, *mtfF* by PCR using primers from Johansen *et al.*, 2009 [2].
The selected ssGPL genes *mdhtA*, *merA*, and *mtfF* were detected in most examined strains with the exception of two MAH isolates and *M. intracellulare.* All MAH and MAA strains, the reference strains of MAS and *M. intracellulare*, and 16 MAP strains (comprising 15 MAP-C strains and one MAP-S strain) revealed the three nsGPL genes *gtfA, rtfA*, and *mtfC*. In 24 MAP isolates no nsGPL gene sequences could be found. One bovine MAP strain lacked only the *gtfA* gene sequence. There was no association between presence or absence of the three nsGPL genes in MAP and a specific MAP genotype. The presence of *gtfA, rtfA*, and *mtfC* in more than one third of the studied MAP strains and in the MAS reference strain is in contrast to previous results of Eckstein *et al.*, 2003 [1].
In conclusion, the concept that MAP is lacking nsGPL genes has to be revised. Further investigations will have to clarify if presence of nsGPL genes is linked to specific membrane characteristics and different interactions of MAP strains with the host.

[1] Eckstein *et al.* (2003) Microbiology 149, 2797-2807;
[2] Johansen *et al.* (2009) BMC Microbiology 9:159 doi:10.1186/1471-2180-9-159.

IBP39

Establishment of a long term cell culture infection experiment for the analysis of intracellular adaptation of *Staphylococcus aureus.*

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Staphylococcus aureus is a Gram$^+$ bacterium that causes diverse diseases ranging from mild skin infections to severe illnesses like pneumonia and sepsis. However, this bacterium is also a commensal in the anterior nares or the skin. Lately, *S. aureus* was accepted as an intracellular pathogen that can survive inside non-professional phagocytic host cells. This intracellular persistence, which was already recorded over weeks (Tuchscherr *et al.*, 2015), enables hiding from the immune system and escaping antibiotic treatments. However, currently established cell culture infection assays allow proteome studies of the early adaption phase of only few hours post infection (p.i.) (Surmann *et al.*, 2015).
Here, we established a cell culture infection experiment which allows the proteome study of internalized *S. aureus* for longer time periods p. i.. Internalization of GFP labeled *S. aureus* HG001 into 16HBE14o- epithelial cells was allowed for one hour and extracellular bacteria were subsequently killed by addition of Lysostaphin. Daily, the epithelial cells were disrupted, and intracellular populations were counted and sorted via flow cytometry. Proteome analysis of sorted *S. aureus* was performed to follow the adaption to the intracellular milieu. In order to investigate the behavior of intracellular bacteria further, time-lapse microscopy was carried out for 48 h p. i.
Counting of infected 16HBE14o-cells and internalized *S. aureus* cells revealed that it was feasible to examine the intracellular bacterial population for up to six days after internalization. Sufficient amounts of intracellular bacteria could be collected until three days p. i. for proteome measurements. The intracellular bacterial titer increased during the first day p.i., a time at which it reached its maximum. Time-lapse microscopy revealed two different subpopulations of internalized *S. aureus* that differed in their replication rates. Thus, rapidly replicating *S. aureus* cells triggered lysis of the hosting epithelial cells, but a second subpopulation of host cells carrying smaller numbers of *S. aureus* was present during the whole time course of the experiment.
The workflow developed will permit the study of intracellular adaptation reactions of *S. aureus* until a few days p. i. The *set-up* will be applied for the comparison of clinical isolates, including community and hospital acquired *S. aureus* strains, in order to elucidate differences in adaptation strategies. Simultaneously, the host cell response to long term infection can be investigated.

Tuchscherr, L., *et al.* (2015) Sigma Factor SigB Is Crucial to Mediate *Staphylococcus aureus* Adaptation during Chronic Infections. *PLoS Pathog* 11: e1004870.
Surmann, K., *et al.* (2015) A proteomic perspective of the interplay of *Staphylococcus aureus* and human alveolar epithelial cells during infection. *J Proteomics* 128: 203-217.

IBP40

The fungal quorum sensing molecule farnesol impairs dendritic cell maturation

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Question: The quorum sensing molecule farnesol controls filamentation of *Candida albicans*. The mechanisms of which farnesol blocks the *C. albicans* yeast to hyphae form is well known; nevertheless, the impact of farnesol on host cells is poorly understood, especially in dendritic cells (DC), which are key regulators of immunity and promote an anti-fungal Th1 immune response. For this work, we investigated the effect of farnesol in dendritic cell maturation by analyzing phenotype and transcriptional response.
Methods: Human monocytes were isolated from buffy coats of healthy volunteers and differentiated into monocyte-derived dendritic cells (moDCs) in the presence of GM-CSF and IL-4. The immunophenotype of moDCs were addressed by FACS and the transcriptome analysis of DC in response to farnesol was performed by a whole-genome expression direct hybridization assay on a bead chip array.
Results: Transcriptional analysis of farnesol-mediated DC maturation revealed a high number of differentially regulated genes involved in cytokine-cytokine receptor interaction, cell adhesion molecules and antigen processing and presentation. Farnesol significantly interfered with the differentiation process from monocytes to DC. Reduced surface expression of key markers for maturation and antigen presentation (HLA-DR CD83, CD86 and CD80) could be observed. Furthermore, farnesol modulates the displacement of CD1 molecules. While CD1a showed almost no surface expression; CD1d, a molecule involved in invariant NKT (iNKT) cell activation, was increased on DC generated in the presence of farnesol. Interestingly, we found increased expression of PPARγ-associated pathways genes, which could explain the CD1a and CD1d expression pattern and might be a potential target addressed by farnesol.
Conclusions: Farnesol is a potent regulator of moDCs phenotype and is able to induce transcriptional rewiring in these cells. Further experiments will be performed to evaluate the possible role of PPARγ activation and signaling in farnesol-treated moDCs.

IBP41

Adaptation of *Acinetobacter baumannii* to desiccation

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Question: The opportunistic pathogenic bacterium *Acinetobacter baumannii* is emerging in intensive care units in hospitals worldwide [1, 2] with recent outbreaks also in German hospitals. *A. baumannii* has the extraordinary capacity to adapt to and survive in dry environments which enables the bacterium to persist in the hospital environment [3], but the molecular basis for this trait is unknown.
Methods: Growth of *A. baumannii* was analyzed in media with high osmolarities. The pool of compatible solutes in the bacteria grown under different conditions was investigated via NMR analysis. In addition, we studied the survival of *A. baumannii* on dry surfaces.
Results: A widespread strategy for the adaptation to low water activities is the uptake or synthesis of compatible solutes, small organic solubles that do not interfere with metabolism and other important cell functions [4]. Our experiments revealed the unusual compatible solute mannitol in *Acinetobacter*. Mannitol is synthesized by an unusual bifunctional enzyme, the mannitol-1-phosphate dehydrogenase/phosphatase.
Conclusion: Adaptation of *A. baumannii* to low water environments involves the synthesis of compatible solutes such as mannitol and glutamate.

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IBP42

Development of a flow cytometry based assay to determine the invasion frequency of *Yersinia pseudotuberculosis* into C2BBe1 cells

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Background: In order to determine the invasion frequency of *Y. pseudotuberculosis* into the adherent epithelial cell line C2BBe1 we developed an assay, which quantitatively and qualitatively reflects invasion frequency. Previously tested protocols using the gentamicin protection assay (GPA), gave only quantitative estimates of invasion, whereas pre-staining of the bacteria with fluorescent dyes and subsequent quenching of stained extracellular bacteria did not lead to satisfactory results due to the low frequency of invasion.
Methods: Surface epitopes of bacteria were first labelled with EZ-Link Sulfo-NHS-SS-Biotin, a biotinylated NHS-ester containing a disulphide bridge. The biotin moiety was subsequently ligated with the fluorescent labelled Avidin derivative Neutravidin. After infection of the intestinal C2BBe1 epithelial cells with labelled bacteria, cells were detached with trypsin-EDTA and subjected to reducing conditions (50 mM DTT). C2bbe1 cells were subsequently subjected to flow cytometry analysis, or counterstained with a specific antibody for immunofluorescence.
Results: Reduction of the disulphide virtually completely removed the fluorescent label from the bacteria. Treatment with DTT did not affect epithelial cell membrane integrity as assessed by propidium iodide staining.
Although labelling of bacteria with Neutravidin reduced invasion frequency to about one third of unlabelled control, the signal of intracellular bacteria could be readily detected with a flow cytometer.
Quantitative estimates were made by comparing and correlating the flow cytometry and GPA results in infections with different Yersinia species; and by comparing the flow cytometry results with microscopy-based quantitation of intracellular bacteria in immunostained infected cells.
Increasing invasion frequencies were observed for the low, medium and highly invasive *Yersinia* species *Y. similis*, *Y. pseudotuberculosis* and *Y. enterocolitica* in the GPA assay, the FACS based assay as well as in the immunostaining assay.
Conclusion: We developed a flow cytometric assay to quantitate invasion frequency of *Yersinia* spec. in intestinal epithelial cells.

IBP43

Effect of itaconate on *Salmonella* Typhimurium

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Itaconate (methylensuccinate) has recently been identified as one of the antimicrobial compounds produced by macrophages upon activation (*1,2*). This compound is a potent inhibitor of the key enzyme of the glyoxylate cycle, isocitrate lyase, which is important for survival of many pathogens within macrophages (*2-4*). Recently, we found that pathogens like *Yersinia pestis* possess an itaconate degradation pathway (*5*). The *rip* (***r***equired for ***i***ntracellular ***p***roliferation) genes involved in itaconate degradation can be found in a number of pathogens including *Salmonella* Typhimurium (strain SL1344). The *ripA*, *ripB* and *ripC* deletion mutants were severely attenuated in *cybb*$^{-/-}$ *nos2*$^{-/-}$ mice, confirming the importance of *rip* operon for the pathogenesis. Furthermore, the corresponding enzyme activities were detected in cell extracts of *S.* Typhimurium grown in the presence of itaconate. Surprisingly, the growth on acetate and itaconate was possible only if the medium contained traces of C_4-C_6 compounds like aspartate, glutamate, or citrate. The reason for that was down-regulation of enzymes involved in anaplerotic reactions responsible for the synthesis of C_4 dicarboxylic acids from acetyl-CoA. Indeed, we found that itaconate not only inhibits isocitrate lyase activity, but also represses the synthesis of the enzyme of the glyoxylate cycle. The glyoxylate cycle operon *ace* contains malate synthase (*aceB*), isocitrate lyase (*aceA*) and isocitrate dehydrogenase kinase/phosphatase (*aceK*), and is under transcriptional control of the repressor IclR. Interestingly, Δ*iclR* strain was capable to grow on minimal medium containing acetate and itaconate only. Note that the *iclR* deletion strain resembles *Y. pestis* natural phenotype, as *Y. pestis iclR* appears to be a pseudogene due to a frame shift (*6*). To explain the effect of itaconate on *ace* transcription, we studied binding kinetics of different metabolites to recombinant IclR; the working model will be discussed. To conclude, our study shows the dual effect of itaconate on the metabolism of *S.* Typhimurium and the relevance of itaconate degradation pathway during infection.

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IBP44

Interaction of the human pathogenic fungus *Aspergillus fumigatus* with cells of the innate immune system

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Aspergillosis is a common infection of immunocompromised hosts with a severe outcome, high mortality rates and yearly increasing incidences[1]. *Aspergillus fumigatus* (*Af*) propagates via small sized conidia and hyphae that invade the lung tissue and encounter innate immune cells, mainly macrophages and neutrophil granulocytes that form the first line of the host's immune defense[2]. However, in the context of immune deficiency the host is not able to efficiently clear a fungal infection. Our studies aim at revealing pathogenicity mechanisms of *Af.* Among others these include strategies of masking conidia from recognition by immune cells, modulation of the maturation of the phagolysosome (PL) and host immune response to increase the intracellular survival and spread the infection.
First, we purified conidia-containing PLs from a co-culture of macrophages and virulent *Af* wild type and non-virulent mutant conidia to determine the proteome via LC-MS.
Second, we selected several mutants that are altered in their surface structure and assayed their ability to induce an immune response. By measuring the oxidative burst and the amount of extruded neutrophil extracellular traps (NETs) we determined the immune response of neutrophil granulocytes. Live-cell imaging of neutrophils challenged with spores aimed at unraveling how the cells detect the pathogen. In addition, recognition of conidia by macrophages was analyzed with a phagocytosis assay.
We successfully purified PLs of macrophages containing wild-type or mutant conidia and identified a number of proteins that were differentially present. Among others, we found subunits of the vATPase V_0 domain and members of the Rab and LAMP family reduced in wild-type conidia-containing PLs.
For several *Af* surface proteome mutants we saw a reduction in the phagocytosis ratio.
The analysis of the PL proteome will help identifying proteins that are targeted by *Af* wild-type conidia to prevent a full maturation of the organelle and fungal degradation.
Analysis of surface mutants provides insights into how the fungus avoids recognition from the immune system and circumvents its clearance in immunocompromised patients.

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IBP45

Structure and dynamics of lipase specific foldase from *Pseudomonas aeruginosa*

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The Gram-negative human pathogen *Pseudomonas aeruginosa* causes a wide range of infections with severe morbidity and mortality cases. This bacterium produces several virulence factors, among them lipolytic enzymes which play a role for host invasion and degradation of host-cell membranes.[1,2] Studies of the extracellular lipase LipA revealed that this enzyme requires a cytoplasmic membrane bound steric chaperon called lipase-specific foldase (Lif) to obtain its catalytic active conformation. Lif binds and activates LipA inside the periplasm directly before LipA is released into the culture medium.
In vitro refolding experiments indicated high affinity (K_d = 5 nM) of the foldase for the inactive, so-called, "pre-native" LipA. Furthermore, LipA

activation is accompanied by an increase of alpha helical structures in the foldase indicating notable dynamics upon the formation of the foldase-lipase complex[3].
Here, we aimed to study the role of foldase for activation and secretion of LipA *in vivo* and *in vitro* by combining biochemical, mutational, structural, single molecule fluorescence and computational methods. Mutational studies revealed foldase residues essential for lipase activation located at the lipase-foldase interface. Single molecule fluorescence resonance energy transfer (smFRET) experiments indicated that roughly 30 % of the foldase exists in a binding-competent state even in the absence of the lipase. Additionally, we have observed hinge-movements in the foldase related to helix α8 and bending of helix α9 both presumably relevant for the release of the lipase by a postulated "push-out" mechanism. NMR data revealed possible interactions of the folding domain with the peptide which links the foldase to the bacterial inner membrane. These intramolecular interactions may play an important role for *in vivo* secretion and activation of lipase.

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IBP46

Proteome-wide thiol redox profiling in *Clostridium difficile* during diamide and bile acids stress

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Question: The spore-forming, Gram-positive human pathogen *C. difficile* has become the most frequent cause of hospital acquired diarrhea and serious intestinal inflammation. An increasing antibiotic resistance of the bacterium and a very high relapse rate call for new antimicrobial strategies. During infection, *C. difficile* is challenged with locally high concentrations of bile acids which have been assumed to cause disulfide stress in bacteria (Cremers *et al.* 2014). Alterations in the thiol proteome could have dramatic cellular consequences since cysteine residues play an important role in enzymatic reactions and can serve as regulatory switches. We pursue to establish an assay to comprehensively track changes in the thiol redox state of cysteines in order to investigate the suggested action of bile acids as instigators of disulfide formation in *C. difficile* 630Δerm**.**
Methods: A differential cysteine labeling protocol based on cysteine alkylation with metabolically labelled iodoacetamide was developed, and its feasibility demonstrated in *C. difficile* by stressing the cells with diamide which is well-known to provoke disulfide stress. Subsequently, the assay was applied to cells stressed with a cocktail of bile acids. Proteins were separated via SDS-PAGE, in-gel trypsinized and analyzed by LC-MS/MS. MaxQuant software was employed to evaluate the redox status of cysteines.
Results: Bioinformatics inspection of the *C. difficile* proteome reveals an extraordinary high content of the amino acid cysteine. The established differential cysteine labeling assay not only allows to determine redox-responsive proteins, but also enables to pinpoint the exact cysteine residues within protein sequences that are prone to oxidation. While diamide induces a massive and global oxidation in the proteome of *C. difficile*, bile acids could not be fixed as general instigators of disulfide stress.
Conclusion: The established method allowed for the first time a global view on the redox status of the cysteine proteome of *C. difficile*, but also supported a comprehensive protein quantification. It can serve as a basis to understand adaptational mechanisms of the bacterium in the intestinal tract, e.g. during the challenge with reactive oxygen and nitrogen species produced by the host.

IBP47

***nagA* and *nagB* are important for growth of *Streptococcus pneumoniae* in the presence of N-acetylglucosamine**

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We have explored the transcriptomic response of *Streptococcus pneumoniae* D39 to N-acetylglucosamine, an important carbon source for its colonization in the nasopharynx and pathogenesis. Transcriptome comparison of D39 wild-type grown in chemically defined medium (CDM) in the presence of N-acetylglucosamine to that grown in the presence of glucose revealed elevated expression of putative N-acetylglucosamine transport and utilization genes. The genes involved in the utilization of N-acetylglucosamine, *nagA*, *nagB* and *glmS*, are among the ones upregulated in the presence of N-acetylglucosamine. *nagA*, *nagB* and *glmS* are putatively regulated by a transcriptional regulator NagR. We predict the putative operator site of NagR in P*nagA*, P*nagB* and P*glmS*, which is further confirmed through promoter truncation experiments. Growth comparison of Δ*nagA*, Δ*nagB* and Δ*glmS* with the wild-type demonstrates that *nagA* and *nagB* are essential for growth in the presence of N-acetylglucosamine. Role of CcpA in the regulation of N-acetylglucosamine is also elucidated through microarray studies.

IBP48

Role of interaction between IncA protein of *Chlamydia trachomatis* and host protein G3BP1 in the increase of c-Myc protein concentration during infection of HeLa cells

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Question: The obligate intracellular bacterium *Chlamydia (C.) trachomatis* is one of the most common sexually transmitted pathogens and frequently found in association with neoplastic changes in cervical epithelium. *Chlamydiae* are characterized by a unique biphasic developmental cycle which proceeds in a vacuolar compartment termed inclusion. Through the delivery of effector proteins either into the chlamydial inclusion membrane or into the cytoplasm of the host cell this microorganisms directly manipulate eukaryotic pathways via protein interactions. The interaction between type III-secreted protein IncA of *C. trachomatis* and human protein G3BP1 was found by ourselves in a yeast two-hybrid screen. The aim of this work was to investigate the functional significance of this protein-protein interaction.
Methods: The interaction between IncA and G3BP1 was verified by GST pull down and co-localization experiments. HeLa cells were infected with *C. trachomatis* or transfected with expression vectors encoding IncA. Reactions of the cells were investigated by Western blot, immunofluorescence and qPCR experiments.
Results: The interaction between IncA of *C. trachomatis* and human G3BP1 was confirmed by GST pull down experiments *in vitro* using HeLa cell lysates. Moreover, using an immunofluorescence approach, we could show, that G3BP1 co-localized with IncA at the periphery of chlamydial inclusions. G3BP1 harbors a phosphorylation dependent RNase activity and acts as posttranscriptional regulator of *c-myc* mRNAs [1]. Infection of HeLa cells with *C. trachomatis* led to an increase of oncoprotein c-Myc concentration. To see whether interaction of IncA with G3BP1 yields a blockade of cMyc mRNA degradation we performed qPCR experiments. To our surprise cMyc mRNA concentration remained unchanged during course of infection. *C. trachomatis* is known to stimulate the Il-6, Il-6 receptor, STAT3 pathway - a further possible cause of the activation of the *c-myc* gene. Performing experiments with the STAT3 specific inhibitor Stattic we could exclude this reason for cMyc increase. However, it is undisputable that IncA somehow influences the cMyc protein concentration, as the sole overexpression of IncA in HeLa and HEK293 cells led to an increase of the oncoprotein concentration.
Conclusion: The increase of cMyc protein concentration during infection of HeLa cells with *C. trachomatis* is in terms of a possible involvement of the pathogen in formation of cervical cancer. The role of IncA and interacting G3BP1 in this process probably seems to be related to the regulation of cMyc protein stability as there are no changes in cMyc mRNA concentration.

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IBP49

Monitoring global protein thiol-oxidation and protein S-mycothiolation in *Mycobacterium smegmatis* under hypochlorite stress.

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Question: Mycothiol (AcCys-GlcN-Ins, MSH) is the major thiol-redox buffer in *Actinomycetes,* including *Mycobacterium* and *Corynebacterium* species. MSH contributes to protection against reactive oxygen species (ROS). We have previously shown that S-mycothiolation occurs in *C. glutamicum* under NaOCl stress (1). Protein S-mycothiolation controls the activities of several redox enzymes that function in detoxification of ROS and methionine sulfoxides, including the thiol peroxidase Tpx, the mycothiol peroxidase Mpx and the methionine sulfoxide reductase MsrA (2-4). Here we investigated the level of protein S-mycothiolation in *Mycobacterium smegmatis* under oxidative stress as well as its NaOCl stress response.
Methods: Fluorescent-label and MS-based thiol-redox proteomics methods (shotgun-LC-MS/MS and OxICAT) were applied for quantification of *S*-mycothiolated proteins in *M. smegmatis* under NaOCl stress. Thiol-metabolomics was used to quantify the level of MSH. The changes in gene expression during NaOCl stress were analyzed using RNA-Seq transcriptomics.
Results: MSH-deficient *M. smegmatis* mutants displayed an increased sensitivity to NaOCl stress. Protein S-mycothiolation was strongly increased in the wild type under NaOCl stress as shown by non-reducing MSH-specific Western-blot analyses while the level of MSH was depleted in the thiol-metabolome. We identified 58 S-mycothiolated proteins in NaOCl-treated cells by liquid chromatography-tandem mass spectrometry (LC-MS/MS). These proteins are involved in energy and fatty acid metabolism, MSH biosynthesis, protein translation, redox regulation and antioxidant functions. The level of oxidation could be quantified for 770 Cys-containing proteins using the OxICAT approach which include also the RseA and RshA anti sigma factors. Inactivation of RseA and RshA by oxidation leads to up-regulation of the SigH and SigE disulfide stress regulons under NaOCl stress as revealed by the transcriptome.
Conclusion: Our results demonstrate that protein S-mycothiolation is a widespread redox modification in *M. smegmatis* under oxidative stress which serves as thiol-protection mechanism and may also regulates protein functions which remains to be elucidated in future studies.

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IBP50

Structural basis for the inhibition of α-carbonic anhydrase by sulfonamides

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Question: Periplasmic α-carbonic anhydrase of *Helicobacter pylori* (HpαCA), an oncogenic bacterium in the human stomach, is essential for its acclimation to low pH. It catalyses the conversion of carbon dioxide to bicarbonate using zinc as the cofactor. In *H. pylori*, *Neisseria* spp., *Brucella suis* and *Streptococcus pneumoniae* this enzyme is the target for sulfonamide antibacterial agents. We have performed structural and functional studies of HpαCA to understand the mechanisms of *H. pylori* pathogenesis and enable its assessment as a target for drug design.
Methods: HpαCA has been purified and crystallized. Diffraction data sets for HpαCA complex with acetazolamide and methazolamide have been collected to 2.0 Å and 2.2 Å, respectively, using the MX1 & MX2 beamlines of the Australian Synchrotron.
Results: We present structural analyses correlated with inhibition data, on the complexes of HpαCA with sulfonamides acetazolamide and methazolamide which reveal that two sulfonamide oxygen atoms of the inhibitors are positioned proximal to the putative location of the oxygens of the substrate in the Michaelis complex, whilst the zinc-coordinating sulfonamide nitrogen occupies the position of the catalytic water. The structures are consistent with acetazolamide acting as site-directed, nanomolar inhibitors of the enzyme by mimicking its reaction transition state. Additionally, inhibitor binding provides insights into the channel for substrate entry and product exit.
Conclusions: This analysis has implications for the structure-based design of inhibitors of bacterial carbonic anhydrases.

IBP51

The *Aspergillus fumigatus* genome-wide deletion library

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The opportunistic human pathogenic mould *Aspergillus fumigatus* is able to cause severe invasive infections in immunocompromised patients. The most powerful tool to study the involvement of certain genes and proteins in virulence of this fungus is to generate targeted gene knock outs and to analyze the loss of function mutants for their phenotypes and virulence-associated traits. Up to now, these deletion strains were generated individually for each gene of interest which is often a time consuming process. To facilitate and accelerate research on *A. fumigatus*, we started to build up a library of deletion mutants for all of the approximately 10,000 genes of *A. fumigatus*.
Prerequisite for a knock-out library are accurately annotated genes. Therefore, the genome of the *A. fumigatus* A1163 derivative Δ*akuB* was re-sequenced and RNAseq data were used for re-annotation. Based on that, gene deletion primers were generated in an automated process and a streamlined workflow combining generation of deletion cassettes by a two-step PCR reaction, protoplast-based transformation, isolation of genomic DNA, and verification of the successful deletion by PCR was developed in 96-well plate format for high throughput application. This process proved to be successful for around 70 % of all genes in the first run. Furthermore, each mutant carries a unique barcode allowing identification of specific strains when pools of mutants are tested. As a first subset, we deleted 132 glycosylphosphatidylinositol (GPI) anchored protein encoding genes and started phenotypic analysis of the resulting mutant strains.
Once completed, the *A. fumigatus* knock out library will provide access to all feasible loss-of-function mutants. Characterization of larger groups of mutants will be possible instead of investigation of individual deletion strains that is costly in terms of time and labor.

IBP52

CRASP1 from *Aspergillus fumigatus* recruits Factor H, FHL-1 and C4BP and inhibits C3b deposition on the fungal surface

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Question: The opportunistic human-pathogenic fungus *Aspergillus fumigatus* is responsible for a wide spectrum of diseases in humans such as hypersensitivity pneumonitis, allergic asthma (AA), allergic bronchopulmonary aspergillosis (ABPA) as well as invasive aspergillosis in immunocompromised patients. *A. fumigatus* conidia activate the alternative pathway of complement and mark the fungal pathogen for opsonophagocytosis by human neutrophils. Conidia are the first fungal cells which invade the human host and which are immediately confronted by the host innate immune system. Evasion from complement attack is crucial for the survival of the pathogen and the onset of the infection. *A. fumigatus* acquires Factor H, Factor H-like protein 1 (FHL-1), Factor H-related protein 1 (CFHR1), and C4BP from the host[1], however, the fungal

complement regulator acquiring proteins (CRASP) remained unknown. Here we identify the first CRASP from *A. fumigatus* (CRASP1).
Methods: To identify factor H acquiring proteins from *A. fumigatus* ELISA and combined ELISA based western blot analysis (CEWA) was used. The binding affinity of *Aspergillus* CRASP1 to Factor H, FHL1 and C4BP, cofactor activity of recruited Factor H, FHL-1 and C4BP, C3b deposition on conidia surface, and phagocytosis of conidia by human neutrophils was studied.
Results: CRASP1 binds FHL-1 via SCR6-7 and Factor H via SCR domains 6-7 and SCR 19-20. CRASP1 bound purified FHL-1 and Factor H retains its cofactor activity and inhibits complement activation. *A. fumigatus* CRASP1 wild type conidia bind 30 % more purified Factor H and also recruit 25 % more Factor H from human serum to the surface than conidia of the CRASP1 knockout strain. Fungal surface recruited factor H results in reduced opsonophagocytosis by human neutrophils.
Conclusion: CRASP1 is the first fungal factor H and FHL-1 binding immune evasion protein from *A. fumigatus*. CRASP1 contributes to immune evasion of *A. fumigatus* by inactivation of human complement and reduced phagocytosis.

Behnsen *et al.* Infect Immun. 2008; 76(2):820-7.

IBP53
Beneficial infections: *Bacillus cereus sensu lato* isolates from root microbiome fight phytopathogenic *Verticillium* species
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The Rhizosphere of plants consists an extremely diverse microbial community including bacteria, fungi and viruses. It has been observed that some bacterial members of the community show an antagonistic effect towards some bacterial and fungal plant pathogens. These organisms play a crucial role in plant health and thus on crop productivity. Soil-borne fungi, like the broad host range tomato pathogen *Verticillium dahliae* and the *Brassicaceae*-associated oil seed rape pathogen *Verticillium longisporum*, can cause vascular wilt diseases. To determine the effect of plant associated *Bacilli* against these important phytophathogens, 267 *Bacillus* strains have been isolated from the rhizospheres of tomato plants using a *B. thuringiensis* enrichment protocol. Twenty strains of the isolates were picked randomly, genome sequenced and phenotypically characterized. All strains have been taxonomically analyzed by multi-locus sequence typing (MLST) (1). Thirteen isolates could be identified as *Bacillus weihenstephanensis* (Bw) strains and seven isolates as *Bacillus thuringiensis* (Bt) strains. All isolates have been investigated for growth inhibition on fungus strains *V. dahliae* JR2 and *V. longisporum* 43.
Ten strains were found to inhibit *V. dahliae* JR2 growth, strikingly all *B. thuringiensis* strains exhibited this phenotype. From the thirteen Bw isolates, exclusively three strains exhibited a mycoid colony morphology and modified the growth. An antagonistic effect on *V. longisporum* 43 was only observed for the three Bw but not for the Bt strains. However, there were non-inhibiting effects observed. Both fungi formed a ring of white air mycelium which was more dominant in *V. dahliae* JR2, and a stronger melanization surrounding all bacterial strains was found in *V. longisporum* 43. Both observed effects have been found to be depend on media simulating plant compartments resp. plant derived substrates. Interestingly, the inhibiting effect of our tomato plant isolated bacteria was stronger on the fungus isolated from tomato.
The genomes of the isolates as well as the genomes of the used reference strains have been screened for fungicidal factors. Fifteen chitinase genes were identified, two variants in each Bt strain and one in the Bw strains. In all non-inhibiting Bw strains no chitinase genes were detected. Further, 106 secondary metabolite producing non-ribosomal peptide syntethase (NRPS)/polyketide syntethases (PKS) gene clusters were identified. However, Zwittermicin A, a known fungicidal NRPS/PKS product, has not been found (2). Bt isolates as well as their toxins have been used for decades in biological crop protection against herbivore insects and nematodes, our new isolates might represent promising candidates to enable the application of Bt against plant pathogenic fungi.

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IBP54
AMIT – a novel algorithm for migration and interaction tracking for high-throughput analysis of phagocytosis assays
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Questions: Time-lapse microscopy is an important technique to study the dynamics of various biological processes. Imaging of biological systems combined with the analysis for functional, dynamical, and morphological aspects is required to increase our understanding of complex processes. In the context of interactions between human innate immune cells and pathogens, algorithms are required for automated high-throughput analysis of time-lapse microscopy videos of confrontation assays.
Methods: We present an automated segmentation and tracking framework for analyzing phagocytosis assays of polymorphonuclear neutrophils (PMNs) confronted with *C. glabrata*. The algorithm is based on our previously developed framework for tracking of non-rigid cells in brightfield microscopy [1] and was extended by (i) a segmentation approach for fluorescently-labeled pathogens and (ii) a state-transition-model and a cross-linking procedure for track interactions between PMNs and fungal cells.
Results: The PMN segmentation approach yields a sensitivity of 99 % and a precision of 95 % in object detection and the segmentation approach for fluorescently-labeled fungal cells yields a sensitivity of 84 % and a precision of 93 %. The phagocytic activity of PMNs is quantified in terms of percentage of phagocytically active PMNs, number of phagocytosis events per PMN, frequency of phagocytosis events, and killing rate of phagocytosed fungal cells. The findings of our automated analysis approach are directly compared with manual and experimental analyses [2,3] and yield high consistency.
Conclusion: AMIT comprises an automated segmentation and tracking framework and paves the way towards quantitative high-throughput analysis of time-lapse microscopy videos of confrontation assays.

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IBP55
A novel high throughput method for quantification of lectin-sugar interactions in the culture media
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Pseudomonas aeruginosa is a Gram-negative opportunistic human pathogen associated with chronic airway infections. Biofilms formed by *P. aeruginosa* contribute to its high resistance against antibiotics thus preventing efficient treatment of infections. Essential factors for biofilm formation and adhesion to human tissues are *P. aeruginosa* lectins LecA and LecB that can bind to surface polysaccharides[1,2]. Hence, *P. aeruginosa* lectins may serve as potential therapeutic targets.
The lectin LecB of *P. aeruginosa* is a carbohydrate-binding protein which forms a biological functional homotetramer and is attached to the cell-surface presumably interacting with the major porin protein OprF[3,4]. Interestingly, a *P. aeruginosa* strain lacking the *oprF* gene secrets LecB into the culture medium. The regulation of *lecB* expression and the mechanism of LecB secretion to the outer membrane remain to be elucidated.
To study the secretion of LecB we constructed a *P. aeruginosa* Δ*lecB*Δ*oprF* double mutant, which was used for expression of *lecB* from plasmid pBBC2 under the control of the weak *lac*-promoter. We showed by SDS-PAGE and Western blot analyses that in this strain LecB is not associated with the cells, but released into the bacterial culture supernatant allowing to monitor and quantify LecB secretion by determining the amount of extracellular LecB.
For quantification of LecB, we established a high throughput screening system which is based on a modified enzyme-linked lectin assay (mELLA). The supernatant obtained from the *P. aeruginosa* PA0I Δ*lecB*Δ*oprF* mutant carrying plasmid pBBC2 is transferred into microtiter plates coated with fucose-modified polyacrylamide. Upon binding to

fucose, LecB is detected after incubation with an anti-LecB antibody and a peroxidase-linked secondary antibody. We have optimized the expression conditions and succeeded to quantify the extracellular LecB in a concentration range of 50 ng - 5 µg/ml.
Using this method, we attempt to identify genes possibly involved in the regulation of *lecB* expression and secretion of the protein. To this end, a transposon library of *P. aeruginosa* PA0I Δ*lecB*Δ*oprF* was constructed using the vector-based transposition system pUTminiTn5-*luxCDABE*. The success of transposition was confirmed by measuring the luminescence of clones as the *luxCDABE* genes are active when the transposon integrates behind an active promoter. So far, the amount of extracellular LecB in several hundred transposon mutant clones was quantified using the modified enzyme-linked lectin assay. Several strains showing significantly affected LecB secretion are currently analyzed using inverse PCR and sequencing to identify transposon insertion sites.

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IBP56
The calcium-binding protein Frq1 is involved in the early pathogenic development of *U. maydis*
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Calcium is an important second messenger in fungi, plants and animals. It is involved e.g. in proliferation, cytoskeletal organization, secretion, apoptosis and pathogenesis. Little is known about calcium signalling in the pathogenic development of the maize smut fungus *Ustilago maydis*. To infect compatible haploid cells have to mate and subsequently form a dikaryotic filament which infects maize. Upon proliferation of *U. maydis* inside the plant the host forms tumours in which the teliospores are developed. During pathogenic development, especially calcium-binding proteins such as calmodulin are of interest, because they are the first proteins in decoding calcium transients.
Therefore, we are analysing small proteins that contain one or several calcium-binding EF-hand domains. Frq1 contains only EF-hands, but no other predicted domains in its 190 amino acid sequence. Deletion strains show reduced efficiency of mating and filamentous growth as well as less severe maize infections. Thus, Frq1 may play a role in establishing the infectious filament and in the early pathogenic development of *U. maydis.*
Analysis of calcium signalling will contribute to the understanding of pathogenic development of *U. maydis*.

IBP57
Redox regulation of hypoxic adaptation in *Aspergillus fumigatus*
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The mould *Aspergillus fumigatus* can cause life-threatening invasive infections in immunocompromised patients. In the site of infection the fungus faces many environmental stresses such as a dramatic drop in oxygen concentration. The ability to tolerate severely hypoxic environments represents an important virulence trait of *A. fumigatus*. Currently, many reports suggest that adaptation to hypoxia may be regulated by elevated production of ROS. In this project we aim to gain more insights into the mechanisms of how *A. fumigatus* maintains redox homeostasis during exposure to low oxygen levels. In particular, we want to reveal proteins that are specifically modified and thus can be responsible for hypoxic adaptation. Our results confirmed increased amounts of intracellular reactive oxygen intermediates in *A. fumigatus* exposed to low oxygen levels. Moreover, by applying gel-free redox proteomic approach we could identify proteins, which get reversibly oxidized by ROS after shifting oxygen content in the culture from 20 % to 0.2 %. For instance, proteins with a putative role in cellular copper ion homeostasis, assembly of respiratory chain complex IV and oxidative stress regulation were modified in one hour of hypoxic cultivation. Further investigation of biological functions of reversibly oxidized proteins will help us to elucidate essential pathways involved in sensing hypoxia by *A. fumigatus*.

MTP01
Conformational dynamics of the sodium/proline transporter PutP: Controlling access to the ligand binding sites
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Question: PutP of *Escherichia coli* is a membrane-integrated transport protein mediating the uptake of L-proline energized by the co-transport of sodium [1]. PutP is crucial for various bacteria-host interactions such as the virulence of *Helicobacter pylori* and *Staphylococcus aureus* [2,3]. Transporters work according to the alternating access mechanism, which implies an alternate opening of the transporter to either side of the membrane thereby exposing the central ligand binding sites to the periplasm or the cytoplasm [4]. Here, we explore the molecular details of the alternate opening and closing process.
Methods: A homology model of PutP [5] proposed domains of the transporter potentially involved in opening and closing of cavities. Cysteine accessibility studies were used to identify cavities in the protein as well as to observe ligand-induced conformational changes. Amino acid substitutions were introduced to identify positions crucial for PutP function, which was tested with transport and binding assays.
Results: Transmembrane domain 6' was found to be part of the inner cavity of PutP and harbors multiple residues important for proline binding at the inner end of this cavity. A ligand-induced closure of this cavity was observed. Furthermore, we showed that external loop 4 (eL4) controls the periplasmic entrance to ligand binding sites [6]. Interactions between Glu311 at the tip of eL4 and the backbone at the end of TM10' are crucial for closure of the periplasmic gate thereby stabilizing an inward-open conformation.
Conclusion: The results support the idea that PutP is *in vitro* most stable in its inward facing conformation. Proline binding induces an occluded conformation. External loop 4 acts as periplasmic gate controlling access to the ligand binding sites on the outside.

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MTP02
Zinc trafficking in *Cupriavidus metallidurans*
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The heavy metal resistant organism *Cupriavidus metallidurans* CH34 accumulates under in non-amended mineral salts medium 63.000 zinc atoms/cell. Chelation of divalent cations with 100 µM EDTA results in a similar number. In contrast, the cells accumulate 125.000 zinc atoms/cell when 100 µM zinc chloride were provided, which can't be raised by adding higher concentrations (1). To avoid toxic effects of high zinc concentrations a strict regulation of uptake, efflux, storage and allocation to client proteins is necessary. In *C. metallidurans,* the zinc uptake regulator Zur represses genes for zinc import and distribution systems at high zinc concentrations (2). Zur interacts with the promotor of *zupT* for the main zinc importer and of $cobW_1$, encoding a zinc chaperon. $CobW_1$ is part of the COG0523-family of P-loop GTPases. Two other members of this family ($CobW_2$, $CobW_3$) are annotated in the genome *C. metallidurans*. Their genes are in a cluster with *zur* and *dksA* for another putative transcription regulator. Expression of the genes of this cluster increases with zinc deficiency but the proteins are already present in the cell when grown in non-amended mineral salts medium. In contrast, the genes in the $cobW_1$ cluster are expressed only under zinc starvation conditions. Common features of the three CobWs are a GTPase domain with a typical Walker A- and Walker B-motif, a metal recognition site and a base recognition motif. The C-terminal region of these proteins is variable and harbors different numbers of metal binding amino acids. The proteins were purified and their metal content determined by ICP-MS. $CobW_3$ binds *in vitro* 5 mol zinc and $CobW_1$ 1 mol zinc per mol protein. The different binding capacities and the different expression patterns leads to the hypothesis, that $CobW_1$ is involved in zinc allocation under starvation conditions and the other two CobWs in zinc storage. With

pulldown experiments this hypothesis was verified and zinc depended proteins, as well as transport proteins could be found.

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MTP03
Relative quantitation of *Escherichia coli* phospholipids by MALDI TOF/TOF mass spectrometry in one step
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The inner membrane of *E. coli* consists of roughly 70-75 % phosphatidylethanolamine (PE), 20-25 % phosphatidylglycerol (PG) and 5-10 % cardiolipin (CL) with mainly three different fatty acid chains, C16:0, C16:1 and C18:1 (de Mendoza & Cronan, 1983). Using MALDI TOF/TOF mass spectrometry, a semiautomatic set-up was designed that allows a relative quantitation of *E. coli* glycerophospholipids, extracted from intact cells, in a single step and enables a detailed analysis of the distribution of head groups, fatty acids as well as a comparison of the fatty acid composition of individual phospholipids.
First, lipid standards of some phospholipids naturally occurring in *E. coli* were used to determine the parameters essential for mass spectrometry (MALDI target plate, matrix, laser intensity, peak resolution, etc.) and to analyze the properties of the different phospholipid species. For quantitation of phospholipid standards, the phosphate content was determined after incineration of the organic material. Whereas the fatty acid composition of a phospholipid has no influence, the variation in head groups has a high impact on the detection level within the MALDI TOF. As reason for this behaviour varying charges of the different phospholipid species (PE: zwitterionic; PG: one negative charge; CL: two negative charges) are discussed. To compensate the different characteristics “flight factors” were defined. Second, the identification of all phospholipids naturally occurring in *E. coli* was performed additionally to the accuracy of the molecular weight by fragmentation of molecules by post source decay (PSD). Choosing the optimized conditions, the m/z of fragments for the individual fatty acids, the separated head group and corresponding intermediates were analyzed. Based on these results, a lipid identification “filter” was created to allow a semi-automatic analysis of individual lipid extracts.
To verify the method, the phospholipid, head group and fatty acid composition of *E. coli* K-12 cells were determined at different growth phases and different growth temperatures. In addition, *E. coli* mutant strains carrying defects in the phospholipid head group biosynthesis were analyzed to confirm lipid classification. Interestingly, some of the mutant lipid extracts showed an enrichment in precursors, which were also identified.
In summary, after extraction of *E. coli* phospholipids with organic solvents, all lipid species were identified by MALDI TOF/TOF mass spectrometry in a single step. Determination of phospholipid concentrations and head group “flight factors” combined with the generation of a lipid identification “filter” now allows a rapid, detailed and comprehensive *lipidome* analysis for *E. coli*.

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MTP04
Super-resolution microscopy reveals bacterial cell-biological traits among Planctomycetes
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In 1924 Planctomycetes were first mistakenly described as fungi. However, soon their bacterial origin was acknowledged but ever since then, many eukaryotic related features were associated with Planctomycetes. For example, they were claimed to comprise a eukaryotic-like membrane compartmentalization a condensed nucleoid and an endocytosis-like uptake mechanism. Despite these traits that are usually associated with Eukaryotes, Planctomycetes were thought to lack the otherwise universal bacterial cell wall component peptidoglycan. However, many of such proposed conspicuous features are currently under debate, while some turned out to be misinterpretations.
Here, we revisit the controversy question of the planctomycetal cell architecture, employing various microscopic techniques together with genetic- and proteomic experiments. Since among Planctomycetes, *Planctopirus limnophila* is genetically accessible and *Gemmata obsucuriglobus* play a major role in planctomycetal research, we focused our studies on these organisms. First we developed additional tools that allow the constitutive cytosolic expression of GFP in *P. limnophila*. In addition, we used dyes such as FM4-64 and DAPI to stain the membranes and the nucleoid of cells. Thus the cytosol was stained green while the membranes were labeled red and the nucleoid blue. Appling Structure Illumination Microscopy (SIM) we were able to show invaginations of the inner cytoplasmic membrane into the cytosol. These observations could be verified using cryo electron tomography. A treatment with high sucrose concentrations could increase these invaginations and shows a similar effect in *P. limnophila* and *E. coli*. This treatment of *E. coli* is known to enlarge the periplasm and thus we conclude that Planctomycetes -other than proposed- do not possess an additional membrane system that divides the cytoplasm into two compartments, but that they comprise an unusual dynamically enlarged periplasm. Using immunofluorescence this hypothesis was further supported by the localization of the ATPases in the middle of the Cell in planctomycetes. Furthermore, we applied different proteomic approaches to analyze the membrane composition of *P. limnophila*.
To analyze the uptake of proteins and other macromolecules we used SIM and d-STORM to visualize their localization at super resolution (up to 50 nm). Applying these techniques we revealed, other than proposed, a non-vesical dependent uptake of GFP and fluorescent labeled dextran into the periplasmic space.
Our findings point towards a more Gram-negative like cell plan of *P. limnophila*, that lacks an eukaryote-like compartmentalization. However, the periplasm seems to be dynamic and is able to store polysaccharides that are likely to be transferred through the outer membrane via an unseen fiber mediated uptake mechanism.

MTP05
Substrate specificity of the Tat systems in *Bacillus subtilis*
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The twin-arginine translocation (Tat) system translocates folded and often cofactor-containing proteins across the cytoplasmic membrane. In *Bacillus subtilis*, two Tat systems exist that lack TatB components and are therefore regarded as minimal Tat systems with bi-functional TatA. Both systems are described to function only for specific substrates. However, when recombinantly produced in *Escherichia coli*, these Tat systems are able to translocate also other Tat substrates. Here we report the examination of their Tat substrate specificity in *B. subtilis* itself. We used a model Tat substrate that is efficiently translocated in proteobacterial TatABC systems and tested its transport in *B. subtilis*. Strains deficient in one or both Tat systems served to examine the exact pathway by which the proteobacterial Tat substrate can be exported out of the *B. subtilis* cell.

MTP06
Structural and functional analysis of KDEL receptors
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KDEL receptors (KDELRs) are responsible for the retrieval of soluble ER-resident proteins and have an important signaling role in maintaining Golgi homeostasis. Thereby KDELRs act as G-protein-coupled receptors and activate G-proteins (Gs and Gq) according to KDEL protein binding in the Golgi. Stimulation of Gq leads to an activation of Src family kinases resulting in tyrosine phosphorylation cascades and control of anterograde plasma membrane trafficking. Gs stimulation on the other hand leads to an activation of PKA and retrograde membrane transport (Cancino *et al.*, 2014). Recent studies have shown that KDELR1 regulates the integrated stress response (ISR) in T-cells by interaction with PP1, a key phosphatase in eIF2α control. Our group and others have recently shown that a minor fraction of KDELRs is also located at the plasma membrane where it enables binding of the neuroprotective factor MANF (Mesencephalic Astrocyte-derived Neurotrophic Factor) which is secreted after

thapsigargine-induced ER stress (Henderson *et al.*, 2013). The presence of KDELRs in the plasma membrane is also increased after thapsigargine treatment, pointing towards a cargo-oriented KDELR distribution. Three highly conserved endocytotic motifs within the receptor sequence additionally indicate an important KDELR function at the plasma membrane.
To analyze KDELR function at the plasma membrane in more detail, the project focuses on KDELR signaling, especially in response to cell surface binding of MANF. Through RNAseq analysis it should be possible to reveal MANF-dependent changes in nuclear gene expression. To examine if these changes are likewise KDELR-dependent, specific KDELR knock-down experiments will be performed in the next step. With the results of these experiments it should be possible to propose a model for KDELR signaling from the plasma membrane and to clarify by FRET microscopy if KDELR signaling involves receptor dimerization.

MTP07
A molecular chaperone dedicated to folding and translocation of reductive dehalogenases
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Reductive dehalogenases (*rdhA*, RDases) are key enzymes involved in anaerobic organohalide respiration (OHR), during which bacteria are able to use chlorinated compounds as terminal electron acceptors. RDases are redox enzymes containing FeS clusters and a corrinoid as cofactors, and are translocated across the cytoplasmic membrane by the Twin-arginine translocation (Tat) system. In members of *Dehalobacter* and *Desulfitobacterium* spp., the product of an accompanying gene, generally named *rdhT*, was recently proposed to play a role as molecular chaperone in the folding of the reductive dehalogenase (1,2). Recently, this finding was applied to heterologously produce active RDases (3). However, the mechanism by which the molecular chaperone acts on the maturation of RDases is not yet solved.
In this study, we investigate the diversity of RdhT chaperones in *Dehalobacter restrictus* and their interaction with the Tat signal peptide of their cognate redox component, as well as their specificity or cross-reactivity towards alternative signal peptides. To this respect, both *in vivo* and *in vitro* experimental approaches are conducted and will be presented.

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MTP08
The membrane proteome of *Escherichia coli* in response to high rate Tat-mediated protein secretion
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Escherichia coli is one of the most extensively used prokaryotic organisms for the industrial production of recombinant proteins. There are two major pathways of protein secretion in bacteria, the general secretion pathway (Sec-pathway) and the twin arginine translocation pathway (Tat-pathway)[1]. Although recombinant substrates are preferably exported to the periplasm via the Sec-pathway, this secretion platform suffers from severe limitations hampering the expression of heterologous proteins. Secretion via the Tat-pathway overcomes one of these limitations by its ability to transport fully folded proteins across membranes[2]. Nevertheless, strong expression of recombinant proteins invariably induces secretion stress which diminishes production efficiency[3]. Up to date the production stress in *E.coli* remains poorly understood.
This study focuses on understanding of alterations in the *E. coli* proteome during secretion stress. In order to obtain a most comprehensive picture of the entire proteome several methods for the separation of inner membrane, outer membrane and periplasmic proteins of *E. coli* have been evaluated (i.e. sucrose gradient centrifugation, extractions by sarkosyl, lithium chloride or glycine-HCl). Extracted subfractions were analyzed by SDS-PAGE and LC-MS/MS, followed by data visualization using Voronoi treemaps. Established protocols were applied to *E. coli* cells overproducing Tat substrates and misfolded derivates of those.
Robust and reliable methods for an enrichment of *E. coli* proteome subfractions have been established. The tracking of proteome alterations in *E. coli* during overproduction stress via the Tat pathway enables a better understanding of production bottlenecks and will enhance the usefulness of *E. coli* as an expression platform.

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MTP09
Characterization of the Tat-system in *Pseudomonas fluorescens*
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Twin-arginine translocation (Tat) systems transport folded proteins across the cytoplasmic membrane of most prokaryotes [1]. These systems are well-known to play crucial roles for pathogenic bacteria and their deletion results in markedly lowered virulence in almost all tested cases. However, their impact on the functional role for beneficial bacteria has not been studied to significant extent. We addressed this aspect in the plant growth promoting rhizobacterium (PGPR) *Pseudomonas fluorescens*. PGPR are thought to contribute to stress control of plants and improve plant growth by three major pathways:
1.) Direct or indirect antagonism against phytopathogens (Biocontrol)
2.) Enhancement of plant nutritional status (Biofertilizers)
3.) Regulation of plant hormones contributing to the reduction of plant stress (Phytostimulators)
Using a mutagenesis and complementation approach, we generated mutant strains that are affected in Tat transport components and tested the impact of such mutations on biological functions of *P. fluorescens*. The data revealed that key pathways of *P. fluorescens* strongly rely on the functional transport of Tat substrates.

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MTP10
Functional characterization of DASS member CitT, an obligate exchanger
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The citrate carrier CitT from *E. coli* is expressed under anaerobic conditions and together with a fermentable cosubstrate confers the ability to grow on citrate. It belongs to the Divalent Anion Sodium Symporter (DASS) family, which includes dicarboxylate transporters from bacteria, plants and mammals. Human members of this family (SLC13) play key roles in the regulation of fatty acid synthesis, adiposity and insulin resistance [1].
CitT was shown to be a citrate transporter which also recognizes succinate and tartrate [2]. The natural transport reaction is thought to be the exchange of citrate (nutrient) for succinate (final product of citrate fermentation). CitT was overexpressed in *E. coli*, purified and reconstituted into liposomes. Different transport assays using radioactively labeled substrates were performed to elucidate the transport mechanism.
Counterflow measurements revealed that CitT is an obligate exchanger which can transport citrate, succinate and fumarate. Intermediates of the citrate fermentation pathway (fumarate, malate and oxaloacetate) are not transported. CitT catalyzes homo- and heteroexchange. In contrast to most members of the family, it does not catalyze unidirectional transport coupled to a sodium (or proton) gradient.
The only predicted charged residue in the transmembrane domain, Asp170 is well-conserved among CitT homologs and is essential for catalysis.

[1] C. Mulligan *et al.*, *J Gen Physiol* 143 (2014), p. 745-759
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MTP11
Unfolding the mechanism of spore wall biosynthesis in *Myxococcus xanthus*
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Question: *Myxococcus xanthus* is a Gram-negative bacterium that differentiates into environmentally resistant spores. During this differentiation process, the entire rod-shaped cell is rearranged into a spherical spore. The vegetative cell peptidoglycan is degraded and functionally replaced by *de novo* synthesis of and assembly of a novel

glycan wall on the surface of the outer membrane. Synthesis and export of this unusual glycan, consisting of Glc and GalNAc (1:17) oligosaccharides and glycine, depends on the *exo* locus which encodes an atypical Wzy-like polysaccharide export pathway (Holkenbrink *et al.*, 2014). Our goal is to biochemically define synthesis of the spore wall glycan. We hypothesize that the first committed step is the transfer of UDP-GalNAc, or a modified UDP-GalNAc, onto the undecaprenyl-phosphate (C_{55}-P) lipid carrier, and propose that this function is fulfilled by the inner membrane protein ExoE. Here, we characterized this function of ExoE.
Methods: ExoE was cloned, expressed, solubilized from isolated membranes and purified. Purified ExoE was incubated with UDP-[^{14}C]-GalNAc and *E. coli* membranes enriched in the undecaprenyl-phosphate (C_{55}-P) lipid carrier. After incubation, the lipid-associated material was extracted, resolved by thin-layer chromatography and detected by Phosphorimaging.
Results: C_{55}-P-P-GalNAc was detected after incubation of ExoE with UDP-[^{14}C]-GalNAc and *E. coli* membranes enriched in the undecaprenyl-phosphate (C_{55}-P) lipid carrier, demonstrating that ExoE functions in the transfer of GalNAc to the undecaprenyl-phosphate. Here, we present a further characterization of ExoE.
Conclusion: *M. xanthus* ExoE plays an essential role in spore wall synthesis by linking GalNAc to the undecaprenyl-phosphate lipid carrier.

MTP12
Time resolved stability of flotillin domains *in vivo* in *B. subtilis*
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Flotillins are associated with functional membrane domains (FMMs), or lipid rafts in pro- and eukaryotes. FMMs are physically and functionally separated parts of the plasma membrane. Due to the different lipid and protein composition of these domains they contribute in supplying a suitable environment for vital cellular processes including protein secretion, signal transduction, transport and cell wall metabolism.
FMMs are known to be highly dynamic and are known to interact, but the molecular details of how prokaryotic flotillins interact with each other are unknown. The question if these domains interact by the exchange of protein, or are stable long lived bodies on a flotillin protein level was addressed. Therefore, two flotillins, FloT and FloA, from *Bacillus subtilis* were tagged with the photo-convertible fluorescent protein Dendra2. Single FMMs were photo-converted and growing cells were imaged over time. Interaction or subunit exchange between the different FMMs was determined by measuring change in Pearson Correlation Coefficient (PCC). PCC is used to determine co-localization of photo-converted and not photo-converted FMMs, a gain in co-localization indicating an exchange of protein between different domains.
The time resolved PCC value does not change for both of the flotillins and for extended periods of time, indicating FMM associated flotillins are not rapidly interchanged between different domains. The method established for correlated co-localization after photo-conversion (CCAP) could be used to address protein complex dynamics in general and on multi- and single cellular levels.

MTP13
Towards the function of the lipid binding protein PA3911 from *Pseudomonas aeruginosa*
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The Gram-negative bacterium *Pseudomonas aeruginosa* is an opportunistic pathogen which is colonizing the lung of patients suffering from the genetic disorder cystic fibrosis. In the chronic state of infection, *P. aeruginosa* grows as a biofilm in the airways of cystic fibrosis patients showing high resistance to antibiotics [1]. A proteomic approach revealed that protein PA3911 from *P. aeruginosa* PAO1 is one of the most up-regulated proteins under anaerobic biofilm conditions which mimic the situation in the cystic fibrosis lung. To date no biological function for PA3911 is described in the literature. From theoretical analysis a potential function as a lipid carrier protein was proposed.
Phosphatidic acid was demonstrated as a ligand of purified PA3911 protein using commercially available membrane lipid strips™ (Echelon® Biosciences Incorporated). Furthermore a theoretical protein structure model [2] was used to identify residues of the putative lipid binding pocket of PA3911. Site-directed mutagenesis and subsequent lipid binding experiments allowed to partially characterize the proposed lipid binding pocket.
The physiological relevance of PA3911 was analyzed with several motility assays (twitching, swimming, swarming). The PA3911 transposon mutant PW7609 [3] showed increased twitching motility when compared to the wild type strain, whereas the swimming and swarming motility were not altered. Besides this, it was demonstrated that strain PW7609 is also impaired in biofilm formation using a standard 96 well microtiter plate biofilm assay. These findings underscore the physiological relevance of PA3911 protein under biofilm conditions.

[1] Lau *et al.* (2005). Modulation of lung epithelial functions by *Pseudomonas aeruginosa*. *Trends Microbiol* 13, 389-397
[2] Biasini *et al.* (2014). SWISS-MODEL: modelling protein tertiary and quaternary structure using evolutionary information. *Nucleic Acids Res.* 42, W252-W258
[3] Jacobs *et al.* (2003). Comprehensive transposon mutant library of *Pseudomonas aeruginosa*. *Proc Natl Acad Sci USA* 100(24), 14339-14344

MTP14
Marine phages as excellent tracers for reactive colloidal transport in porous media
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Question: Here we evaluate marine phages as specific markers of hydrological flow and reactive transport of colloidal particles in the Earth's critical zone (CZ). Marine phages and their bacterial hosts are naturally absent in the CZ, and can be detected with extremely high sensitivity. In the framework of the DFG Collaborative Research Center AquaDiva, we asked the following questions: (1) Are marine phages useful specific markers of hydrological flow and reactive transport in porous media? (2) Which phage properties are relevant drivers for the transport of marine phages in porous media?
Methods: Seven marine phages from different families (as well two commonly used terrestrial phages) were selected based on their morphology, size and physico-chemical surface properties (surface charge and hydrophobicity). Phage properties were assessed by electron microscopy, dynamic light scattering and water contact angle analysis (CA). Sand-filled laboratory percolation columns were used to study transport. The breakthrough curves of the phages were analyzed using the clean bed filtration theory and the XDLVO theory of colloid stability, respectively. Phages were quantified by a modified high-throughput plaque assay and a culture-independent particle counting method approach.
Results: Our data show that most marine tested phages exhibited highly variable transport rates and deposition efficiency, yet generally high colloidal stability and viability. We find that size, morphology and hydrophobicity are key factors shaping the transport efficiency of phages. Differing deposition efficiencies of the phages were also supported by calculated XDLVO interaction energy profile.
Conclusion: Marine phages have a high potential for the use as sensitive tracers in terrestrial habitats with their surface properties playing a crucial role for their transport. Marine phages however, exhibit differences in their deposition efficiency depending on their morphology, hydrophobicity and availability.

MTP15
Characterization of proteins involved in DNA processing and transfer encoded within the GGI of *Neisseria gonorrhoeae*
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Question: *Neisseria gonorrhoeae* is a Gram-negative β-proteobacterium that belongs to the genus *Neisseria*. The genus comprises 11 species of which only *N. gonorrhoeae* and *Neisseria meningitidis* are human pathogens, causing diseases like gonorrhea and meningitis, respectively. A main feature of *N. gonorrhoeae* is its natural competence, where the DNA provided for uptake is either released by autolysis or by a unique Type-IV-Secretion System (T4SS). This T4SS is encoded on a 57-kb large likely horizontally acquired genetic island (GGI), which is present in about 80 % of all clinical isolates of *N. gonorrhoeae*. This T4SS belongs to the small

group of contact-independent T4SSs and is the only known system that secrets DNA directly into the extracellular milieu. Next to its role in DNA transfer, the secreted DNA also plays an important role in attachment to surfaces and biofilm formation.
Conjugative T4SS consist of proteins which form the transport channel (also called mating pair formation (MPF) complex) and of DNA processing and transfer proteins (Dtr). The MPF complex of the T4SS encoded on the GGI resembles the MPF of the F-plasmid. The putative Dtr proteins are encoded within an operon that encodes, next to the relaxase TraI and the coupling protein TraD, the small membrane protein Yaa and the putative accessory protein Yaf. These proteins resemble proteins often found together with relaxases of the large Mob_H family. In contrast to relaxases of other Mob families, no members of the Mob_H relaxases have currently been characterized. Here we will present an initial characterization of the role of these proteins in the secretion of the ssDNA.
Methods and results: Analysis of deletion mutants of *yaf*, *traI*, *traD* and *yaa* revealed that TraI and TraD are important for DNA secretion, but that no effects could be observed for the deletion of *yaf* [1], [2]. Remarkably deletion of *yaa* resulted in a strong increase of DNA release [2]. Here we will present our initial attempts to further characterize these proteins.
Conclusion: Relaxases of the Mob_H family form one of the largest families of relaxases. Currently the mechanism by which Mob_H relaxases process DNA and how the DNA is further targeted is unknown. Characterization of the relaxase and other proteins involved in DNA processing and transfer encoded within the GGI of *N. gonorrhoeae*, helps to understand the mechanism of DNA secretion by the T4SS of *N. gonorrhoeae* and helps to understand DNA transport facilitated by other members of the Mob_H family.

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[2] E. Pachulec, K. Siewering, T. Bender, E.-M. Heller, W. Salgado-Pabon, S. K. Schmoller, K. L. Woodhams, J. P. Dillard, and C. van der Does, *PLoS One*, vol. 9, no. 10, Oct. 2014.

MCP01
Semi-automated statistical quantification of initial colonization of bacteria on different materials under standardized conditions
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The formation of biofilms on different materials provokes high costs in industrial processes, as well as in medical applications. Therefore, the interest in development of new materials with improved surfaces to reduce bacterial colonization rises. In order to evaluate the quality and safety of these new materials, it is highly important to ensure world-wide comparable tests that are relying on statistical evidence. The only way to reach this statistical safety is through a high-throughput screening under standardized test conditions.
We developed a flow through system for cultivation of biofilm-forming bacteria under controlled conditions with a total capacity for testing up to 32 samples in parallel [1]. Quantification of the surface colonization was done by staining the bacterial cells with a fluorescence marker, followed by epifluorescence microscopy. More than 100 images of each sample were automatically taken and the surface coverage was estimated with the free open source software gmic (http://gmic.eu), followed by a precise statistical evaluation. Overview images of all gathered pictures of the whole material coupon were generated to illuminate the colonization characteristics of the selected bacteria on certain materials.
With this method, differences in bacterial colonization on different materials can be quantified in a statistically validated manner. The innovative and solid test procedure will support the design of improved materials for medical and industrial applications such as implants, ship hulls, pipelines, heat exchangers, aquaculture equipments, photovoltaic-panels and fundaments of wind power plants.

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MCP02
Extracellular matrix structure governs invasion resistance in bacterial biofilms
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Many bacteria are highly adapted for life in communities, or biofilms. A defining feature of biofilms is the production of extracellular matrix that binds cells together. The biofilm matrix provides numerous fitness benefits, including protection from environmental stresses and enhanced nutrient availability. Here we investigate defense against biofilm invasion using the model bacterium *Vibrio cholerae*. We demonstrate that immotile cells, including those identical to the biofilm resident strain, are completely excluded from entry into resident biofilms. Motile cells can colonize and grow on the biofilm exterior, but are readily removed by shear forces. Protection from invasion into the biofilm interior is mediated by the secreted protein RbmA, which binds mother-daughter cell pairs to each other and to polysaccharide components of the matrix. RbmA, and the invasion protection it confers, strongly localize to the cell lineages that produce it.

MCP03
Cell-cell communications in *Bacillus subtilis* mixed-species biofilms
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Biofilm development in diverse bacteria has been shown to respond to multiple environmental signals like small molecules secreted by microorganisms. These signals have been usually considered to be self-generated, i.e. quorum-sensing, but can also be produced by other organisms living in the vicinity, thus creating an interspecies communication network. *Bacillus subtilis* is a Gram-positive model bacterium for studying biofilm formation. It differentiates into several subpopulations of specialized cell types in response to different environmental cues thus making it an ideal model for studying complex signaling networks. Multiple soil bacteria can produce small signaling molecules that influence biofilm formation by *B. subtilis*. We aim to identify such organisms and to characterize the chemical nature and signaling pathway of novel signaling molecules that are able to modify the architecture of *B. subtilis* biofilms.Bacteria isolated from soil samples were screened for their ability to produce signaling molecules able to modify the structure of *B. subtilis* biofilms. Five soil isolates were selected for further characterization due to their ability to modify *B. subtilis* complex colony structures. These bacteria were identified through 16S rDNA sequencing. Four of the isolates were identified as either Lysinibacillus sp. or *Bacillus pumilus*, which are closely related to *B. subtilis* and thus may share similar signaling mechanisms. However, one bacterium was identified as Acinotebacter sp. which is a Gram-negative organism and thus likely to affect *B. subtilis* biofilm development via a distinct mechanism. The culture supernatants of selected bacteria were submitted to chromatography, enzymatic, and biochemical analysis to discern the nature of the signaling molecules. The purine derivative hypoxanthine was identified as a signaling molecule produced by one of the *Lysinibacillus* sp. isolates. *B. subtilis* is able to recognize and respond to several signaling molecules produced mainly by members of closely related genus but also from non-related organisms that may share the same ecological niche. Hypoxanthine is one such molecule that has shown to increase wrinkle formation in complex colony biofilms of *B. subtilis*. The signalling pathway of hypoxanthine on *B. subtilis* is being studied through the use of knock-out mutants and fluorescent gene-expression reporter fusions.

MCP04

The role of the phosphodiesterase NbdA in NO-induced biofilm dispersal of *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is an important opportunistic human pathogen causing a variety of nosocomial infections including pneumonia, sepsis, catheter and urinary tract infections. The bacterium has become a model system for biofilm research because of its resistance to conventional antibiotics, host antimicrobial effector mechanisms and its ability to form biofilms. Dispersal is considered as the last step of the biofilm life cycle being a process used by bacteria to transfer from sessil to motile lifestyle. Changes in c-di-GMP levels have been shown to be associated with biofilm detachment in a number of different bacteria. The signalling molecule nitric oxide (NO) induces biofilm dispersal through stimulation of c-di-GMP (bis-(3‘-5‘)-cyclic dimeric guanosine monophosphate) degrading phosphodiesterase (PDE) activity. We previously characterised the membrane-bound proteins MucR and NbdA (NO-induced biofilm dispersion locus A) which both share an identical domain organisation consisting of MHYT-GGDEF-EAL regarding their role in NO-induced dispersal. Inactivation of *mucR* impaired biofilm dispersal in response to glutamate and NO while deletion of *nbdA* only negatively affected biofilm detachment upon exposure to NO. The relevance of NbdA in NO-induced dispersal was further supported by increased PDE activity but reduced c-di-GMP levels upon exposure to NO in wild type biofilms in comparison to biofilms of *nbdA* mutant. Moreover, we demonstrated *nbdA* to be transcriptionally activated after addition of NO. Biochemical analyses of recombinant protein variants lacking the membrane-anchored MHYT-domain revealed NbdA being an active PDE. In contrast, MucR showed diguanylate cyclase and PDE activity *in vitro* [1]. *P. aeruginosa* strains lacking *nbdA* and *mucR* were phenotypically characterised to further elucidate the specific role of MucR and NbdA.

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MCP05

Characterization of ExeM, an active extracellular nuclease required for biofilm formation of *Shewanella oneidensis* MR-1

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Biofilms are the predominant lifestyle among all bacteria. While providing cells with an increased ability to withstand physical stress as well as antibiotics and starving conditions it may also increase virulence of many pathogenic species. Extracellular DNA (eDNA) is a ubiquitous component of bacterial biofilms serving as an important structural part of the matrix. How eDNA is produced, modulated and degraded (e.g. to release cells from the biofilm) is still sought to be understood.
It has been shown that extracellular nucleases are able to degrade eDNA in both planktonic cultures and bacterial biofilms to induce biofilm dispersal, for structural modulation of the biofilm matrix, utilization of DNA as nutrient source, control of horizontal gene transfer, and escape from neutrophil extracellular traps. We have previously demonstrated that the extracellular nuclease ExeM (SO_1066) is a major contributor for biofilm formation of *Shewanella oneidensis* MR-1, as the deletion of *exeM* resulted in large amounts of accumulated eDNA under biofilm conditions. Furthermore, addition of purified MBP-ExeM resulted in inhibition of biofilm formation.
Here, we further explore the characteristics of ExeM with respect to function and ability to influence biofilm formation in *S. oneidensis* MR-1. We performed *in vivo* and *in vitro* studies on various mutant versions of the protein and determined activity and co-factor requirements. In addition, the results strongly indicate that at least a significant amount of the protein locates to the inner membrane due to a C-terminal membrane anchor which may require further processing by a rhombosortase for effective further transport and release. The study provides first more in-depth insights into the activity and transport of this well-conserved nuclease.

MCP06

Local c-di-GMP signaling by a network of interacting GGDEF/EAL domain proteins involved in *E. coli* biofilm control

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c-di-GMP is a ubiquitous bacterial second messenger that in general promotes biofilm formation. *E. coli* K-12 has 29 proteins with GGDEF/EAL domains, which include 12 producers of c-di-GMP (diguanylate cyclases or DGC) and 13 degraders (phosphodiesterases or PDE). The multiplicity and sometimes high target specificity of these enzymes has led to the idea of 'local' signaling involving direct protein-protein interactions (1). With the PdeR/DgcM/MlrA module having been demonstrated as a prototypical example of a locally acting c-di-GMP control, in which a DGC and a PDE directly interact with each other and with a target protein (2), the question arose whether other DGCs and PDEs are involved in similar local actions.
Using the BacterioMatch-Two-Hybrid system, we systematically investigated interactions among all 29 GGDEF/EAL domain proteins of *E. coli* K-12. Surprisingly, we did not find additional specific DGC/PDE pairs involved in single complexes, but rather observed that a subset of the GGDEF/EAL domain proteins has even multiple interaction partners. Interestingly, in all these interactions, at least one of the partners belongs to the central switch module (DgcE/PdeR/DgcM/MlrA) known to play a key role in the control of the biofilm regulator CsgD and therefore the production of the biofilm matrix components curli and cellulose. Among other GGDEF/EAL domain proteins, however, no interactions were observed.
We conclude that with respect to direct protein-protein interactions, the complement of GGDEF/EAL domain proteins fall into three classes: (i) core components of the central c-di-GMP switch module that control biofilm matrix synthesis (PdeH, DgcE, PdeR, DgcM), (ii) accessory components that may conditionally modulate the output of this switch by direct interactions with the core switch proteins, and (iii) DGCs and PDEs with enzymatic activity only that act independently of other GGDEF/EAL domain proteins to affect the cellular c-di-GMP level which is sensed by PdeR as a key component of central switch module.

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MCP07

Investigation of the biofilm development regarding the EPS formation of *gfp*-expressing *Halobacterium salinarum* R1

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Introduction: Biofilms are sessile communities of microorganisms within a matrix of extracellular polymeric substances (EPS). Based on a lot of advantages like the resistance against environmental influences, the biofilm formation is the dominant mode of life. The EPS formed by bacteria consists of different substances such as carbohydrates, proteins, lipids, or extracellular DNA (eDNA) [1]. In contrast the formation of archaeal biofilms as well as the single constitutes of EPS compounds are not yet known. One method to visualization of biofilms and EPS by confocal laser scanning microscopy (CLSM) is the tagging of cells with a fluorescent reportergene (*gfp*) and the staining of different EPS compounds with fluorescent dyes.
Objective: The aim of this study was to investigate biofilm formation with regard to the EPS development of *gfp*-expressing *Hbt. salinarum* R1.
Methods: The *E. coli / Hfx. volcanii* shuttle plasmid pP2JB87 containing a gene encoding a high salt tolerant GFP [2] was used to transform *Hbt. salinarum* R1. The sessile cells were grown to different stages and EPS compounds were stained with different fluorescent dyes. The biofilm structures formed by the *gfp*-expressing *Hbt. salinarum* R1 cells were visualized by CLSM.
Results: The biofilm development investigated by CLSM showed a primary attachment of cells followed by growth of bulky tower-like microcolonies. Extracellular substances, containing glycoconjugates with terminal α-D-mannosyl and α-D-glucosyl groups as well as N-acetyl-D-glucosamine and sialic acids, were found with a defined arrangement inside the microcolonies. The presence of eDNA was proven as early as after one day of biofilm formation. An increase of the amount of EPS was observed with the ageing of the biofilm.
Conclusion: *Gfp*-expressing *Hbt. salinarum* R1 cells in combination with EPS specific fluorescent dyes can be used to investigate the biofilm

structures and the EPS composition by CLSM. It is possible to observe defined arrangement of different EPS compounds inside the microcolonies and an increase of the EPS during the biofilm development.

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[2] Reuter, Ch. J.; Maupin-Furlow, J. A. (2004). *AEM* 70 (12): 7530-7538

MCP08
Proteomic profiling of *Clostridium difficile* biofilms
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Question: Bacterial infections are often related to the formation of mono- and mixed species biofilms where cells are embedded in a complex matrix, which causes an increased resistance to various stresses, in particular antibiotics and the host immune system. *Clostridium difficile*, an anaerobic gut pathogen, has been isolated from multispecies biofilms and is able to form mono- and mixed-species biofilms *in vitro*. Understanding *C. difficile* biofilm physiology on a global scale is an important prerequisite to fight *C. difficile* infections.
Methods: A comprehensive label-free proteomics analyses (GeLC-MS/MS) comparing the whole-cell proteome of planktonic and biofilm-grown cells was employed to identify proteins differentially expressed during biofilm formation and to define biofilm-associated regulatory networks of the *C. difficile* reference strain 630Δerm. Biofilms were either grown as colony-biofilms on membrane filters or as aggregated cells in liquid medium.
Results: According to our comparative proteome analysis of *C. difficile* 630Δerm grown planktonically or in two biofilm-setups, the majority of the proteins involved in biofilm formation are regulated by alternative sigma factors, i.e. SigH, Spo0A, CodY, and CcpA. In both biofilm models various proteins involved in sporulation, fermentation and toxin production were strongly expressed compared to planktonic cells. The biofilm expression profiles are strongly dependent on the biofilm setup. In aggregated cells proteins involved in motility, alteration of the cell surface (i.e. CapD, MshA, SunS), drug-efflux (i.e. GlnQ, YbhF and DrrA) were found to be highly abundant, whilst in colony biofilms proteins involved in anaerobic sulfite reduction (AsrABC) and lactate utilization were found to be strongly expressed when compared to their planktonic counterparts. We suggest colony biofilms as an ideal model to investigate starvation processes, whilst aggregated cells are better suited to investigate cell-to-cell interactions and signaling in dense populations.
Conclusion: The results indicate (I) a significant difference in the physiology of the biofilms grown in two model-systems and (II) that biofilm formation is a complex process, in which sporulation, toxin-production and motility can be massively effected by the sessile lifestyle.

MCP09
Impact of lactose and low temperatures on biofilm formation of bacteria isolated from milking machine biofilms
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Biofilms in milking machines are a source of recurrent contamination of raw milk with potential spoilage organisms and/or pathogens. Biofilm formation at low temperatures enables bacteria to persist in storage tanks, where milk is usually held at about 4 °C. The effect of lactose, the main carbohydrate of milk, was assessed as a supporting or inhibiting factor for biofilm formation in this study.
Bacterial isolates from a milking machine were identified by fatty acid analysis and 16S rRNA gene sequencing and tested for biofilm formation in polystyrene microplates. Biofilm forming potential was compared in tryptic soy broth (TSB) containing glucose or lactose, after incubation at 30 °C and 4 °C, respectively. Biofilm formation was quantified by a standard test based on crystal violet staining and measurement of absorption.
Lactose enhanced the biofilm formation for strains of the species *Kocuria salsicia*, *Macrococcus caseolyticus*, *Gordonia paraffinivorans* and *Brevundimonas vesicularis*, while the biofilm forming ability decreased for *Bacillus pumilus*, *Raoultella terrigena*, *Empedobacter falsenii* and *Pseudomonas gessardii*. Strain-specific differences in the reaction to lactose were observed for *Acinetobacter guillouiae* and *Stenotrophomonas maltophilia*.
Incubation at 4 °C enhanced the biofilm forming ability of *Curtobacterium plantarum*, *Pseudomonas koreensis* and *Pseudomonas poae*. No effect or a slightly increased biofilm formation at 4 °C was observed for most of the *Acinetobacter guillouiae* isolates, but only in TSB containing glucose. Exchanging glucose by lactose led to a decreased biofilm formation at 4 °C, but not at 30 °C. Despite being able to grow at 4 °C, strains of the species *Pseudomonas gessardii*, *Raoultella terrigena* and *Acinetobacter johnsonii* showed a decreased biofilm forming ability compared to 30 °C.
Biofilms in milking machines on dairy farms may affect raw milk quality and safety. In our study, we were able to identify and characterize bacterial genera present in milking machine biofilms. Testing the effect of lactose and low temperatures on in-vitro biofilm formation is an important first step towards the identification of factors stimulating the process of biofilm formation in the dairy environment.

MCP10
The major biofilm regulator RemA is responsible for transcriptional induction of the uptake systems for the osmoprotectant glycine betaine in *Bacillus subtilis*
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B. subtilis is a soil living organism that colonizes its habitat as vegetative cell, as highly resistant endospore or as a multicellular biofilm. A major challenge in the soil habitat are frequent changes in the osmolarity. *B. subtilis* counteracts high salinity induced osmotic stress through the uptake of compatible solutes. Glycine betaine is such an osmostress protectant and *B. subtilis* imports it through the OpuA-, OpuC- and OpuD transporters. Recently Winkelmann *et al.* (Mol. Microbiol. (2013) 88, 984-997) described RemA as a new major regulator essential for biofilm formation and found that the *opuA* operon was also a direct target of the RemA activator protein.
Question: Which role plays the biofilm regulator RemA in the transcriptional activation and osmoregulation of the compatible solute uptake system OpuA under vegetative and osmostress growth conditions?
Methods: We used footprint analyses with purified RemA protein to identify binding sites in the *opuB* and *opuC* regulatory regions. To understand RemA dependent transcriptional regulation of the *opuA*-, *opuB*- and *opuC* operons we analysed their promoter activities with reporter fusions integrated into the *B. subtilis* wild type and a corresponding *remA* mutant, respectively.
Results: We found that in the absence of RemA the activity of the *opuA*-, *opuB*- and *opuC* promoters are dramatically reduced in comparison to the wild type. Interestingly the promoters remain osmotically inducible at a lower level. Footprint analyses mapped five to seven RemA binding sites within the *opu* promoter regions, respectively, and we characterized their contribution to the *opuA/B/C* promoter activations.
Conclusion: The major biofilm regulator RemA serves as a general activator for transcription of the operons for the glycine betaine uptake systems OpuA, OpuB and OpuC. Although RemA is not involved in osmoregulation of the operons it ensures transcription of the compatible solute transporter operons under both vegetative and biofilm conditions.

MCP11
Testing for *in vitro* synergism and anti-biofilm activity of ampicillin/ceftaroline against enterococcal isolates
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Question: Enterococci are responsible for difficult-to-treat infections, such as recurrent endocarditis and relapsing bacteremia. Biofilms play a major role in endocarditis and are related with treatment failure and increased lethality. Biofilms are microbial, matrix-embedded communities that can be formed on organic and inorganic surfaces exhibiting strongly increased tolerance to antibiotics and resistance to the immune response.
Ceftaroline is a new broad-spectrum cephalosporin with enhanced activity against methicillin-resistant *Staphylococcus aureus* (MRSA) [1]. A recent clinical study showed treatment success of enterococcal endocarditis with the combination ampicillin and ceftriaxone, a precursor of ceftaroline [2]. Currently, few data indicating some synergism between ampicillin and ceftaroline have been published, but the effectiveness of ceftaroline and ceftaroline in combination with other antibiotics in enterococcal biofilms is still elusive.
Methods: In order to investigate a potential synergism between ampicillin and ceftaroline, checkerboard analyses were performed testing the activity of the antibiotic combination on planktonic bacteria. In order to test anti-biofilm activity, the minimal biofilm eradicating concentration (MBEC)

was analysed in a 96-well plate set up using confocal laser scanning microscopy and an in-house cell-count algorithm for analysis of 3D biofilm images.
Results: The checkerboard testing revealed that in 3 out of 19 *E. faecium* strains (16 %) ampicillin and ceftaroline showed synergistic effects, but above of therapeutically achievable concentrations. In contrast, in 14 out of 19 *E. faecalis* strains (74 %) synergy at therapeutic concentrations could be observed. Preliminary data on *E. faecalis* biofilms treated with ampicillin/ceftaroline for 24h indicated a matrix destabilizing effect of the combination leading to loose biofilm structures.
Conclusion: The combination ampicillin/ceftaroline might be an alternative option for treatment of *E. faecalis* infections, the most clinically abundant species among enterococci. In contrast, this combination remains ineffective in *E. faecium* infections. As both antibiotics are b-lactams and target the cell wall synthesis, it might be more effective to combine ceftaroline with an antibiotic targeting other cell processes, e.g. aminoglycosides targeting protein synthesis. A possible synergism has to be analysed.

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MCP12

Panoramic view on *Staphylococcus aureus* biofilm physiology

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Question: *Staphylococcus aureus* represents a dangerous opportunistic bacterial pathogen, which is able to cause a wide range of diseases like skin abscesses, bacteraemia and sepsis. In contrast to a planktonic lifestyle associated with these infections, *S. aureus* is also able to form biofilms on host tissues and implants leading to chronic infections found in osteomyelitis, endocarditis and cystic fibrosis (CF) patients. Despite the facts that up to 80 % of human bacterial infections are biofilm-associated and Staphyloococci are recognized as the most frequent causes of biofilm-associated infections, biofilm physiology of *S. aureus* is still largely unexplored. Furthermore, co-infecting bacterial pathogens affect *S. aureus* biofilm physiology, but so far interactions during poly-microbial infections, e.g. in CF, are poorly characterized.
Methods: In order to shed light on *S. aureus* biofilm physiology, we compared protein and metabolic profiles of planktonic and biofilm-associated *S. aureus* cells using *state-of-the-art* omics technologies. Additionally, we analysed mixed-species biofilms composed of *S. aureus* and *Pseudomonas aeruginosa* representing a prominent pathogen in CF patients, which tends to outcompete *S. aureus* during infection.
Results: As a starting point we have established a flow-through system suited for the cultivation of high amounts of biomass needed for proteomic and metabolic analyses. Biofilm cultivation using this system allowed us to subsequently analyse the proteome of different subcellular fractions and to determine the utilized and secreted metabolites during biofilm growth. Our proteome analyses revealed significant differences in protein expression profiles of planktonic and biofilm-grown *S. aureus* as well as mono- and mixed-species *S. aureus* biofilms.
Conclusion: We have used two complementary omics technologies to elucidate *S. aureus* biofilm physiology and to unravel molecular interactions during polymicrobial infections. Our data contribute to a better understanding of biofilm-associated *S. aureus* infections, an essential prerequisite for the development of novel antimicrobial therapies.

MCP13

Redox regulation of PdeC, a c-di-GMP-specific phosphodieasterase with an N-terminal periplasmic CSS domain in *Escherichia coli*

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Bacterial macrocolony biofilms contain matrix-producing and matrix-free cells in distinct zones which is a prerequisite for the colonies buckling up into high ridges and intertwined wrinkles (1, 2). In *E. coli* K-12 strains the production of matrix components such as amyloid curli fibres and cellulose is promoted by the second messenger c-di-GMP, which is synthesized by 12 diguanylate cyclases (DGC) and degraded by 13 phosphodiesterases (PDE) (3, 4). A central c-di-GMP-driven switch module (DgcE/PdeR/DgcM) increases the cellular c-di-GMP level in slowly growing and stationary phase zones of macrocolony biofilms and thereby turns on the expression of the biofilm regulator CsgD. CsgD then activates the curli genes as well as *dgcC*, which encodes a DGC specifically required for cellulose synthesis (4, 5).
In addition, most of the other DGCs and PDEs are expressed but do not contribute to this scenario under standard lab conditions, suggesting that these enzymes are present in an inactive form ready to be activated by specific signals perceived by their various N-terminal sensor domains. PdeC is one of six members in *E. coli* of a novel subfamily of transmembrane PDEs characterized by an N-terminal CSS domain linked to an EAL domain. Two highly conserved Cys residues (one in the CSS-motif) in the periplasmic domain are involved in disulfide bond formation promoted by the DsbA/DsbB system under standard growth conditions. This oxidized form of PdeC is enzymatically inactive. Mutations that eliminate disulfide bond formation or treating cells with reducing agents result in strong activation of PdeC, which is accompanied by proteolytic processing. As a result, matrix production is down-regulated and macrocolony biofilms loose their intricate 3D morphology. In summary, our results indicate that CSS-PDEs perceive redox signals via the extracytoplasmic CSS domain which controls enzymatic activity on the cytoplasmic side of the inner membrane. In addition, we identified PdeC as a novel DsbA/DsbB substrate.

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MCP14

Reproducible biofilm cultivation of chemostat-grown *Escherichia coli* and investigation of bacterial adhesion kinetics on biomaterials using a non-constant-depth film fermenter

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Background: Biomaterials-associated infections are primarily initiated by the adhesion of microorganisms on the biomaterial surfaces and subsequent biofilm formation. Understanding the fundamental microbial adhesion mechanisms and biofilm development is crucial for developing strategies to prevent such infections. Suitable *in vitro* systems for biofilm cultivation and bacterial adhesion at controllable, constant and reproducible conditions are indispensable.
Aims: This study aimed (i) to develop an *in vitro* system for the reproducible long-term cultivation of biofilms at constant and low shear conditions using a non-constant depth film fermenter and (ii) to use this system to elucidate bacterial adhesion kinetics on different biomaterials, focusing on biomaterials surface nanoroughness and hydrophobicity.
Methods: Chemostat-grown *Escherichia coli* were used for biofilm cultivation (up to 9d) on titanium and investigating bacterial adhesion as function of time (1h to 38h) on titanium, poly(styrene), poly(tetrafluoroethylene) and glass.
Results: The biofilms showed a typical four-stages-development including bacterial adhesion, microcolony formation and early and late maturation. Using chemostat-grown microbial cells (single-species continuous culture) minimized variations between the biofilms cultivated during different experimental runs. Bacterial adhesion on biomaterials included an initial lag-phase I followed by a fast adhesion phase II and a phase of saturation III. With increasing biomaterials surface nanoroughness and increasing hydrophobicity, adhesion rates increased during phases I and II. The influence of materials surface hydrophobicity seemed to exceed that of nanoroughness during the lag-phase I, whereas it was *vice versa* during adhesion phase II.
Conclusion: This study introduces the non-constant-depth film fermenter in combination with a chemostat culture to allow for a controlled approach to reproducibly cultivate biofilms and to investigate bacterial adhesion kinetics at constant and low shear conditions. The findings will support adequate testing of biomaterials surface modifications eventually preventing biomaterial-associated infections.

MCP15

Sediments and epilithic biofilms in surface waters are reservoirs for coliphages

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Biofilms are ubiquitous in aqueous environments and may act as reservoirs for pathogenic microorganisms. Accumulation of hygienically relevant bacteria in aquatic biofilms has been studied in detail before. However, little is known about the occurrence of viruses in biofilms. The aim of this study was to elucidate the distribution of somatic coliphages as potential surrogates for human enteric viruses between water, sediment and epilithic biofilms in a river environment. Moreover, the occurrence of *Escherichia coli* as a commonly used faecal indicator and host for coliphages was assessed. Samples of water, sediment and epilithic biofilms were collected weekly in the period from July to September 2015 at three different sampling sites along the river Ruhr in Essen, Germany. *E. coli* was assessed using the Colilert-18/Quanti-Tray/2000 system in a most probable number (MPN) format. Somatic coliphages were quantified by a plaque assay according to DIN EN ISO 10705-2. Detection of coliphages and human enteric viruses via quantitative real-time PCR is still in progress. *E. coli* was detected in all water samples with a mean concentration of 1.6 MPN/ml, while the mean concentration in sediment and epilithic biofilms was 1.9×10^3 MPN/g and 5.2×10^3 MPN/g, respectively. For coliphages, preliminary results show mean concentrations of 1.0×10^{-2} pfu (plaque-forming units)/ml in water, and elevated amounts of 8.2×10^2 pfu/g and 4.9×10^2 pfu/g in sediment and epilithic biofilms, respectively, using the plaque assay. In conclusion, the results show that not only faecally-derived *E. coli*, but also somatic coliphages accumulate in river biofilms compared to the bulk water. This indicates that surface water sediments and epilithic biofilms may play an important role as a reservoir for bacteriophages of faecal origin and thus possibly also for human enteric viruses in surface water environments.

MCP16

Antibiotics-loaded blue fluorescent polymeric particles for tracking in LIVE/DEAD stained pathogenic biofilms

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Question: A promising strategy to treat biofilm-embedded microorganisms might be the encapsulation of antibiotics into microparticles (MPs) and nanoparticles (NPs). To visualize such particles in bacterial biofilms live/dead-stained by SYTO9 (green) and propidium iodide (red), we developed a blue-fluorescent polymer. The aim of this study was to investigate the penetration properties and the effectiveness of particle-encapsulated antibiotics in *Burkholderia cepacia* biofilms.
Methods: 7-amino-4-methyl-3-coumarinylacetic acid (AMCA) was cross-linked to PLGA by EDC that was conformed by size exclusion chromatography and thin layer chromatography. The NPs and MPs were prepared by using a modified solvent evaporation method [1]. The Tobramycin-loaded NPs and MPs were produced by double-emulsion-evaporation method [2]. The particles were analyzed by photon correlation spectroscopy and scanning electron microscopy.
Biofilms of *B. cepacia* were grown for 24 h and subsequently treated with the particles for 24 h. After live/dead-staining, the biofilm-embedded bacteria and the particles were analyzed by confocal-laser-scanning-microscopy.
Results: AMCA-PLGA demonstrated a high and stable fluorescence over a time range of 72 h at 4 °C, 20 °C and 37 °C. The particles were clearly visible as spherical blue structures (MPs) or blue dust (NPs) within the biofilms indicating sufficient penetration properties. Tobramycin (Tb) was successfully encapsulated in particles and showed superior effects against biofilm-embedded bacteria in comparison to the free drug.
Conclusions: Biodegradable polymers such as PEG-PLGA exhibit a great potential as micro and nano-carrier systems for therapeutics and therefore might be suitable to encapsulate antibiotics like Tb to improve their deposition and efficacy in deeper biofilm layers. AMCA represents an excellent fluorescent labelling for visualization of PEG-PLGA-based NPs and MPs.

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MCP17

Anti-biofilm activity of novel nano-silver against *Bacillus subtilis*

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Question: Are silver nanoparticles prepared through different chemical reduction method using two different stabilizers performing anti-biofilm activity against *Bacillus subtilis*?
Methods: Glass slides were covered by novel nano-silver as anti-adhesive surface against bacteria as a route for preventing bacterial-surface attachment (biofilm). Nano-silvers were successfully synthesized by standard citrate chemical method and novel bio-chemical method in a range of 20-30 nm as examined by Dynamic Light Scattering Spectroscopy. Cleaned glass slides were covered by epoxy then coated by the synthesized nanoparticles, separately, and characterized by Atomic Force Microscope. The anti-adhesive activity was tested against fluorescently labeled *Bacillus subtilis* bacteria using Confocal Laser Scanning Microscopy.
Results: Results approved the ability of the synthesized nanoparticles to reduce biofilm development (as an anti-adhesive agent) with different degrees.
Conclusion: Stabilizing agent has a significant effect on the anti-adhesive of silver nanoparticles

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MCP18

Matrix composition and community structure analysis of natural cave biofilms

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Bacterial biofilms secrete extracellular polysaccharides, which can serve as a scaffold for additional biogenic substances (carbohydrates, proteins, lipids and nucleic acids) and inorganic particles that form the matrix of biofilms. The bacterial community defines the composition and structure of the secreted extracellular polymeric substances (EPS). In return, the structure of the matrix determines fluxes and gradients in the immediate proximity of the embedded cells. Therefore the production and distribution of extracellular polymeric substances affects the interactions and dynamics of natural microbial assemblages.
In this study, we addressed the following research questions: (i) Does the composition of the community and the matrix correlate? (ii) Can we observe a stratification of the community composition and matrix characteristics?
We describe a natural microbial community forming a massive cave biofilm composed of secreted and accumulated substances. The subaerial biofilms received inputs of low levels of dissolved organic carbon (DOC) from seepage water and methane released into the cave. The bacterial community composition was analyzed using amplicon sequencing combined with Fluorescence *In Situ* Hybridization (FISH) to reveal spatial patterns within the biofilms. In addition, cryosections of the samples were prepared and different depth layers were examined. A lectin-binding assay was used to characterize the composition of glycoconjugates.
Our results uncover a diverse bacterial community composed of several methanotrophic lineages such as *Methylococcaceae* but also a high abundance of *Pseudomonadaceae* and *Planctomycetaceae*. FISH probes targeting the abundant subgroups of the *Proteobacteria* and methanotrophic *Gammaproteobacteria* revealed distinct patterns within the biofilms. Glycoconjugates were predominantly stained by the Fucose-specific lectin *Aleura aurantia* (AAL). We visualized a unique network of bacterial cells sheathed with thick layers of carbohydrates.
The ecology of biofilms is determined by the interrelation of the taxonomic composition and the extracellular matrix. The EPS provides a

physical and chemical barrier, which creates microenvironments shaped by the exudation of metabolic products as well as the influx of new substances. We provide first evidence for a highly diverse community forming snottites at near neutral pH, by accumulating great amounts of carbohydrates.

MCP19

The role of c-di-GMP in biofilm formation of the filamentous cyanobacterium *Nostoc punctiforme*

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In cyanobacteria various second messengers are described, from cyclic nucleotides to gases [1]. The signaling molecule c-di-GMP is a ubiquitous second messenger in bacterial species including cyanobacteria. C-di-GMP is produced by diguanylate cyclases (DGCs) with GGDEF domains and degraded by phosphodiesterases (PDEs) with EAL domains. It binds to a large range of effector components and impacts several processes, including biofilm formation and EPS production [1, 2]. C-di-GMP stimulates the biosynthesis of extracellular polymeric substances (EPS) in biofilms and regulates the transition between a motile and a sessile life style in many bacteria [2].In cyanobacteria many proteins containing regulatory domains for synthesis or degradation of c-di-GMP are associated with light-responsive domains. It was shown, that this secondary messenger is directly involved in the regulation of cyanobacterial phototaxis in the unicellular non-diazotrophic cyanobacterium of the genus *Synechocystis* [3]. In our study we focus on c-di-GMP function and EPS production associated with biofilm formation in *Nostoc punctiforme*, which is known to produce EPS in biofilms and liquid culture. By cloning a part of the *cph2* gene (5-6) from cyanobacterium *Synechocystis* sp., that carries the GGDEF domain for production of c-di-GMP, a c-di-GMP overproducing *N. punctiforme* strain was constructed. The role of this second messenger in EPS production, heterocyst differentiation and biofilm formation was demonstrated. Furthermore the photoreceptor activity was analyzedand the results were compared to results in *Synechocystis*. Additional functions of c-di-GMP in filamentous nitrogen-fixing cyanobacteria e.g. of the genus *Nostoc* are unknown, though c-di-GMP-related genes were identified in the genome sequence of *Nostoc punctiforme and will be investigated*.

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MCP20

Bacterial life in biofilm-like habitats at the top of the oceans

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The uppermost top of any oceanic water body is defined as the sea-surface microlayer (SML). It forms the interfacial boundary layer between the ocean and the atmosphere. Thus, it has a profound function for marine biogeochemistry and climate regulation, such as the air-sea exchange of climate-relevant gases, heat, and particles. The SML is a very dynamic habitat due to the influence of wind, but extremely smoothened sea surfaces (i.e. slicks) are observed frequently. Organisms in the SML experience strong physical and chemical forcing, e.g. UV radiation and accumulation of pollutants, compared to their planktonic counterparts in the underlying bulk water (ULW). Consequently, it is known that bacterial communities in the SML can differ strongly from the ULW. There is evidence that under calm meteorological conditions (low wind speed) bacterial communities in the SML are especially distinct from the ULW. Along with recent findings that gel-like particles, e.g. transparent exopolymeric particles (TEP), are commonly enriched in the SML, the idea of the gelatinous nature of the SML is rising and especially slicks could be considered as biofilm-like habitats. However, systematic studies on slicks are scarce.
To study the biofilm-like nature of the SML, we investigated slick and non-slick sea surfaces in the Pacific Ocean, the South China Sea and the Baltic Sea and compared these to samples taken from the ULW. We analyzed TEP as a proxy for extracellular polymeric substances. The bacterial communities were analyzed by flow cytometry and 16S rRNA gene fingerprints to determine their abundance and community composition, respectively. Moreover, bacterial activity was studied by measuring the uptake of different carbon substrates.
We found that the SML was generally enriched in TEP compared to the ULW and that highest enrichments were always found in slick samples. Similarly, bacterial numbers and activities were highest in slicks. Compared to non-slick samples, the bacterial community composition in slicks was increasingly different from bulk water communities. This differentiation was generally well related to the enrichment of TEP in the SML.
Overall our results indicate, that the enrichment of TEP most likely resembles a biofilm-like matrix at the sea surface. This TEP-matrix creates a specific environment for its bacterial inhabitants, enhancing bacterial growth and activity, which is comparable to other known biofilm habitats. Therefore, we conclude that slicks feature biofilm-like properties with the excessive accumulation of particles and microorganisms embedded in a TEP matrix.

MCP21

Biofilm stability is affected by environmentally relevant low concentrations of silver nanoparticles

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Silver nanoparticles (AgNP) are widely used as antimicrobial agent in numerous forms of products used in daily life (e.g. in various textiles, cosmetics and sanitary products). Although antimicrobial effects of AgNP are supposed in general, there still is a significant lack of knowledge about their actual fate in the environment and potentially adverse effects upon aquatic habitats [1]. In the context of the DFG research group INTERNANO, project BIOFILM performed a series of experiments to investigate the effects of exposure to environmentally relevant low concentrations of citrate stabilized AgNP upon freshwater biofilms. This presentation will focus on one essential biofilm driven ecosystem function described as biofilm adhesion: during biofilm development, microbes produce extracellular polymeric substances (EPS) that glue cells to the underlying surface as well sediment grains to each other, increasing their resistance towards erosion. This process has been recently described as biostabilization [2] providing an important ecosystem service in sediments. Thus, biofilm adhesiveness has great ecological significance as a sink for pollutants and contaminants with increased resistance versus remobilization into the free water column and connected aquifers. Monospecies biofilms of *Aquabacterium citratiphilum* were cultivated on glass slides and treated with AgNP (diameter 40nm, citrate stabilized). In doing so, an environmentally relevant, sub lethal concentration was applied as well as one with lethal effects upon bacteria. Three controls were established: untreated, treatment with $AgNO_3$ and $CuSO_4$. To investigate the different levels of impairment, biofilm productivity was determined measuring EPS carbohydrates and protein contents. Furthermore, biomass and biofilm structure were analysed as well as biofilm functionality, in this case stability, using a non-destructive adhesion measurement method with high temporal and spatial resolution (MagPI[3]).
We report here for the first time the statistically significant effect of low-dose exposure of AgNP on biofilm cohesiveness, and simultaneously determined EPS carbohydrate composition and biofilm protein content. Alteration of biofilm stability as shown by MagPI will finally be discussed against the recently elucidated reduction of biofilm stability and viscosity by microgravimetry and microrheology, respectively.

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MCP22

***In vitro* assessment of industrial biofilms in metal working fluid systems**

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Water miscible metalworking fluids (MWF) are commonly used in several industries during processing of metal work pieces. Despite, the addition of biocides to MWFs some microorganisms (MO) are able to survive and are even able to form biofilms in the MWF tanks and machine pipelines.

Then, these biofilms are the source of contamination, after the MWF got changed. Through the metabolization of MWF compounds the working conditions are changing, which influences the metalworking process. Also, microbial contamination is a potential health risk of the machine operator. A longer using time of MWF would safe running costs of the metalworking machine. The aim of this work was to develop an *in vitro* model in analogy to real machine systems, which allows the generation of realistic biofilms to test the impact of a number of physico-chemical parameters on the biofilm formation. Interacting parameters as, metal specification, operating temperature, mixing, nutritional addition and time on the biofilm formation have been analyzed by fluorescence- and atomic force microscopy. Therefore, stripes with a defined size, consisting of glass, elemental copper, alloyed aluminum as well as alloyed stainless steel have been placed into small chambers. The chambers were filled with MWF, which was inoculated beforehand with a defined bacteria consortium. Apart from the influence of the parameters on the biofilm formation, a possible correlation between the percentage of viable MO in the biofilm and the colony forming units in the MWF was investigated. Within this work, for the first time, realistic *in vitro* biofilms in MWF could be generated. Furthermore, parameters were defined that influence biofilm formation in MWF systems.

MCP23

Application of low pH sulfidogenic bioreactors to selectively recover metals from acidic process waters

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Acidic, sulfate- and metal-rich waters are generated by different industrial processes, including mining of metals and mineral processing. A novel technique that promotes both, selective recovery of potentially valuable metals and removal of sulfate from acidic liquors, has shown great potential [1]. The system uses versatile and robust consortia of novel, acidophilic sulfate-reducing bacteria (SRB) to produce H_2S which reacts with chalcophilic metals (e.g. Cu, Zn) to form insoluble metal sulfides. While this has previously successfully been applied to precipitate CuS and ZnS, present in relatively low concentrations in iron-rich mine waters [1,2], here we describe results obtained with highly metal-rich, acidic solutions produced from bioleaching a copper concentrate in order to selectively precipitate different metal sulfides.

The pregnant leach solution (PLS) was characterized by very low pH and high concentrations of some metals (Fe^{3+}, Cu^{2+}, Zn^{2+}) and sulfate, and significant amounts of Ni. To recover the chalcophilic metals as sulfides *via* controlled biomineralization, a pH-controlled, continuous flow bioreactor, housing novel acidophilic SRB, was set up. The 2L bioreactor was operated effectively at pH 2.5 - 4 fed with synthetic PLS supplemented with glycerol and yeast extract. By moderating the pH, it was possible to control which metal(s) precipitated and those which remained in solution, as the metal sulfides concerned have different solubility products. Sulfate and metal concentrations in the feed solution were gradually increased and pH and glycerol concentration adjusted. Flow rates and metal concentrations were used to determine the efficiency of the process, and the microbial community was monitored using molecular techniques.

Soluble Fe^{3+} was initially precipitated as the mineral schwertmannite by controlled addition of alkali. The SRB system was adapted to the increased sulfate concentrations in the PLS which resulted in osmotolerant species becoming increasingly dominant. ZnS was precipitated within the bioreactor by adjusting the system pH, and CuS and NiS were selectively recovered in separate, offline vessels flushed with excess H_2S from the sulfidogenic bioreactor. The rate of glycerol oxidation thereby influenced the hydraulic retention time of the system and the feed glycerol concentration determined the amount of excess H_2S being produced.

This study broadens the applicability of the described sulfidogenic systems to more complex mine and process waters for a selective recovery of valuable, chalcophilic metals and removal of sulfate.

[1] Ñancucheo & Johnson: Microb. Biotechnol. Vol. 5 (2012), p. 34
[2] Hedrich & Johnson: *ES&T.* Vol. 48 (2014), p. 12206

MCP24

Groundwater microbial communties response to simulated spills of hydraulic fracturing-related fluids

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The exploitation of shale gas requires hydraulic stimulation (fracking), i.e. the injection of fluids at high pressures into the formations, to create fractures and fissures, and thus to release gas from the source rock. Up to 40 % of these fluids flows back to the surface, together with reservoir waters. To assess the response of groundwater microbial communities to a potential spill and their resilience, laboratory experiments under *in situ* conditions were conducted using groundwater samples. Microcosms containing R2 broth medium or groundwater spiked with either single frac chemicals, frac fluids, artificial reservoir water, NaCl, oil, or different mixtures of reservoir water and frac fluid (to simulate flowback) were incubated in the dark. Classical microbiological methods and molecular analyses were used to assess the effects in the microbial abundance and community structure. Microbial communities were quite halotolerant and their growth benefited from low concentrations of reservoir waters or salt, but they were negatively affected by higher concentrations of formation waters, salt, biocides or frac fluids. Changes on the microbial community structure could be detected by T-RFLP. Single frac components like guar gum or choline chloride were used as substrates, while others like triethanolamine or light oil distillate hydrogenated prevented microbial growth in groundwaters. Ongoing work will provide information on potential transformations of frac or geogenic chemicals by groundwater microbiota and their lifetime.

MCP25

Heavy metal release by fungal induced black slate degradation

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Microorganisms are important factors in the degradation of rocks. Especially fungi promote the biological decomposition of lithic substrates. These processes are of high ecological relevance because they increase carbon dioxide concentration in the atmosphere and cause damages at historical buildings. Other major difficulties are waste dumps. Once the rock material has been lifted to the earth`s surface, microbial activity may release embedded heavy metals from rocks. The filamentous fungus *Schizophyllum commune* is a widespread basidiomycete and has the ability to degrade wood. In doing so, it secretes many enzymes like unspecific oxidases such as laccases, which are responsible for lignin degradation. Many enzymes involved in wood decomposition are assumed to be implicated in organic carbon release from rocks like black slates. In this study, the connection between fungal caused rock degradation and heavy metal release is investigated. Furthermore, a laccase overexpressing strain and a control are compared to verify the contribution of laccases on black slate decomposition. For these experiments, the fungal strains were grown in liquid medium with and without grounded black slate for up to 14 days. Afterwards, metals in the medium were analyzed using inductively coupled plasma mass spectrometry (ICP-MS). The results show a significant involvement of *S. commune* on the release of e.g. Fe, Pb, and U. However, in some cases the metal concentration in the medium is higher in the absence of the fungus. This indicates that these metals are bioavailable, possibly essential for the fungus, and so taken up by it. Considering the role of laccases, the overexpressing strain sometimes shows higher release of metals compared to the control and *vice versa*. The results suggest a high ecological importance of *S. commune* as a rock-degrading fungus. It decomposes the slate to use containing organic carbon and nutrients for its energy generation and nutrition. During the process of biological weathering, it could be shown, that metals are released to the surrounding. In nature, this could cause serious problems in matters of soil and water contamination. Further experiments will be performed to test the bioavailability of released metals.

MCP26
Sequestration and drift of heavy metals and radionuclides under influence of ectomycorrhiza
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Ectomycorrhiza (ECM) as mutualistic interaction can easily colonize dense mineral-organic soil formations. Due to their capabilities to accumulate elements, ECM fungi can act as biological filters and therewith provide scavenger functions for ECM tree partners and surrounding organisms. So ECM fungal fruiting body tissue shows high bioconcentration factors: *Paxillus involutus* 66 for Cs, *Pisolithus tinctorius* 40 for Pb and 28 for U. In microcosms with ECM clear reductions of Al, Cs and Pb concentrations were measurable in the soil mineral substrate, depending on soil substrate composition and pH. Additionally intracellular immobilization of toxic elements by cytosolic Glutathione S-transferase (GST) activity appeared. So mycelium showed higher GST activity under influence of high elemental values of Ni, Sr and Pb. Furthermore increased Pb values with up to 18 mg/g Pb occurred in guttation fluids of ECM fungi, even more from co-cultures. Aquaporines (AQP) are potential drivers. So AQP inhibitors acetazolamid and silver affected an inhibition of AQP transported water resulting in decreased exudation of guttation fluids by fungal mycelium. Therewith areas as secondary mineral enriched fields, like post-mining areas, are interesting for extraction processes of solvable minerals ensured by microorganisms such as fungi.

MCP27
Inhibition of acidophilic microbes in model systems for decommissioning of *in situ* bioleaching of sulfide copper ores
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Deeply buried complex ore bodies in the European Earth crust are expected to feed the growing hunger for base metals by industry, because easily minable deposits became rare. Conventional mining would include removal of overburden, hauling of the ore to the surface and grinding it to processable powders meaning high capital demands and potentially high environmental burdens. The European EU Horizon 2020 project BioMOre aims to avoid costs and hazards of open-pit mining by development of a new combination of *in situ* leach mining technology and a ferric iron lixiviant generated in bioreactors with acidophilic iron-oxidizers. The general principal of the new technology will be tested in a block leaching pilot *in situ* in the Rudna Mine (KGHM, Poland). Indirect ferric iron bioleaching of fractured deposits will be subsequent to an initial leaching with a mineral acid solution to dissolve e.g. carbonates and acid-extractable metal sulfides. The injected lixiviant from the injection wells percolates through the fractured ore, catalyzing the oxidative dissolution of metal-containing minerals under oxygen-limiting or anoxic conditions, and the metalliferous solution generated is pumped to the land surface and processed via hydrometallurgy. Autotrophic iron oxidizers from the bioreactors present in the lixiviant can colonize the deep buried geological formations. Inhibition of these allochthonous microbial populations subsequent to the depletion of the deposits is one of the challenges for the decommissioning phase of the BioMOre-project.
Shake flask and percolator experiments inoculated with a ferric iron-generating bioreactor adapted to black shale and sandstone ore from Rudna Mine (Poland) have been established at 35^{0}C and pH 1.4, and were fed with 50 mM ferrous sulfate. The inhibition efficiency of chloride, formate, acetate, SDS and benzoic acid was examined in different set-ups. T-RFLP, qPCR, FISH, microcalorimetry and ATP-measurements were used to monitor the effects on members of the acidophilic mixed culture in combination with chemical determinations.
T-RFLP results corroborated the predominance of *Leptospirillum* and *Acidithiobacillus* species in the bioreactor culture. The high efficiency of metal sulfide oxidation by biologically generated ferric iron has been proven as high metal concentrations have been determined in the circulating leach-solution of the percolator systems. Inhibition of metabolic activity of iron-oxidizing acidophiles was achieved in the presence of 300 µM formate and 400 mM chloride. The bacteriostatic effects of SDS and benzoic acid have also been confirmed. Contrary to expectations, acetate revealed very low inhibition effects with concentrations higher than 10 mM.
The results show the feasibility of inhibition of *in situ* bioleaching activity relevant for decommissioning subsequent to the metal depletion of deposits.

MCP28
Applied electrochemistry in biohydrometallurgy
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Bioleaching is the extraction of metals from ores by the use of microorganisms acting as biocatalysts in the mineral dissolution process. Applied bioleaching (biomining) is of great economic importance for the recovery of high value metals such as Cu, Au, U, Ni, Co and Zn. However, some minerals such as primary copper sulfides are difficult to leach. This is most probably due to the formation of a passivating layer (consisting of elementary sulfur, sulfides and/or hardly soluble iron precipitates) which covers the mineral surface. In this context electrochemistry is an important tool of mineral and metallurgy research. And electrochemical techniques become increasingly established to understand and to control the mineral dissolution during bioleaching [1, 2]. Thus, our aim is to combine bioleaching with analytical electrochemistry to investigate changes in the electrochemical properties of the mineral-liquid-phase. Further we aim at the application of preparative electrochemical techniques to improve the bioleaching process for the recovery of valuable and rare metals.
A three electrode arrangement was used to investigate mineral dissolution of leached and not leached material. Two types of working electrodes were fabricated to study their electrochemical behavior by various electrochemical techniques.
We observed mineral passivation with massive mineral electrodes as same as with mineral carbon paste electrodes made from a copper sulfide mineral. These electrode systems are suitable to investigate, for instance how mineral passivation can be avoided and how to improve bioleaching by galvanic effects between different minerals.
Electrochemical methods are feasible tools to shed more light on mineral dissolution and on the interaction between leaching bacteria and the mineral surface. However, we are still in progress to establish electrochemical methods into the bioleaching process for an improved metal recovery.

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[2] Zhao, Hongbo; Wang, Jun; Qin, Wenqing; Hu, Minghao; Zhu, Shan; Qiu, Guanzhou (2015): Electrochemical dissolution process of chalcopyrite in the presence of mesophilic microorganisms. In: *Minerals Engineering* 71, S. 159-169.

MCP29
Ecological and industrial aspects of the interaction of radionuclides with bacterial S-layer proteins
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Radionuclides interact with biomass in various ways. In general, the different interaction mechanisms can be assigned to two physiological aspects, first a radiological toxicity and second a chemical toxicity. Bacteria and archaea developed very early in evolution a multifunctional cell envelope called surface-layer (S-layer) which protects bacteria in extreme environments. This layer is a para-crystalline protein lattice formed by the self-assembly of secreted proteins on the cell wall of bacteria and archaea. S-layers are often glycosylated and phosphorylated and their lattice formation depends often on bivalent cations such as calcium. In case of bacteria living in radionuclide and heavy metal contaminated environments, S-layers are able to act first of all as scavenger for reactive oxygen species (ROS) formed by either radiolysis of water or Fenton reaction. The inactivation of the radicals is achieved by intermolecular crosslinking of tyrosine residues of the protein monomers. Secondly, S-layer proteins are able to selectively bind several radionuclides such as uranium and curium but also other toxic elements such as arsenic. By restraining these metals on the surface of the cell a toxication of the organism is prevented. Interestingly, the mechanism and binding behavior is different for different elements and is highly dependent on pH. Whereas the hexavalent uranium is bound by several

surface exposed functional groups and is easily released at acidic pH, the trivalent curium substitutes calcium and is only released at pH 2.0 or below. The stable and selective curium complexation is especially interesting as lanthanides are considered as chemical analogous for trivalent actinides. Thusly, S-layers bind also rare earth elements very effectively, being highly interesting for the development of metal selective filter materials for their enrichment and recovery.

MCP30

Impact of mofette activity on microbial communities in soil and in the lacustrine bottom of the Laacher See, Germany

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Mofettes are cold, geogenic exhalations with extremely high CO_2 concentrations and occur in the surface near tectonic faults, where the gas ascends from the earth's lower crust or upper mantle. Their effects on the local ecosystem are widely unknown, but along with the implementation of carbon capture and storage and its potential of leakage, mofettes present a natural simile. The high CO_2 concentrations result in mostly hypoxic, extreme habitats. Presumably, microbial activity will have a noticeable effect on these locations as well as show adaptations. To understand the microbial associated processes, knowledge of the abundance and diversity of the participating microorganisms is essential. The influence of geogenic CO_2 release in the shallow lacustrine and terrestrial environment on microbial communities was tested with a focus on methanogenic archaea and methylotrophic bacteria. To describe the bacterial diversity and abundance, 16S rDNA gene sequencing was used to perform phylogenetic analyses. Sediment samples from a terrestrial and a lacustrine gas vent as well as control sites with not enhanced CO_2 concentrations in soil at the eastern part of the Laacher See were used. The structure of the bacterial communities and predominant genera are affected by CO_2 vents. We observed methanotrophic bacterial genera indicating the presence of methanogens as well. We assume that noticeable amounts of methane should be produced, but will be immediately consumed by methanotrophic bacteria because of the low methane concentration in the vent.

MCP31

Actinomycetes from a heavy metal contaminated site

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We isolated actinomycetes from a metal contaminated site situated in a former uranium mining area. The substrate of the test site Gessenwiese near Ronneburg, Germany, represents an extreme habitat with low pH, high salt concentrations and free heavy metal ions that lead to adaptations to cope with these harsh conditions. Actinomycetes are well known for their ability to produce a broad range of bioactive secondary metabolites. In our actinomycete isolates from the extreme habitat, we see the potential for application to bioremediation of polluted sites, to study distribution of environmental resistances and sources for new drugs. The activation of genes for new metabolites can be facilitated through stress like heavy metals. At the same time, screening for growth promoting features is performed for application in microbially aided phytoremediation. Of about 300 isolated actinomycetes, antimicrobial activities and plant growth promoting features including nitrogen fixation, phosphate mobilization, phytohormone and siderophore production, as well as heavy metal resistance was screened. A number of isolates showed the potential to act as plant growth promoting bacteria by phosphate and iron mobilization and the production of antibiotics and auxins. In a preliminary screening, two out of ten tested strains gave evidence of the production of active compounds only visible under metal treatment. The transfer to a microfluidic system at nanoliter scale would enable a faster screening and cultivation with minimal cost of media and reagents as well as the possibility to work in a wide concentration range for adding stress factors like metal ions.

MCP32

Olivine dissolution by a model biofilm: biological impact and analytical methodology considerations

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Subaerial biofilms (SAB) on rocks are ubiquitous in nature and actively participate in weathering of exposed terrestrial surfaces and soil formation (Gorbushina 2007). Due to the enormous amounts of variables that belong to a biological process, the quantification of biogenic influence is only possible by using well-controlled and simplified laboratory models. A recently developed *in vitro* system to study colonisation of rocks models a complex SAB community through two key participants - a symbiotic cyanobacterium and a rock-inhabiting fungus. With the aim of gaining more insight on the impact of rock-inhabiting biofilms on mineral weathering we studied the impact of this model biofilm on olivine dissolution rates.

Forsteritic olivine was incubated in batch reactor flasks with and without a model biofilm consisting of the cyanobacterium *Nostoc punctiforme* and the rock-inhabiting ascomycete *Knufia petricola,* and submerged in a growth solution (pH 6). The flasks were incubated for 30 days under 25 °C, 90 µmol photons/m^2s and were shaken at 150 rpm. qPCR was performed to quantify the cell growth of both organisms, BET to gather the specific surface area of the olivine and ICP-OES to follow up the change of concentration of the leached out metals.

Our results show that our model consortium -especially *K. petricola*- does increase the dissolution rate of olivine. The pH increased from the initial 6 to around 7.2 for all setups. Initially Mg was preferentially released over Si (Mg/Si of 3.5), until after two days the ratio starts equilibrating around stoichiometric dissolution. During this timeframe the dissolution rate drops by nearly two orders of magnitude, just as observed by Daval *et al.*, (2011). The difference in dissolution rates between the biologically influenced and abiotic setups is initially non-existent, but increases over time. After 30 days the setup with *K. petricola* gives a dissolution rate of 1.08×10^{-15} moles/cm^2s, compared to 9.23×10^{-16} moles/cm^2s for the abiotic setup.

We expect this study to increase awareness on the impact of microbiology on mineral weathering. Additionally it is a starting point for other, more sophisticated experiments using for instance flow through or drip flow reactors or other minerals.

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Gorbushina, A. A. (2007). "Life on the rocks." Environ Microbiol 9(7): 1613-1631.

MCP33

***Thermodesulfobium* sp. strain 3baa – acidophilic sulfate reducing bacterium isolated from a mine pit lake**

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Most sulfate reducing prokaryotes (SRP) which have been described so far were isolated from neutral-pH environments and were determined to be neutrophilic. However, in recent years the presence and activity of SRP were also found in low-pH environments - especially those generated by sulfuric acid. Though many strains obtained from these acidic habitats showed no growth below pH 5, to date three validly described moderately acidophilic sulfate reducing bacteria exist: i) *Thermodesulfobium narugense* $Na82^T$ [1], ii) *Desulfosporosinus acidiphilus* $SJ4^T$ [2], and iii) *Desulfosporosinus acididurans* $M1^T$ [3]. The sulfate reducer examined in this study, strain 3baa, was isolated from acidic sediments (pH 2.6) of pit mine lake 111 in Lusatia, Germany. For strain purification, a modified role tube technique with semisolid, artificial pore water medium [4] posed to pH 3 was applied. H_2 and CO_2 served as pH-independent electron donor

and carbon source, respectively. 16S rRNA gene analysis indicated 98 % sequence similarity to *Thermodesulfobium narugense* Na82^T isolated from a pH-neutral hot spring (pH 6.9, 58 °C) in Japan. Like the type strain, strain 3baa is rod-shaped (0.3×2 - 3 µm) and exhibits a Gram-negative cell wall structure. Similarly, strain 3baa appears to be strictly chemolithoautotrophic in respect to the electron donors/carbon sources tested. As one of the major questions was whether we deal with an acidophilic or rather acidotolerant organism, we performed batch growth experiments in the range of pH 2 - 7 at approximately 0.5-unit intervals. Sulfate concentrations and pH were monitored over time and cell numbers were determined by epifluorescence microscopy using nucleic acid stain SYBRGreen I. Growth occurred in the range of pH 2.6 - 6.6, while no growth was observed at an initial pH of 2.0 or 6.9. Sulfate reducing activity increased with decreasing initial pH with the highest rate of 1.4 µmol ml^{-1} d^{-1} at pH 2.6. In contrast, cell counts of the culture liquid demonstrated highest yields in the intermediate range of pH 4.2 - 5.6 and lowest yields at pH 2.6 and 3.1. At low pH, we observed precipitate formation at the solid-liquid interface. Investigation of submersed glass and polycarbonate carriers by confocal laser scanning microscopy revealed a densely populated surface with numerous microcolonies and larger mineral aggregates covered with cells. From these results we conclude that strain 3baa is an acidophilic sulfate reducer. High sulfate reduction rates may present a mechanism of pH homeostasis in an acidic environment. This as well as the role of biofilm formation and mineral precipitation for growth at low pH needs further investigation and will be addressed in the near future.

[1] Mori *et al.* (2003) Extremophiles 7:283-290.
[2] Alazard *et al.* (2010) Extremophiles 14:305-312.
[3] Sánchez-Andrea *et al.* (2015) Extremophiles 19:39-47.
[4] Meier *et al.* (2012) FEMS Microbiol Ecol 79:69-84.

MCP34

Growing at the solid-liquid interface: biofilm formation of a sulfate reducing bacterium in an acidic and metal rich medium – an ultrastructural investigation

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Sulfate reducing bacteria are promising candidates for the remediation of acidic metal rich waste waters. They produce alkalinity and hydrogen sulfide which reacts readily with certain metal cations to form insoluble sulfide minerals. Chemolithoautotrophic *Thermodesulfobium* sp. strain 3baa was recently isolated from sediments of an acidic mine pit lake. When it was grown in artificial pore water medium (APWM) [1] posed to low pH (pH 2.5 - 3.5), the formation of precipitates strongly adhering to solid surfaces was observed. The overall aim of this study was to visualize and analyze the structure of this layer at high resolution applying scanning (SEM) and transmission electron microscopy (TEM) accompanied by EDX micro-analysis. We wanted to address the following questions: i) At what point do bacterial cells colonize the solid surface and do they produce an extracellular polymeric matrix? ii) What kinds of inorganic precipitates are formed and in which chronological order do they appear? iii) How are cells and precipitates localized in relation to each other? Strain 3baa was grown in batch cultures containing APWM posed to pH 3 and H_2/CO_2 in the headspace. A polycarbonate carrier was introduced to support biofilm growth. In 7-day intervals, the carrier were removed and the culture liquid was analysed for pH, sulfate (IC), dissolved Al, Fe and Mg (AAS), and cell counts (SYBRGreen I). For SEM, biofilms were fixed, dehydrated and coated with 20 nm Au. For TEM and ultrathin sectioning, samples were embedded in Spurr resin. Carriers removed during or at the end of exponential growth phase of planktonic cells exhibited only few randomly dispersed cells. The carrier surfaces were coated by a thin Al-rich layer. Additionally, Al-rich spherical structures of different sizes were observed increasing in number and forming larger aggregates with time. These were also found on cell surfaces with some cells completely covered. Only after planktonic cells had long reached stationary phase, the carriers became more densely populated and minerals rich in Fe and S appeared. Three different types of Fe- and S-rich minerals were observed depending on their specific location to the gas-liquid interface. Cells were found within as well as on top of mineral aggregates. Extracellular polymeric substances were not observed. TEM revealed Al-rich spheres to be closely associated with cell surfaces, however, no intracellular precipitates were detected. From our observations we can conclude that cells only have a strong affinity to the solid surface when Al and/or sulfide precipitates are present. The production of an extracellular polymeric matrix does not seem a prerequisite to form an adhesive biofilm. It may be suggested that Al-rich precipitates take over this role. There was no indication that mineral precipitation has an inhibitory effect on cell growth or activity.

[1] Meier *et al.* (2012) FEMS Microbiol Ecol 79:69-84

MCP35

Forest strata drive spatial structure of archaeal and bacterial communities and microbial CH_4 cycling in neotropical bromeliad wetlands

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Question: Several thousand of tank bromeliads per hectare of neotropical forest create a unique wetland ecosystem that harbors diverse communities of archaea and bacteria and emit substantial amounts of methane. Since there is only little information about the microbiology within bromeliad wetlands, we studied the spatial distribution and potential functions of microbial communities and methane (CH_4) cycling and their drivers in tank bromeliad wetlands.
Methods: We selected tank bromeliads of six different species and two functional types (terrestrial and canopy) in a neotropical montane forest of Southern Ecuador and sampled organic tank slurry. Archaeal and bacterial communities were characterized using terminal-restriction fragment length polymorphism (T-RFLP) and Illumina MiSeq sequencing, respectively, and linked with physico-chemical tank-slurry properties. Additionally, we performed tank-slurry incubations to measure CH_4 production, stable carbon isotope fractionation and pathway of CH_4 formation.
Results: Archaeal and bacterial community composition in bromeliad wetlands were dominated by methanogens and by *Alphaproteobacteria and Bacteroidetes*, respectively, and did not differ between species but between functional types. Hydrogenotrophic *Methanomicrobiales* were the dominant methanogens among all bromeliads but also characteristic for canopy bromeliads. Aceticlastic *Methanosaetaceae* were characteristic for terrestrial bromeliads. Complementary, hydrogenotrophic methanogenesis was the dominant pathway of CH_4 formation. The relative contribution of aceticlastic to total produced CH_4 increased in terrestrial bromeliads and led to a concomitant increase in total CH_4 production. *Rhodospirillales* and *Clostridiales* were characteristic for canopy bromeliads. *Planctomycetales* and *Actinomycetales* were characteristic for terrestrial bromeliads. While nitrogen concentration and pH explained 32 % of the archaeal community variability, 29 % of the bacterial community variability was explained by concentrations of nitrogen, acetate and propionate.
Conclusion: Bromeliad functional types, associated with different forest strata, and their constrained environmental characteristics, e.g light availiability and tank organic matter quality and quantity, may be most important drivers for spatial distribution of archaeal and bacterial communities and microbial CH_4 cycling in neotropical forest canopies.

MCP36

Gastric bypass surgery markedly perturbs the community structure and the functional composition of the intestinal microbiota

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Roux-en-Y gastric bypass surgery (RYGB) is performed to help reduce body weight to less harmful levels in severely obese patients. Following RYGB, patients only eat small amounts of food and nutrients are not efficiently digested in the small intestine, both leading to a reduction in overall caloric intake. Globally this operation is performed over 150,000 times each year. Since the impact of this surgical rearrangement of the gastro-intestinal tract on the intestinal microbiota has only been investigated to a small degree, our research question was: How does RYGB affect the community structure and the functional composition of the intestinal microbiota?
In this study we investigated in a rat model the effect of RYGB on the microbiota from the ileum as well as the colon and compared these to body weight matched animals with sham surgery. From the two gut

localities, we investigated the microbiota inhabiting the mucus layer and the intestinal lumen separately. To resolve the community structure in regard to taxonomy and enzymatic functionalities 16S rRNA gene sequencing and metaproteomics was performed.
The results reveal profound changes in the taxa distribution and the enzymatic functional capacity of the microbiome in the ileum as well as the colon after RYGB. For example, for taxa distribution we observed in the ileum and colon greater prevalence of Actinobacteria especially Bifidobacteriales after RYGB with Firmicutes at lower abundances. Enterobacteriales was also more prevalent in the colon of RYGB then in the control. An example for changes on a functional level in the ileum was that the relative numbers of Actinobacteria proteins involved in amino acid metabolism or carbohydrate metabolism were higher in RYGB. In the colon proteins from Clostridia belonging to the function of carbohydrate metabolism or the function energy production were seen at lower levels in RYGB whereas proteins from Actinobacteria which are involved in carbohydrate metabolism or cell motility were observed at higher relative numbers.
With these results and further research it may be possible to design specific diets or medical interventions to limit the perturbation in the microbiota following RYGB or to remove specific pathogenic taxa inhabiting the intestinal tract after surgery.

MCP37
Accurate microbiota profiling: defining a core lung microbiome in mice
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The compositional variation of microbial communities in the lungs of healthy individuals is not completely described so far nor is it known how microbial communities in the lung establish during development. Therefore, the objective of this study is to investigate the development of a healthy lung microbiome and potential impacts of slight environmental changes. We hypothesise that (i) changes in microbial composition over time are highly dynamic and substantial diverse in healthy individuals, (ii) with time the lung environment imposes selection pressure on microbes, which leads to a stabilized core microbiota, and (iii) even small environmental changes have a clear impact on the lung microbiome.
To address these questions we used a mouse model and studied the development of the lung microbiome over time from birth to the adult stadium (Fig. 1). Genetically identical female animals (Balb/c) were either held under standardized conditions or weekly exposed to external, used litter from C57B/6 mice. Lung tissue was sampled at five different time points for 16S rRNA gene amplicon sequencing using the Illumina MiSeq platform and the data were analyzed with the software package QIIME (http://qiime.org).
As expected, the microbial community changes dynamically with high individual diversity between mice up to 18 weeks. Reaching this age, the core microbiome seems to stabilize and to be dominated by certain taxa (e.g. *Ochrobactrum sp.*). No significant treatment effect due to the added litter could be observed.
These data provide a solid basis for further investigations of the lung microbiome. Considering the dynamic development of a core lung microbiome with age, important issues such as the role of certain taxa within the lung environment or severe external impacts like the use of antibiotics on the lung microbiome, as well as major microbiome functions need to be explored.

MCP38
Impact of the presence or absence of calcium, phosphorus and phytase in chicken gut microbiota
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Question: Gut microbiota affects the host's individual development due to its role in assimilation and nutrient uptake. The aim of this study was to elucidate the effect of supplementing diets with calcium, phosphorus and microbial phytase in the microbial community of the gastrointestinal tract.
Methods: 1064 chickens allocated in 56 pens were fed with a commercial diet until day 14. From day 15 to 26, pens were randomly assigned to 8 dietary treatments, consisting of a maize-soybean basal diet supplemented with: calcium (0 or 3g/kg), phosphorus (0 or 2g/kg) and *E. coli*-derived phytase (0 or 1500 FTU/kg). Mucosa and content samples from 57 individual birds, were taken on day 26, from crop, ileum and caeca. Total nucleic acids were extracted and subjected to 16S Illumina amplicon sequencing.
Results: Replicates of the same diet showed a high microbial composition variability. The average similarity within replicates was 30-82 % in crop, 18-49 % in ileum and, 16-37 % in caeca. At genus level, a cumulative effect of each diet on each section was observed on mucosa samples ($p=0.003$). The same effect was not occurring in the content samples, nevertheless diets showed a statistical difference in the crop and caeca ($p<0.02$). Particularly, the content of the crop showed that diet with calcium was dominated by *Lactobacillus taiwanensis*, with an average abundance of 80 %. This species was also present in the diet with added phosphorus, calcium and phytase (65 %). In case of ileum mucosa, an uncultured *Ralstonia* showed higher abundance (~ 30 %) in diets without any supplementation, with phytase or with calcium, when compared with diets with phosphorus and phytase, and with phosphorus and calcium (~ 13 %). Uncultured *Clostridiaceae* was present on diets with phosphorus. Also it was found that diets with phosphorus and calcium increased the abundance of *Parvimonas*. The caeca was the most diverse section, and showed differences between content and mucosa. Diet with phytase and no calcium and phosphorus addition decreased the abundance of *Anaeroplasmataceae*. Species such as *Faecalibacterium praustnitzii* and *Pseudoflavonifractor capillosus,* known as butyrate producers, were found in content and *Paenibacillus thailandensis,* a species with xylanase activity was detected in mucosa.
Conclusion: Diets had relatively minor effects on changes in microbial community, this might be because of the high variability observed among birds analyzed for each diet and section. With this, it was found that *Lactobacillaceae* dominated crop and ileum sections while caecum showed to be more diverse.

MCP39
The trustworthy and the misprized – trace element deprivation switches methanogenic key players in anaerobic digestion
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Trace elements play an essential role in all organisms due to their functions in enzyme complexes. In biogas reactors, control and supplementation of trace elements lead to stable and efficient methane production processes while trace element deficits cause process imbalances. The biogas process involves diverse bacterial communities but only few different methanogenic archaea. Among the latter, *Methanosarcina* is regarded as the 'trustworthy' and robust methanogen due to its metabolic versatility and dominance in many anaerobic digesters. Other methanogens such as *Methanoculleus* are less abundant in well supplied reactors. The effect of trace element deficits on the efficiency of methane production is known but the underlying metabolic mechanisms and the adaptation of the affected microbial populations to such deficits are not yet fully understood. We investigated the microbial community dynamics and process changes induced by trace element deprivation. Two lab-scale continuous stirred tank reactors R1 and R2 fed with stillage and supplemented with trace elements and a commercial iron additive were operated in parallel for 72 weeks to gain two comparable systems. Then the feeding regime for R2 was changed by omitting trace element supplements and reducing the iron additive. For the whole experimental time of 93 weeks, various process parameters (methane yield, concentrations of trace elements, organic acids and ammonia nitrogen, pH) and the composition and activity of the microbial communities were monitored. Hydrogen sulfide, ammonia nitrogen and acetate concentrations increased in the trace element depleted reactor R2 compared to the control reactor R1, whereas specific methane production did not significantly change. T-RFLP fingerprinting of *mcrA* genes and their transcripts revealed that *Methanosarcina* and *Methanoculleus* dominated the methanogenic communities in both reactors. However, the activity ratio of these two genera was shown to depend on trace element supplementation. *Methanosarcina* dominated the well supplied anaerobic digester, pointing at acetoclastic methanogenesis as the dominant methanogenic pathway. Under trace element deprivation, *Methanoculleus* and thus hydrogenotrophic methanogenesis was favored whereby *Methanosarcina* was not overgrown by *Methanoculleus*. Multivariate statistics revealed that the decline of Co and Ni as well as Mn, Mo and W most strongly influenced the shifting proportions of *mcrA* transcripts originating from both genera. Deprivation of Co might lead to shortage of

corrinoid cofactors required for acetoclastic and methylotrophic methanogenesis, thus favoring the hydrogenotrophic pathway. Moreover, *Methanosarcina* relies on [NiFe] hydrogenases while the 'misprized' *Methanoculleus* uses Ni-free hydrogenases and has a lower hydrogen threshold. Therefore, *Methanoculleus* takes the lead under trace element deprivation.

MCP40

Unique islands – microbial community composition and the potential of methane oxidation in tank bromeliad substrate of *Werauhia gladioliflora*

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Question: The densely arranged leaves of tank bromeliads create water bodies, distinct from the environment, collecting rainwater and leaf litter which is decomposed by a diverse community of aquatic organisms. Several thousand of tank bromeliad individuals per hectare of tropical forest create a unique canopy wetland ecosystem which significance for the neotropical methane cycle has been recently recognized. Methane (CH_4) formation, the final step in anaerobic degradation of organic material, is mediated in tank bromeliads by methanogenic archaea. Nevertheless, only little is known about the microbial diversity and community composition in tank bromeliads. We hypothesized that each individual bromeliad harbors its own microbial community being affected by environmental parameters creating unique chemical tank-slurry properties. We further hypothesized that methanotrophic bacteria inhabit tank bromeliad substrates, potentially able to oxidize produced CH_4.
Methods: Therefore, we investigated chemical tank-slurry properties (pH, carbon, nitrogen, oxygen and fatty acid concentrations) as well as the microbial community composition in eight individual tank bromeliads of the species *Werauhia gladioliflora* collected in a Costa Rican secondary forest. Microbial community profiling (terminal-restriction fragment length polymorphism) and quantitative analyses (qPCR) were conducted for bacterial and archaeal 16S rRNA genes as well as for the functional groups of methanotrophs and methanogens, using *pmoA* and *mcrA* marker genes. The potential of CH_4 oxidation was monitored during incubation experiments.
Results & Conclusion: We observed significant differences in the microbial community composition between individual plants. Every bromeliad tank seem to be an unique island with respect to its resident microbial community that is affected by chemical tank-slurry properties. The decrease of CH_4 concentration during the incubation of tank-slurry under aerobic conditions further indicated that not only CH_4 is formed but can be oxidized in bromeliad tanks as well.

MCP41

Effects of different dietary calcium-phosphorus and protein sources on bacterial community composition in the gastrointestinal tract of growing pigs

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Question: During the last decade, there has been a growing interest in feeding strategies stimulating a balanced gut microbiota which has an essential impact on animal health. A modulating effect on gut microbiota has been proven for certain dietary macro-elements. In this study, we investigated in a two factorial approach the influence of two dietary calcium-phosphorus levels and two protein sources on the bacterial composition of porcine gut.
Methods: Each experimental diet (soybean meal or pea based diet, supplemented with either a low or high calcium-phosphorus level) was fed to six pigs for six weeks. Feces were collected once per week. Digesta samples from ileum, jejunum, caecum and colon were taken at the end of experiment. DNA was extracted and the structure of bacterial communities was characterized by terminal restriction fragment length polymorphism (T-RFLP). To give phylogenetic assignment to single TRFs clone libraries of 16S rRNA gene from fecal and ileum digesta samples were constructed.
Results: Bacterial communities from fecal samples clustered into three main similarity groups and showed significant differences (P = 0.001) during course of the experiment. The microbiota from digesta samples clustered closely to small or large intestine origin. In caecum we found significantly (P = 0.001) different community structures in respect to the dietary calcium-phosphorus content, but not to protein source (P = 0.028). Individual TRFs contributing to these differences were identified and their phylogenetic identity assigned to clone library entries.
Conclusion: With this study on dietary effects on porcine gut microbiota we found a change in fecal bacterial communities as adaptation to environmental change in porcine gut. Composition of bacterial communities in porcine caecum is rather shaped by level of dietary calcium-phosphorus than fermentable substrate. Deeper insights into phylogenetic structure and functional activities of active bacterial communities contributing to the observed shift in bacterial community may be gained by Illumina amplicon sequencing and metaproteomic analysis.

MCP42

Adaptation of the pig´s fecal microbiota in response to different diets

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Question: Diet is one of the major environmental factors shaping the gut microbiota composition and activity. Nowadays, modulation of the dietary protein and carbohydrate composition together with pre- and probiotic supplementation are the most promising strategies to promote a healthy pig´s gut microbiota. However, it is still being discussed how and how long the microbiota adaptation process lasts, which is of a great importance for all nutritional studies.
In this study, terminal restriction fragment length polymorphism (T-RFLP) analysis together with a metaproteomic approach were used to determine the length of the adaptation period for the microbiota to restore its structural balance after the change from a basal diet to an experimental diet. In addition, the microbial response to diets with two different levels of CaP and two protein sources was investigated.
Methods: Pigs were randomly grouped and kept at four different diets varying in the level of supplemented CaP and the protein source (pea and soy bean). Fecal samples from three animals per diet were collected at seven different time points during the whole experiment. Changes on the microbial community structure were investigated via 16S rDNA using T-RFLP approach. One dimensional-nanoLC-ESI-MS/MS approach was also used to analyze the pig´s fecal metaproteome. The MS/MS data were analyzed through a two-step database search strategy, using both Proteome Discoverer and MaxQuant software for a qualitative and quantitative metaproteome characterization.
Results: T-RFLP measurements of the pig´s fecal microbiota showed significant shifts (p prior to the feeding of the experimental diets was different (p <0.05) from the community structure assessed after the experimental diets were fed. Here, a further distinction was observed between the microbial composition defined in the early points of time of the experiment and the fecal microbiota composition of the late time points (i.e. after the metabolic adaptation).

Conclusion: We report insights on the temporal changes of the gut microbiota after a dietary change. The results highlight the strong effect of the diet composition on the structure and activities of the fecal microbiota.

MCP43

Characterization of microbial communities from first stage reactors of two-phase agricultural biogas plants

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Hydrolysis and acidogenesis process steps as a component of large scale biogas plants are more and more used in practice. About 10 % of the agricultural biogas plants in Germany are equipped with hydrolysis and acidogenic process units. In frame of the joined project "AcEta (efficient hydrolysis and acidogenesis)" the aim of this study is the characterization of microbial communities within first stage reactors of agricultural biogas plants.
The idea behind the physical separation of acid-forming and methane-producing microbial groups in two phase reactors is to create the ideal condition for each microbial conversion step in the biogas producing process. Up to date populations in the first stage of two phase agricultural biogas plants are not well characterized. In this study five first stage reactors from different agricultural biogas plants were investigated. To characterize the microbial communities several methods were used. As cultivation independent methods 16S rDNA libraries were constructed and analyzed by amplified rDNA restriction analysis and sequencing. Also real time PCR quantification of different bacterial and archaeal groups was applied. Microorganisms which were able to grow aerobically were identified with colony PCR and MALDI-TOF-MS analysis. The MALDI-

TOF-MS method also is used to build a database which will enable a fast identification of these microorganisms.
Practical investigations have shown that wrongly designed hydrolysis and acidogenesis process steps are quite common. In combination with the results from the project partners which perform kinetic experiments in lab scale, compilation of process data and the elaboration of design recommendations, the urgently needed basic information for the reliable design and efficient operation of hydrolysis- and acidogenesis process steps will be determined.

MCP44
Quorum sensing controls phenotypic heterogeneous expression of the autoinducer synthase gene *traI* via copy number control of pNGR234*a* in the plant symbiont *S. fredii* NGR234
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Sinorhizobium fredii NGR234 is a plant symbiont that is able to nodulate over 112 different plant genera. Its genome encodes for two autoinducer (AI) systems regulating genes involved in the cell envelope biosynthesis, motility, cofactor metabolism and secretion related genes[1]. The NGR234 6.9 Mb genome consists of three replicons, a chromosome, a megaplasmid pNGR234*b* and a symbiotic plasmid pNGR234*a*[2]. The pNGR234*a* is a *repABC*-like plasmid and the region flanking oriV is highly similar to the *A. tumefaciens* Ti plasmid for which copy number regulation has been reported in response to altered AI concentrations[3]. qPCR analyses of copy numbers of pNGR234*a* of single AI deletion mutants *S. fredii* NGR234r*traI* and r*ngrI* and the corresponding double AI-synthase deletion mutant indicate that the partial or complete lack of AI molecules affects the copy number of the pNGR234*a* replicon. Similarly, mutations in the anti-activator protein TraM and/or the LuxR regulatory proteins NgrR and TraR result in an altered copy number of pNGR234*a*. In general, the copy number was altered in response to mutations affecting the AI regulons and by the addition of external AI molecules. The increased copy number basically eliminates the previously described phenotypic heterogeneous expression of the *traI* gene[4]. Using RNAseq we now provide first evidence that links the copy number control of pNGR234*a* and the phenotypic heterogeneity with two novel ORFs identified on the symbiotic replicon. We have designated these ORFs *cncA* and *cncB* and both have previously not been reported. The *cncB* gene encodes for a 51 aa protein and *cncB* for a 143 aa protein. Both proteins are unique to broad host range rhizobia and not present in *A. tumefaciens* or closely related bacteria affiliated with the rhizobiales. Altogether our data indicate that *S. fredii* has evolved a different regulatory mechanism for copy number control of its symbiotic plasmid compared to other rhizobial and agrobacterial species.

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MCP45
First evidence linking alpha-hydroxy-ketone-like quorum sensing in *Janthinobacterium* and *Duganella* with *Fusarium graminearum* growth suppression
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Genome sequencing of eleven *Janthinobacterium* and *Duganella* isolates in combination with phylogenetic and genomic analyses of publicly available genome data sets showed that only three out of 22 investigated strains harbor genes for the N-acetyl-homoserine lactone synthesis and no strain for the AI-2 synthesis. Instead, all but one strain carry a single gene cluster involved in the biosynthesis of an alpha-hydroxy-ketone-like autoinducer molecule. This signal molecule was previously designated janthinobacterial autoinducer-1 (JAI-1) (1), in analogy to the CAI-1 and LAI-1 autoinducer of *Vibrio* and *Legionella* (2). The JAI-1 synthase gene, *jqsA,* was identified in a highly conserved gene cluster together with the cognate receiver and sensor protein *jqsR* and the response regulator *jqsS*. Genome wide RNA-seq studies using *jqsA* deletion mutants of each a *Duganella* and *Janthinobacterium* representative identified up to 41 genes controlling gene expression in a QS-dependent way. Among the most strongly regulated genes were two clusters involved in the violacein and cell wall biosynthesis. Interestingly, most regulated genes carried a highly conserved palindromic JAI-1 motif within the putative promoter regions. Further tests indicated that all strains are capable to suppress growth of the plant pathogen *Fusarium graminearum* in laboratory growth assays, regardless of the violacein biosynthesis capacity. Most striking, co-incubation studies of the janthinobacterial isolate HH102 and its *jqsA* deletion mutant together with the plant pathogen *F. graminearum* provided first evidence for a QS-dependent interaction with this pathogen.

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MCP46
Mapping of the allelochemical-induced cell aggregation of key bacteria found in pelagic iron-rich aggregates
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Small groups of iron oxidizing bacteria (FeOB) and iron reducing bacteria (FeRB) dominate pelagic iron-rich aggregates ('iron snow') formed below the redoxcline in an acidic lignite mine lake. We isolated *Acidithrix,* the dominant FeOB, and *Acidiphilium*, the dominant heterotrophic FeRB, from iron snow aggregates and investigated interspecies chemical cross talk through cell-free supernatant exchange experiments and metabolomics profiling to elucidate potential allelochemicals mediating their interaction. Supernatant exchange experiments revealed supplementation with *Acidiphilium* cell-free supernatants resulted in faster rates of Fe(II) consumption (from 3.39 mM day^{-1} to 5.72 mM day^{-1}) as well as precipitation of brownish, insoluble iron (III) species in *Acidithrix* incubations. In addition, macroscopic cell aggregates of *Acidiphilium* were observed after 5 days following supplementation with *Acidithrix* cell-free supernatant. GC/MS analysis of bacterial extracellular products and metabolomics profiling suggested that 2-phenylethylamine (PEA) produced by *Acidithrix* is the chemical that triggers aggregation of *Acidiphilium* cells. *Acidiphilium* cultures supplemented with PEA triggered faster growth and cell aggregation, suggesting PEA produced by *Acidithrix* functions as the allelochemical which signals *Acidiphilium* to associate with iron snow. These results indicate interspecies chemical interactions between key organisms in pelagic iron snow aggregates can help the organisms to colonize, shape and transform the distinct spatial network they inhabit within the redoxcline.

MCP47
Interplay of global regulators of *Staphylococcus aureus* during the chronic osteomyelitis
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Staphylococcus aureus is a major human pathogen that can cause chronic and difficult-to-treat infections. *S. aureus* persistence in host tissue is linked to the bacterial ability to change to small colony variants (SCVs), which are adapted phenotypes for long-term intracellular persistence. The switching process between the aggressive wild-type phenotypes and persisting SCVs is very dynamic, but the underlying mechanisms are largely unknown. Our recent data from cell culture experiments indicated that regulatory mechanisms are involved in this dynamic switching process that involve downregulation of the quorum-sensing system *agr* and upregulation of the stress-related transcription factor SigB during bacterial persistence [1, 2]. In this work we investigated, whether these dynamic adaptation processes can be measured *in vivo*, as well. We have established a hematogenous murine osteomyelitis model with the *S.*

aureus strain 6850 that develops to chronicity over the course of 2 month and closely resembles the human disease [3]. Firstly, we tested 6 other *S. aureus* strains in this model and identified two clinical isolates that caused chronic osteomyelitis with bone deformation such as strain 6850, whereas the other strains tested also persisted in bone tissue without causing deformation processes. During persistence all tested *S. aureus* strains developed SCVs. Further on, we analysed the role of the global *S. aureus* regulators Agr and SigB in bacteria recovered from bone tissues of mice during the infection process. With all strains tested we found that *agr* is silenced and that the SigB-system is highly upregulated during persistence. These results could be reproduced with *S. aureus* isolates recovered from patients` specimens of chronic osteomyelitis Our findings clearly demonstrate that silencing of *agr* and high *sigB* expression is a common feature of chronic infection processes.

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MCP48

Staphylococcus aureus requires less virulence to establish an infection in diabetic organisms

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S. aureus is the most frequent pathogen responsible for diabetic foot infections that can develop to chronicity and are extremely difficult to treat. In this study, we define the bacterial degree of virulence that is required to establish an invasive tissue infection in diabetic organisms [1, 2]. To this end, we collected staphylococcal isolates from diabetic and non-diabetic foot ulcer and characterized the virulent capacity by functional assays, e.g. host cell invasion and cytotoxicity. In general, isolates from diabetic patients exhibited less virulence than isolates from non-diabetic patients, but nevertheless were able to establish severe infections. We even detected non-invasive isolates in deep diabetic tissue, although the strains were measured as almost a-pathogenic in cell culture models. Testing defined isolates in diabetic and non-diabetic murine footpad infection models revealed that in diabetic host organisms bacteria can persist at higher numbers than in non-diabetic tissue. Although the bacterial tissue loads were not different, low-virulent strains induced less inflammation and swelling than strains with a high virulent capacity. Taken together, our results demonstrate that in diabetic hosts not only high-, but also low-virulent staphylococcal strains can cause persisting tissue infections that are characterized by less inflammation, but a high bacterial tissue load.

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MCP49

Discovery of small molecule inhibitors of multicellular development in social amoeba

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The social amoeba *Dictyostelium discoideum* represents one of the earliest branches of the common ancestor of all eukaryotes. It is thus an ideal model organism to study general eukaryotic communication and cellular mechanisms. Since it can exist both in a single-cell and a multicellular state, *D. discoideum* serves as a paradigm in understanding the onset of multicellularity. Differentiation and multicellularity in this protist have evolved in a setting where they are surrounded by food sources (*e.g.* bacteria), as well as predators. As a result of this, interactions between these ecologically-connected organisms are undoubtedly orchestrated by signaling molecules, and the study of these interactions can provide a platform for the discovery of new natural products.

The social amoeba *D. discoideum* typically preys on bacteria, yet it can also serve as a food source for the related dictyostelid *D. caveatum*.[1] This feature was first described 30 years ago and has since been subject to further investigations. Importantly, *D. caveatum* can only feed on *D. discoideum* if the latter is present in a pre-culminant state. Previous studies have shown that *D. caveatum* secretes a factor that effectively freezes *D. discoideum* in a pre-culminant state, inhibiting the formation of the multicellular fruiting body and allowing *D. caveatum* to phagocytose its prey.

While preliminary experiments clearly show that a small diffusible molecule is the responsible morphogenesis inhibitor, its structure, biosynthesis, and mode of action remain elusive.[2] We utilize bioassay-guided fractionation to attempt to isolate and elucidate the structure of the small molecule(s) responsible for the inhibition of multicellular development in *D. discoideum*.

In the light of this ecological interaction, a deep understanding of the fundamental molecular communication and signaling processes can be gained. Understanding how small molecules selectively interfere with the early eukaryote *D. discoideum's* association machinery will provide us with insight for deciphering and modulating basic cellular mechanisms.

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MCP50

Functional and chemical analysis of fungal symbionts and antagonists of fungus-growing termites

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Question (working hypothesis): Termites of the genus *Macrotermitinae* cultivate a specific and mutualistic food fungus (*Termitomyces sp.*) for nourishment in so called "fungus gardens".[1] These nutrient-rich environment is prone to invasion by parasitic and pathogenic fungi. We hypothesize that the symbiotic food fungus *Termitomyces sp.* contributes to the defense of system by e.g. secretion of antimicrobial small molecules, which selectively target garden parasites and other invading species. Furthermore, we hypothesize that garden parasites can successfully counteract and invade the system once the garden homeostasis is unbalanced.

Methods: We started with the isolation of a broad range of symbiotic and associated fungi isolated from different termite nests of the genus *Macrotermitinae*. Then, we performed challenging assays pairing fungal symbionts and garden parasites to investigate the production of specific antifungal compounds. Subsequently, the produced antimicrobial secondary metabolites were analyzed using HPLC/LC-HRMS/NMR. In future, we will investigate the expression levels of the respective compounds using different transcriptomic and metabolomic methodologies.

Results: 1. Genome-mining of the fungal symbiont *Termitomyces sp.* revealed PKS and NRPS cluster encoding for potential secondary metabolites.[2] The expression of the respective compounds is now investigated. 2. Metabolomic studies of the fungal antagonist (*Pseudo*)*xylaria* sp. revealed also a broad spectrum of new secondary metabolites

Conclusion: We explore specific and well-described microbial interactions in combination with other multidisciplinary dereplication processes to find new chemical entities and to study many important aspects of the ecology and evolution of symbiotic associations.[3]

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MCP51

Eat or be eaten – predator-prey interactions of *D. discoideum* and amoeba-pathogenic bacteria

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Bacteria are constantly exposed to a multitude of threats: bacteriophages can infect and kill bacteria; amoebae, nematodes, insects, and plants can prey on prokaryotes, and competitor strains fight for the same resources. In order to survive in this battlefield, bacteria have evolved highly effective defense mechanisms.[1,2] Because killing and deterring the antagonists are powerful ways to thrive in this environment, bacteria display a great diversity of toxins and antibiotics that selectively act on their enemies. Amoebae are voracious and ubiquitous predators to bacteria that cause constant depletion of huge bacterial reservoirs. This puts both organisms under strong evolutionary selection pressure: the bacteria have evolved mechanisms to prevent grazing and the amoebae must counteract or surmount these mechanisms in order to survive.[3]

We focus on the interactions between the eukaryotic soil amoeba *Dictyostelium discoideum* and amoeba-pathogenic soil bacteria. In particular, general defense strategies of *D. discoideum* against pathogenic bacteria are investigated that rely on the degradation of both bacterial amoebicides and communication signals. The chemical warfare in this predator-prey relationship is examined using molecular biology approaches, various bioassays (killing assay, plaque assay), and by chemical analysis of culture extracts, both from the amoeba and the bacteria, as well as from co-cultivation experiments. Subsequent bioassay-guided fractionation of bioactive extracts from amoebal or bacterial cultures eventually allows identifying natural products with antibacterial, amoebicidal or cytotoxic properties that orchestrate the coexistence of the competitors in nature.

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MCP52

Two ways of *Pseudomonas* quinolone signal degradation in clinical isolates of the emerging pathogen *Mycobacterium abscessus*

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Question: The opportunistic pathogen *Pseudomonas aeruginosa*, which colonizes the lung of patients suffering from cystic fibrosis, regulates its virulence via complex quorum sensing (QS) systems. One of them utilizes the alkylquinolones (AQs) 2-heptyl-3-hydroxy-4(1*H*)-quinolone (*Pseudomonas* quinolone signal, PQS) and 2-heptyl-4(1*H*)-quinolone (HHQ) as signal molecules [1].

M. abscessus is an emerging pathogen causing pseudotuberculous lung disease in patients with cystic fibrosis. To analyze the potential of different *M. abscessus* strains to interfere with *P. aeruginosa* QS, ten clinical isolates, six of which harbor *aqd*-genes homologous to the genes for AQ degradation of *Rhodococcus erythropolis* BG43 [2], were tested for their ability to convert HHQ and PQS.

Methods: AQ concentrations in organic extracts of culture samples were analyzed by HPLC and LC/MS.

Results: Cell suspensions (OD 3.5) of clinical *M. abscessus* isolates which have a specific gene cluster homologous to the *aqd*-genes of *R. erythropolis* BG43 [2] convert 20 µM HHQ or PQS within 2h (OD 3.5). HHQ is hydroxylated to PQS, followed by cleavage of the heterocyclic ring to *N*-octanoylanthranilic acid which subsequently is hydrolyzed to anthranilic acid. Clinical isolates not harboring the *aqd*-cluster can degrade PQS albeit much more slowly (at OD 3.5: degradation incomplete after 72h). Intermediates which have the same UV/vis spectrum as PQS but different retention times in HPLC separations are transiently formed. So this way of degradation might proceed via reactions involving modification of the of the alkyl chain.

Conclusion: The emerging pathogen *M. abscessus* possesses two pathways for degrading the *P. aeruginosa* QS signals PQS and HHQ. One corresponds to the way *R. erythropolis* BG43 degrades PQS and HHQ. The other one presumably occurs via formation of other intermediates, which let us suggest, that the alkyl chain is modified.

M. abscessus may have the potential to interfere with *P. aeruginosa* QS by inactivating the AQ signals HHQ and PQS and hence reduce the virulence. It will be very interesting to analyze the interactions between *P. aeruginosa* and *M. abscessus* and investigate whether the ability to inactivate *P. aeruginosa* quorum sensing signals contributes to co-colonization competitiveness.

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MCP53

Elucidation of specific interaction between the fungus *Aspergillus nidulans* and Streptomycetes

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Streptomycetes, a species-rich group of actinobacteria, are well-known producers of a plethora of secondary metabolites. Of particular interest are the two species *Streptomyces rapamycinicus* (NRRL 5491, 1) and *Streptomyces iranensis* HM35 (DSM 41954, 2, 3), which (i) exhibit a high similarity at the genomic level, (ii) produce the immunosuppressant rapamycin and (iii) specifically induce secondary metabolite formation in filamentous fungi, *e.g.* in the important model fungus *Aspergillus nidulans* the orsellinic acid (*ors*) gene cluster (4) and in the human pathogenic fungus *Aspergillus fumigatus* the fumicycline gene cluster (5). The latter feature provides an excellent model system to elucidate the underlying molecular mechanisms how a silent fungal secondary metabolism gene cluster can be activated by a bacterium. To identify the primary bacterial signal we generated a random Tnp(a) transposon insertion mutant library of *S. iranensis*. For this purpose the transposon vector pTNM (6) and the existing protocol were modified, thus we accomplished successful conjugation of pTNM_kan into *S. iranensis* as well as the generation of a *S. iranensis* mutant library. By screening the mutant library in 48-well plates, we found promising mutants lacking the activation of fungal secondary metabolite formation. To verify the involvement of the identified genes, an applicable strategy for targeted gene deletion in *S. iranensis* was developed. First results of the generated direct gene deletion mutants and further characterizations thereof, will be presented.

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MCP54

Bacteria induce pigment formation in the basidiomycete *Serpula lacrymans*

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Question: The basidiomycetes *Serpula lacrymans* and *Paxillus involutus* produce atromentin-derived pigments that are involved in Fenton redox cycling and were shown to have increased production from nutritional cues[1,2,3]. We addressed the question if there are other environmental cues like microbial communication that can cause induction of these widespread pigments.

Methods: *Serpula lacrymans* was co-incubated with one of three diverse terrestrial bacteria, *Streptomyces iranensis*, *Bacillus subtilis* and *Pseudomonas putida*. Analyses on both a genetic and chemical level were accomplished with qRT-PCR and analytical chromatography.

Results: We show that the atromentin gene cluster of *S. lacrymans* was up-regulated and that secreted pigments accumulated, respectively, during co-incubation with a bacterium. Each co-incubation condition caused a similar early pattern of gene up-regulation, however, *S. lacrymans* with *Streptomyces iranensis* showed prolonged elevated expression whereas with *B. subtilis* and *P. putida* the expression level subsided.

Conclusion: We concluded that because a diverse set of bacteria caused pigment induction and production in the basidiomycete *Serpula lacrymans* there is a common signal elicitation from habitat-sharing microbes.

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MCP55

Using fluorescent *Enterococcus mundtii* to study the variation in its gene expression, spatially and temporally, in the gut of *Spodoptera littoralis* larva

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Insects harbor a plethora of microorganisms within their gut as their normal flora. They are not only non-pathogenic to them, but also have roles to play in the insect growth, development and immunity. There lies a complex interaction within the microflora and, between the host and the microorganisms. This leads to variations in the microbial population with the development of the insect. Some get eliminated, others are added, whereas, some dominate throughout the insect life cycle [1].
The gut microbiota of *Spodoptera littoralis,* a Lepidopteran insect of family Noctuidae, has been elucidated. The core community consists of *Enterococci, Lactobacilli and Clostridia.* The variation andselection of one bacterial species over the other is quite evident throughout the life-cycle. By the time the larva reaches the 5th instar stage, *Enterococcus mundtii* and *Clostridia sp* persist and dominate. Also, there is a difference in the presence and abundance of bacteria among the fore, mid and hind gut of the larva. [1,2].
The class IIa antimicrobial peptide- mundticin KS [3], produced by *E. mundtii,* acts against the competing bacteria, and exercises its predominance (Unpublished). This way, they help preserve the host gut microbiota, reduce infection in the host and enhance its health. A method of fluorophore-labelling of *E. mundtii* has been developed to show that it is the persistent and metabolically active species in the gut microbiota (Unpublished). This intrigues us to find out the importance of this symbiotic species.
Thus, the **aim** of my project will be to isolate the fluorescent *E. mundtii* from various regions of the larval gut, across its developmental stages, and study the variation in their gene expression. The Next generation method of RNAseq will be used to sequence the transcriptome.
Methods:
Fluorophore labelling of bacteria and feeding the larvae; Dissection of Guts of *S. littoralis* larvae; FACS to isolate Fluorescent bacteria; RNA extraction; RNA seq
Thus, on analyzing the transcriptome of *E. mundtii* over the stages of *S. littoralis* larval development, we might **conclude** its specific role as a symbiont and its importance as a dominating gut microbiota. This approach can be extended to other relevant gut bacteria.

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MCP56

Native root-associated bacteria protect their host plant from a fungal sudden-wilt disease via synergistic mechanisms.

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Plants maintain extensive microbial associations whose functions remain largely unknown. In 2011, our model plant *Nicotiana attenuata* suffered sudden tissue collapse and black roots, symptoms similar to a *Fusarium-Alternaria* disease complex, when grown in its native habitat, the Great Basin Desert, Utah, USA. To find potential remedy for this sudden wilt disease, 3 different protection strategies (fungicide, soil amendment and seed inoculations with native root-associated bacterial and fungal strain isolated from previous experiments) were tested in the field. A field trial with more than 900 plants in field plot showed that only the inoculation treatment with a mixture of five native bacterial isolates significantly reduced disease incidence and mortality. Similar disease reduction rates were obtained from a second field trial during the following year, demonstrating the robustness of the plant protection effect by bacterial treatment (Santhanam *et al.,* 2015). In general, beneficial bacterial mutualist protects their host plants via different mode of actions such as production of antimicrobial and biofilm, competition for nutrients and colonizing ecological niches. Further investigations under *in vitro* conditions, on the potential mechanism of disease suppression revealed that the mixture of bacterial isolates complementing their biocontrol traits via synergistic mechanisms such as biofilm formation, competition of nutrients- siderophore production, colonizing ecological niches and production of the antifungal compound surfactin. Native plants, perhaps like most eukaryotes, develop opportunistic mutualisms with prokaryotes which help them to protect from phytopathogens.

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MCP57

The metabolic gene *gbuA* is essential for inducing QS-regulated virulence factors at low population density and nutrient limitation in *Pseudomonas aeruginosa*

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Question: The ubiquitous bacterium *P. aeruginosa* employs the same set of quorum sensing (QS)-regulated virulence factors for attacking organisms of different kingdoms like other microbes, plants, and humans. QS is defined to be cell density-dependent, but in *P. aeruginosa* QS is co-regulated by certain environmental cues, mostly nutrient limitation [1]. We investigated the QS-dependent expression of virulence factors with a model system consisting of a bacterial co-culture with *P. aeruginosa* and the opportunistic pathogen *Aeromonas hydrophila* using chitin as sole growth substrate [2]. In this model system growth of *P. aeruginosa* is dependent on the QS-regulated parasitic exploitation of the chitinolytic properties of *A. hydrophila*. During parasitic growth with *A. hydrophila*, the QS-regulated virulence factor pyocyanin is crucial for growth of *P. aeruginosa*. The production of pyocyanin is thereby already induced at a low population density as a consequence of limited nutrient availability.
In this study we aimed at identifying novel genes that are involved in QS-regulated pyocyanin production under co-culture conditions.
Methods: Transposon mutagenesis of *P. aeruginosa* was carried out and mutants were screened for reduced production of pyocyanin in co-cultures with *A. hydrophila*. Promising mutants were further characterized physiologically and genetically.
Results: We identified the gene *gbuA* (PA1421) that encodes a guanidinobutyrase (GbuA) catalyzing the conversion of 4-guanidinobutyrate (4-GB) to 4-aminobutyrate and urea and that is regulated by GbuR. *GbuA* and *gbuR* deletion mutants did not produce pyocyanin in co-cultures with *A. hydrophila* and showed reduced pyocyanin production in single cultures. Transcriptional promoter-*lacZ* fusions revealed that in a *gbuA* mutant transcription of the operon *pqsABCDE*, which is involved in the QS system based on 2-alkyl-4(1H)-quinolone signals, and of two operons required for pyocyanin biosynthesis, *phzA1G1* and *phzA2G2*, were reduced during growth in both single and co-cultures. Addition of the QS signals HHQ and PQS, which are produced by PqsABCD, as well as overexpression of PqsE restored pyocyanin production in a *gbuA* mutant in co-culture. The effect of *gbuA* deletion on pyocyanin production in single cultures could be enhanced by the addition of 4-GB.
Conclusion: These results suggest that the intracellular accumulation of 4-GB leads to a reduced transcription of genes of the AQ system and, thus, a reduced transcription of genes for pyocyanin biosynthesis. The metabolic context of 4-GB formation and degradation is currently under investigation. Our results indicate that nutrient availability can dominate QS-dependent virulence factor production via a so-far unexplored metabolic pathway.

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MCP58 – withdrawn

MCP59

Identification of secondary metabolites with roles in interactions between *Chlamydomonas reinhardtii* and other microorganisms

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Approximately half of global carbon fixation is performed by aquatic photosynthetic organisms, which are mostly algae and cyanobacteria.[1] As primary producers they play a key role in their habitats. So far, little is known about the interaction of microalgae with other microorganisms, and the modes of interaction.[2] To get a closer view into this topic, we started to study the interactions of the unicellular microalga *Chlamydomonas reinhardtii* living in freshwater and wet soil with other microorganisms. We quantified the growth rates of axenic and mixed cultures of *C. reinhardtii* and different bacteria. To analyze the chemical nature and function of secondary metabolites in these interplays, metabolic profiles were compared between axenic and mixed cultures using liquid chromatography-mass spectrometry (LC-MS). Also, MALDI imaging will be used to study the interaction interface. First results and environmental aspects of current experiments will be presented.

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MCP60

***Quorum Sensing* signaling molecules produced by bacteria modulate behaviors of marine eukaryotic organisms**

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The cues that trigger habitat selection and metamorphosis of marine organisms have been matter of debate for decades, and biofilms play a crucial role in this process. The goal of this work was to evaluate the effect of *Quorum Sensing* signaling molecules (QSSMs) on migration and metamorphosis of *Hydractinia symbiolongicarpus* larvae (Cnidaria: Hydrozoa), which grow on gastropod shells occupied by hermit crabs. Sequencing of 16S rRNA gene from cultivable bacteria associated to shells was performed. Also, using AHLs-based sensors and cross-streaking assays, QSSMs produced by bacteria were detected, extracted and analyzed by thin layer chromatography. Finally, tests to determine the effects of extracts were made using larvae. Ten bacterial species were isolated from shells and QSSMs were detected in three: *Shigella flexneri*, *Microbacterium liquefaciens* and *Kocuria erythromyxa*, producing four different AHLs or similar molecules: 3-oxo-C6-AHL, 3-oxo-C8-AHL, 3-oxo-C10-AHL and 3-oxo-C12-AHL. Biofilms and QSSMs extracts from these species induce a positive chemotactic effect on larvae, what was not observed in mutant strains unable to produce QSSMs. Furthermore, QSSMs induce larval attachment to the substrate at high rates activating initial steps of metamorphosis; however, attached larvae did not proceed to metamorphosis to a primary polyp after 24 hours. These results suggest that QSSMs are strong chemotactic signals and also, that other molecules might act synergistically with them to induce complete metamorphosis. The effects of these molecules on the developmental dynamics of *H. symbiolongicarpus* represent an example of inter-domain chemical communication and might be used in strategies directed to restore affected coral reefs through coral nurseries methods.

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MCP61

Chemical signals that control mating in the benthic diatom *Seminavis robusta*.

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Question: As major primary producers and basis of aquatic food webs, diatoms shape the marine environment. Several studies indicate that chemical cues mediate their life cycle and mating, but only recently the first diatom pheromone was identified in the benthic diatom *Seminavis robusta*. This proline derived diketopiperazine L-diproline mediates the chemoattraction of the mating partners. However, evidence suggests that more signaling molecules are involved in sexual reproduction.
Since the natural environment of benthic diatoms is very divers, other organisms might interfere with their chemical communication. It is therefore of great interest, how specific pheromones are perceived and whether co-existing species can actively interfere the signaling process.
With our study we further investigate the pheromone system of *S. robusta* by i) identification of novel infochemicals, ii) determination of pheromone specificity and iii) by evaluating the role of co-existing bacteria.
Methods: i) A metabolomics-guided approach was used to identify novel pheromone candidates of *S. robusta*. The exometabolomes of the two mating types were analyzed by LC-MS and upon comparison of their metabolic profiles pheromone candidates were found and verified in bioassays.
ii) For structure-activity-relationship studies derivatives of L-diproline were synthesized. Their bioactivity was then analyzed in attraction assays.
iii) Production and degradation of L-diproline in axenic, non-axenic diatom cultures as well as bacterial cultures was analyzed by GC-MS and UPLC-MS.
Results: i) We found signaling molecules that induce a cell-cycle arrest in the pairing cells and the production and perception of the attraction pheromone in *S. robusta*. Thereby, candidates for these sex-inducing-pheromones of both mating types were identified with one being a sulphated, polyhydroxylated compound.
ii) Our results indicate a surprisingly low stereospecificity of L-diproline perception, suggesting that the reception of the attraction pheromone might be promiscuous. Structure activity assays reveal conserved and flexible structure elements in L-diproline analogs.
iii) Comparing axenic and non-axenic cultures, we observed a different efficiency in the mating rate, indicating that the bacterial community can influence the sexual cycle of diatoms. However, we still have to understand how bacteria can metabolize and degrade L-diproline or if they interact with other important metabolites involved in diatom life cycle.
Conclusion: In this study we used an elaborated analytical work flow to identify novel infochemicals of a benthic diatom. Using synthetic diproline analogs the promiscuity of the attraction pheromone receptor was proven. Furthermore, our findings suggest an influence of bacteria on sexual reproduction. Our results point to a cost-efficient multistep-pheromone system in *S. robusta*.

MCP62

Co-cultivation of *Serratia odorifera* 4Rx13 and *Bacillus subtilis* B2g leads to quantitative changes in the volatile profile compared to mono-cultivated strains

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Bacteria emit diverse and complex blends of volatiles, of those the majority is still unknown. Because of their physico-chemical properties the bacterial volatiles present ideal info-chemicals in interaction processes with insects, plants and fungi. Although it was shown that bacterial volatiles beneficially or deleteriously influence plant growth and/or the growth of fungi, their ecological significance is poorly understood.
Serratia plymuthica 4Rx13 is a rhizobacterium that releases at least 100 volatiles including sodorifen, several alcohols, terpenoids and sulfur compounds (1). Bioassays demonstrated that the volatile blend of *S. plymuthica* 4Rx13 can dramatically inhibit the growth of plants (i.e. *Arabidopsis thaliana*, *Physcomitrella patens*) and phytopathogenic fungi (*Rhizoctonia solani*) (2, 3). Thereby, our recent research focused on the functional characterization of volatiles emitted from *S. plymuthica* grown as single bacterial strain. Single bacterial strains do not occur in multi-organismic ecosystems e.g., the rhizosphere or the human body. Furthermore, it is now well established that the production and/or secretion of primary and specialized metabolites changes due to competition or cooperation with other organisms. For instance, first data suggest that also the production of antifungal volatiles changes in microbial communities (4). Therefore, our hypothesis is that the emission of volatiles by *Serratia plymuthica* 4Rx13 alters due to bacteria-bacteria interactions.
To test this hypothesis *S. plymuthica* 4Rx13 was co-cultivated with *B. subtilis* B2g on solid medium. Within 30 days of co-cultivation the headspace volatiles were analyzed using solid phase micro extraction-gas chromatography/mass spectrometry (SPME-GC/MS).
No qualitative differences in the volatile profile have been detected between co-cultivated *S. plymuthica* 4Rx13/*B. subtilis* B2g and mono-cultivated strains. Quantitatively, we observed significant changes in the

volatile profile. While in the early stage of co-cultivation the volatile amount has been lower compared to the mono-cultivation, in the late stage the ratio changed to a higher amount of volatiles in the co-cultivation compared to the mono-cultivation.
We are presently investigating further co-cultivations with other interaction partners as well as the effect of the culture condition on the interaction and in turn on the volatile emission.

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MCP63

Detection and characterization of the quorum-sensing g-butyrolactone molecules produced by *Rhodococcus jostii* RHA1 (RJBs)

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g-Butyrolactones are signalling molecules that have been described and extensively studied in *Streptomyces*[1,2]. These molecules are part of the g-butyrolactone system which regulates secondary metabolism, e.g. antibiotic biosynthesis. g-Butyrolactones bind to a receptor protein (TetR regulators) and thereby regulate gene expression of antibiotic biosynthesis pathways. AfsA is the key enzyme involved in catalysing the first step of g-butyrolactone biosynthesis, the condensation of a glycerol derivative with a fatty acid derivative[3].
An AfsA homologue had been detected in different actynomycetes species, including 4 different *Rhodococcus* strains[4]. No data was available about its function in the *Rhodococcus* genus. In this work we analysed the available *Rhodococcus* assembled genome sequences for the presence of an AfsA homologue, which was found in 12 of the 13 analysed strains. These genes share an amino acid identity of 33 %-44 % with *Streptomyces griseus* AfsA. We studied the possible synthesis of g-butyrolactones by *Rhodococcus jostii* RHA1. We found that this *Rhodococcus* strain is producing molecules which activate a g-butyrolactone specific reporter system developed for *Streptomyces*[5]. This system relies on binding of g-butyrolactones to the TetR regulator of *Streptomyces coelicolor* (ScbR). Activation of this reporter system shows that *R. jostii* RHA1 produces one or more g-butyrolactone-like molecules that have structural similarity to the ones found in *Streptomyces*. LC-MS analysis indeed showed that a molecule is produced by *R. jostii* RHA1 with the same mass and retention time as 6-dehydro-SCB2, an isomer of A-factor, the g-butyrolactone produced by *Streptomyces griseus*[3] and a predicted precursor of one of the described g-butyrolactones from *S. coelicolor*, SCB2[6].
We also showed that the *R. jostii afsA* gene is essential for g-butyrolactone production by constructing a gene deletion mutant. The *R. jostii* Δ*afsA* strain failed to produce g-butyrolactones. We also constructed an *afsA* overexpression strain; its analysis indeed showed that this resulted in a higher production of g-butyrolactones.
We report for the first time that the genus *Rhodococcus* produces g-butyrolactones and that the *afsA* homologue gene is essential for the production of ***R**. **j**ostii* g-**b**utyrolactones (RJBs).
Streptomyces and *Rhodococcus* both are found predominantly in soil. Such g-butyrolactone molecules in fact may be involved in communication between these two genera of actinobacteria in their natural environment.

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3 Kato *et al.*, 2007, *Proceedings of the National Academy of Sciences of the USA of America,* 104(7), pp. 2378-2383.
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5 Hsiao *et al.*, 2009, *Methods in enzymology,* 458, pp. 143-157.
6 Martin-Sanchez *et al.* Manuscript in preparation.

MCP64

New insights in plant-endophyte communication: maytansine as an example

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Question: Studies on microbe-host interactions in plant and animal systems aimed at understanding the role of these associations and their utility in pharmaceutical and agricultural sectors are gaining impetus [1]. Several recent studies have lent evidence to the fact that certain so-called "plant metabolites" are actually biosynthesized by associated endophytic microorganisms. For example, we investigated the central role of chemical crosstalk in plants and endophytes, using the important anticancer and cytotoxic compound maytansine, in *Putterlickia verrucosa* and *Putterlickia retrospinosa* plants [2]. This extremely interesting outcome provided the scientific basis to investigate the actual producer(s) responsible for maytansine biosynthesis in *Maytenus* plants, which has been an open question since its discovery from this plant genus in the 1970s.
Methods: Endophytic communities harboring different tissues of *Maytenus serrata* originating from Cameroon were investigated using a combination of high performance liquid chromatography high-resolution mass spectrometry (HPLC-HRMS^n), matrix assisted laser desorption ionization imaging high-resolution mass spectrometry (MALDI-imaging-HRMS), and genome mining techniques to elucidate the source and sites of maytansine biosynthesis.
Results: Using a combination of bioanalytical methods as well as by molecular profiling, it was revealed that the host plant along with its cryptic endophytic microflora produces the biosynthetically unique core structural moiety mC_7N, called 3-amino-5-hydroxybenzoic acid (AHBA) that serves as the unique starter unit for maytansine biosynthesis via the aminoshikimate pathway. However, the biosynthetic step of halogenase-mediated incorporation of chlorine, which is missing in the host plant, is accomplished by the culturable stem endophytic community. Thereafter, the biosynthetic steps downstream of halogenation till formation of maytansine are achieved by the host plant.
Conclusion: Taken together, it was particularly interesting to note that only the culturable stem endophytic community of a selected *M. serrata* plant had coevolved a means to produce maytansine jointly with the host plant, including trafficking of the precursors from and to the host plant. Our results provide a scientific handle to delve into further details of plant-endophyte communication not only on the transcriptome level but also the metabolome level to pinpoint regulation of host-endophyte communication, and to determine qualitative and quantitative pattern of localization and trafficking of endophytes with regard to shared biosynthesis of maytansine.

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Kusari, S.; Lamshöft, M.; Kusari, P.; Gottfried, S.; Zühlke, S.; Louven, K.; Hentschel, U.; Kayser, O.; Spiteller, M. *J. Nat. Prod.*2014, 77, 2577-2584.

MCP65

***N*-acyl-homoserine lactones produced by the endofungal bacterium *Rhizobium radiobacter Rr*F4 play a vital role in the biological activities of *Rr*F4**

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The Alphaproteobacterium *Rhizobium radiobacter* F4 (*Rr*F4) was originally isolated from the plant growth-promoting basidiomycete fungus *Piriformospora indica* (syn. *Serendipita indica*) that forms a tripartite Sebacinalean symbiosis with a broad range of host plants. Interestingly, the isolated bacterium showed biological activities widely comparable to those exhibited by *P. indica* (Sharma *et al.*, 2008; Glaeser *et al.*, 2015), but the mechanism by which these are achieved is not fully understood. Chemical analysis showed that *Rr*F4 produces a spectrum of different *N*-acyl-homoserine lactones with acyl chains of C8, C10, and C12 as well as hydroxyl- or oxo-substitutions at the C3 position.
To assess the impact of *Rr*F4-produced *N*-acyl-homoserine lactones (AHLs) on its beneficial activities, the AHL-negative mutant *Rr*F4NM13 was used. In contrast to *Rr*F4 wild-type (WT), the mutant failed to promote growth of wheat seedlings. Furthermore, bacteria-induced systemic resistance to the bacterial pathogen *Xanthomonas translucens* pv. *translucens* was reduced in *Rr*F4NM13-treated plants compared to control plants treated by *Rr*F4 (WT). Moreover and consistent with the above findings, growth promoting activity exerted by *Rr*F4 in *Arabidopsis thaliana* was greatly abolished with *Rr*F4NM13. Together, our results suggest that AHLs are critical factors of *Rr*F4's beneficial activity.

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Glaeser SP, Imani J, Alabid I, Guo HJ, Kumar N, Kämpfer P, Hardt M, Blom J, Rothballer M, Hartmann A, Kogel KH (2015). Non-pathogenic *Rhizobium radiobacter* F4 deploys plant beneficial activity independent of its host *Piriformospora indica*. *ISME.J.* doi:10.1038/ismej.2015.163.

MCP66 – withdrawn

MCP67– withdrawn

MCP68

Strategies to study the pathogenicity of the apple canker fungus *Neonectria ditissima* and visualization of pathogenic growth *in planta*

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Neonectria ditissima is the causal agent of apple canker, a devastating disease in all major apple-growing areas of Europe, North America, Chile, Australia, New Zealand, Japan and South Africa. Within the last decades European canker has become a serious economic problem to farmers. The biology of *N. ditissima* as well as the resulting disease symptoms have recently been reviewed (Weber 2014), but little is known concerning molecular mechanisms of plant/pathogen-interactions and the contribution of either phytotoxins or secreted enzymes as putative virulence or pathogenicity factors.
The project aims to identify and characterize factors responsible for disease development. In this context we will address the question whether disease development and pathogenic growth are promoted by either phytotoxin production or enzyme secretion or both?
In order to identify phytotoxic compounds produced by *N. ditissima* and to elucidate the mechanisms of pathogenicity we established a set of diagnostic tools, i.e. a fruit-infection-assay by which disease symptom development and infection strength in fruits can be monitored. Furthermore this assay is used for the identification of antagonistic microorganisms and fungicides *in vivo*.
To visualize pathogenic growth, a GFP-expressing mutant strain of *N. ditissima* was generated by developing the first known method for genetic manipulation of *N. ditissima* conidia via *Agrobacterium tumefaciens*-mediated transformation. The generated fluorescent-labelled mutant strain can be used to visualise pathogenic growth *in planta*.
Furthermore, we will present initial findings concerning directed molecular manipulation of *N. ditissima*. We are able to generate "loss-of-function"-mutants of genes encoding proteins of interest putatively contributing to virulence or pathogenic differentiation.

Weber, R.W.S., 2014. Biology and control of the apple canker fungus *Neonectria ditissima* (syn. *N. galligena*) from a Northwestern European perspective. *Erwerbs-Obstbau*, 56(3), pp.95-107. Available at: http://link.springer.com/10.1007/s10341-014-0210-x [Accessed November 19, 2015].

MCP69

Characterization of small regulatory RNAs in the plant pathogen *Agrobacterium tumefaciens*

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In recent years, the number of identified small regulatory RNAs (sRNAs) in various bacteria has grown rapidly. Most of the characterized sRNAs exhibit their regulatory function by base pairing with their target mRNA leading to altered translation or stability of their target [1]. Despite evolving bioinformatic prediction tools the identification of sRNA targets remains a major challenge.
In the model organism *Agrobacterium tumefaciens*, which is known as a plant pathogen transferring part of its own DNA into the plant cell, over 650 sRNAs have been discovered [2, 3, 4]. To date, only the two sRNAs RepE, influencing the replication of the Ti plasmid [5], and AbcR1, regulator of various ABC transporters [6, 7], have been functionally characterized.
We report studies of the *trans*-encoded sRNA C10 from the circular chromosome of *A. tumefaciens*. Deletion of C10 causes pleiotropic effects regarding growth, motility and virulence. Furthermore, bioinformatic predictions lead to identification of putative targets involved in peptidoglycan biosynthesis and chemotaxis.

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MCP70

Factors affecting optimal growth of *Xanthomonas campesteris* pv. *vesicatoria* in the apoplast of pepper plants.

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Xanthomonas campesteris pv. *vesicatoria* (*Xcv*) is a phytopathogenic gram-negative γ-proteobacterium, that causes bacterial spot disease on pepper (*Capsicum annuum*) and tomato plants. Upon invasion this bacterium colonizes in the intercellular space (apoplast) of the plant. In response, plants limit the avaibility of nutrients, oxygen, iron etc and also produce reactive oxygen species (ROS) as a host defense to restrict bacterial growth.
For successful establishment of a stable bacterial biofilm in the plant apoplast, *Xcv* needs nutrients; however, little is known regarding what these substrates in the plant apoplast might be. Moreover, it is also unclear how this bacterium fulfills its nutrient requirement and to what extent other factors influence bacterial growth in the apoplast.
Iron, is also a crucial trace element required for many biological processes. Intracellular iron homeostasis must be maintained for cell survival and protection against the toxic effects of excessive iron. A further important question, therefore, is how the bacterium maintains iron homeostasis under the potentially iron-limiting conditions of the plant apoplast.
To address these questions biomolecular analysis of the organic acid, sugar and amino acid content of the plant apoplast needs to be studied. Our results suggest that AcnB (aconitase), a FeS (iron-sulphur cluster)-containing enzyme is important for optimal growth and survival of *Xcv* in pepper plants, which might be required for the utilization of citrate as carbon source and could also help to protect the bacterium against oxidative stress. The genome of *Xcv* encodes a further two Acn enzymes, AcnA and AcnA2. It has been shown for other bacteria that Acns are bifunctional proteins: as well as functioning as metabolic enzymes that use citrate, they are also post-transcriptional regulators, controlling the activities of iron and citrate transporters. *Xcv* encodes two citrate transporters, CitH and Cit, and has a *fur* gene as a regulator of iron uptake systems. Therefore, insight into the role of these components will be important to understand how *Xcv* establishes itself in the plant apoplast. We describe the analysis of the pepper apoplast metabolome and citrate transport mutants to determine how important citrate is for bacterial growth and iron homeostasis *in planta*.

MCP71

Sponge holobiont integrity under stress – a stable microbiome subset facilitates antibacterial defense in *Haliclona* sp.

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Sponges host highly diverse microbial communities that contribute substantially to the evolutionary success of the holobiont - the unit composed of the host and its symbionts. However, this close association can be imbalanced by environmental stressors. Sponge microbiomes fulfill integral functions for sponges regarding nutrition, maintenance, and bioactivity-derived defense and the structure of those microbiomes can be allocated into three clusters based on specificity: core, variable, and species-specific (Schmitt *et al.* 2012). Yet, the meaning of this community structure and its implication on holobiont functionality are not fully understood. The aim of this study was to link defense capacity as a proxy for basic functionality to the microbiome structure. Thus, we performed a four-week stress experiment with antibiotic exposure and light exclusion on a high microbial abundance sponge of the genus *Haliclona* to impact the bacterial microbiome and to test for antimicrobial defense in the shifted microbiome. Bacterial community shifts were investigated by amplification of bacterial 16S rRNA gene fragments and subsequent denaturing gradient gel electrophoresis (DGGE) and Illumina amplicon

sequencing. Composition and dissimilarities were assessed using diversity indices, similarity percentage breakdown analysis (SIMPER) and non-metric multidimensional scaling (NMDS). Both stressors strongly influenced the sponge and its bacterial community but certain taxa remained associated and were defined here as the stable subset of the *Haliclona* sp. microbiome. Compared to the native microbiome, 17 of 25 phyla were affiliated to this stable subset and these taxa occurred primarily with relative abundances of 0.05-0.5 % and attributed to 28 % in total. The six most abundant phyla in the native *Haliclona* sp. microbiome *Proteobacteria* (46 %), *Gemmatimonadetes* (14 %), *Verrucomicrobia* (11 %), *Bacteriodetes* (9 %), *Acidobacteria* (9 %), and *Chloroflexi* (5 %), were also most abundant in the microbiome subset, which additionally showed a high abundance of *Firmicutes*. Antimicrobial defense activity was tested in an *Aliivibrio fischeri* based bioluminescence assay and remained intact in the compromised host. We attribute this defense capacity to the tightly associated bacteria of the stable subcommunity and conclude that basic functionality in stressed holobionts is provided by disposable agents of a resilient microbiome sub-community. This study sheds further light on the sponge microbiome structure in relation to antibacterial defense and can serve as a basis for future investigations of microbiome functions in sponge holobionts.

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MCP72

Microplastics reduce growth and survival rates of scleractinian corals and alter microbial communities in ambient water

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Microplastics (plastic fragments <5 mm in diameter) are found globally in marine ecosystems (Moore 2008). Coastal ecosystems, such as coral reefs, are particularly threatened by these contaminants, which mainly originate from terrestrial sources. It has been shown that scleractinian corals can mistake microplastics for prey and ingest it (Hall *et al.* 2015). However, little is known about its effects on health and survival of the coral holobiont - a unit comprised of the coral itself and its microbial symbionts. Thus, we examined the influence of microplastics (size 35-650 µm, concentration 0.1 g l^{-1}) on six scleractinian coral species in a four week lab experiment. We studied the uptake of the particles histologically and documented coral growth using 3D scanning and subsequent analyses of 3D models. As microbial communities play an import role in marine ecosystems, especially for the integrity of the holobiont, we also documented changes in bacterial numbers in the water column, using SYBR Green I staining and cell counting through epifluorescence microscopy. Additionally, this technique was used to study bacterial colonization of microplastic particles. We proved both uptake and rejection of plastic particles by living corals, as well as significantly reduced growth rates (3.1 % vs 0.4 %) together with severe declines in coral health and survival as reaction on microplastic contact. Particles were colonized with bacteria and our results indicate that microplastics reduce the number of free-living bacteria. We conclude that particle handling is energetically costly for the coral, resulting in lower growth rates. The decline in health might be caused through pathogen transmission, with particles acting as vectors. Further analyses will follow, addressing bacterial community composition of the coral holobiont as well as the interference with microplastic-attached and water-associated bacterial communities. This study is one of the first proving negative impact of microplastics on corals and builds the foundation for understanding the relationship between microplastic-associated bacteria and marine organisms.

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MCP73

Root endophytic Basidiomycetes isolates from permanent grassland harbour Mollicutes-related endobacteria and promote wheat growth

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Beneficial plant-fungal interactions play an important role in the ability of plants to survive changing environmental conditions. Diversity and composition of endophytic fungal communities associated with roots of both monocotyledonous (*Arrhenatherum elatius*) and dicotyledonous (*Gallium album*) plants in the permanent grassland of the „Environmental Monitoring and Climate Impact Research Station Linden“near Gießen, Germany was evaluated by microscopy and DNA sequencing of the 18S rRNA-ITS sequences. We characterized two endophytic fungi that are related to Basidiomycota. Using the 16S ribosomal DNA as a phylogenetic marker also revealed that the fungi contained *Mollicutes*-related endobacteria. Interestingly, the fungal isolate from *G. album* exhibited strong growth promotion activities in wheat roots and shoots. We discuss the agronomic potential of the novel Basidiomycetes with regard to known previously discovered fungal endophytes.

MCP74

The hidden allies – microbes associated with the gut of a specialist beetle

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The symbiotic gut microbes are of crucial importance for insects feeding on suboptimal diets. They can embrace functions ranging from digestion of recalcitrant compounds, such as cellulose, to protection of the host against pathogens [1]. In the recent years, the european forest cockchafer (*Melolontha hippocastani*) has been established as a model organism to study insect-microbe associations. During its life cycle, this specialist beetle is able to succed in two well differenciated ecological niches: the larvae live underground feeding on plant roots, while the adults emerge to feed on leaves. However, withstanding this abrupt shift of the diet and the metamorphosis process, eight taxonomic classes of gut symbiotic bacteria are consistent throughout the insects' whole lifecycle [2]. In view of this community stability, we combined *in vivo* stable isotope probing with 454-pyrosequencing of 16S rRNA genes (Pyro-SIP) in order to determine which bacterial phylotypes are the most metabolically active during the larval and adult stages of the insect. Our approach unmasked a shift in the active fraction of gut bacteria, and revealed the orders Lactobacillales and Burkholderiales as key symbionts of, respectively, larvae and adults.
Furthermore, parallel investigations of the larval digestive tract led to the discovery of novel structures called "pockets". The pockets, present only in larvae, are attached at both sides of the distal segment of the gut, the hindgut chamber. 454-pyrosequencing coupled with qPCR analyses showed that their bacterial community significantly differs from the colonizers of the surrounding hindgut wall. Interestingly, a Burkholderiales representative (*Achromobacter* spp.) was found as the most abundant genus within the pockets. By means of Raman Micro-spectroscopy and Gas Chromatography - Mass Spectrometry, we demonstrated that some of the pocket bacteria are able to accumulate poly-*3*-hydroxybutyrate (PHB), which suggests that the origin of these symbionts might be environmental [3]. Moreover, culture dependent experiments unveiled a possible involvement of the pocket microbes in hosts' nitrogenous waste recycling, although, as disclosed by the abovementioned Pyro-SIP approach, their main role might not be played until the adult stage.

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MCP75
Symbiotic function of a rhizobial efflux system and its associated transcriptional regulator
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Efflux pumps enable bacteria to remove toxic chemical compounds from their cell. Rhizobial genome sequences reveal the presence of a number of efflux systems belonging to different families. In the alfalfa symbiont *Sinorhizobium meliloti* strain 1021, 14 efflux systems have been identified (1). In transcriptome analyses, the genes *SMc03167* and *SMc03168* – the deduced proteins are similar to the multi drug resistance proteins EmrB and EmrA of *E. coli*, respectively – were reported to be inducible by luteolin, a plant signal known to induce nodulation genes (2). Using a transcriptional *emrA-gusA* fusion, we demonstrated that the gene is inducible by several flavonoids, strongest by apigenin but also by quercetin, which is not an inducer of nodulation genes. This suggests that the gene is not regulated directly by NodD, which is the activator of nodulation genes. Upstream of *emrA*, a TetR-type regulator (EmrR) is encoded. EmrR binds to palindrome-like sequences within the *emrA-emrR* intergenic region (3). By creating translational *emrR-lacZ* fusions, we determined the likely translational start site of *emrR*. This revealed that *emrR* is also inducible by apigenin. After integration of the *emrR-lacZ* fusion into an *emrR* mutant background, the fusion was no longer inducible by apigenin, however, the expression level in the non-induced strain was significantly higher than in the wild type background. This suggests that EmrR acts as a repressor, which regulates the transcription of *emrAB* and of its own gene. Interestingly, a mutation of *emrR* but not of *emrA*, impaired symbiosis with alfalfa (3, 4 and unpublished results). This might indicate that a proper regulation of *emrAB* is essential for the interaction of *S. meliloti* with alfalfa. To answer this question we used reporter gene fusions of *emrA* and *emrR* and studied their expression in nodules of alfalfa and *Medicago truncatula*. Preliminary results indicate that EmrA is expressed in the infection zone of alfalfa nodule. Subsequent experiments will focus on more detailed analyses of the expression of the efflux system during an indeterminate nodule development.

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MCP76
Bacteria-induced growth and morphogenesis in the green marine macroalga *Ulva* (Chlorophyta)
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Under axenic conditions the marine macroalga *Ulva mutabilis* (Chlorophyta) is developing into callus-like colonies consisting of undifferentiated cells and abnormal cell walls. Normal growth and morphogenesis can be completely recovered by the combination of specific bacteria isolated from *Ulva´s* surface or by the application of partly purified morphogenetic compounds released by these bacteria [1,2]: *Roseobacter* sp. (MS2) among other isolated alpha- and gamma-proteobacteria induces cell division and growth of algal blade cells. However, it was observed that rhizoid growth did not occur and cell wall distortions remained until *Ulva* was also co-cultivated with *Cytophaga* sp. (MS6). Interestingly, the motile *Roseobacter* sp. establishes a biofilm around the primary rhizoid cells of *U. mutabilis* [1], whereas it is still not clear how the *Cytophaga* sp. is selected by the alga.
Using this standardized tripartite model system, we explore various aspects on the cross-kingdom cross-talk including (i) chemotaxis of bacteria, (ii) biofilm formation and (iii) the bacteria-induced cell differentiation and morphogenesis. Whereas bacteria are releasing morphogenetic compounds, *Ulva* releases chemotactic compounds to attract the motile *Roseobacter* sp. and provides nutrients to set-up a mutualistic relationship. Using bioassay guided approaches and explorative exo-metabolite profiling; we aim to decipher the chemosphere, which is defined as part of the biocoenose, where the organisms interact with each other via infochemicals [2].
In addition, a stable macroalgal transformation system was developed which paves the way to study the underlying regulatory mechanism of *Ulva´s* morphogenesis [3]. A first collection of insertional mutants revealed morphotypes comprising atypical thallus formations and/or altered algal attraction patterns of the associated bacteria. Now, we aim to identify and functionally characterize the affected genes that are involved in signaling pathways mediating the bacteria-induced algal morphogenesis. The combined approaches in chemistry and genetics will thus shed light on the molecular and cellular mechanisms in seaweed development.

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MCP77
Unravelling shipworm symbiont physiology – the bacterial endosymbiont TN10130 of the wood-boring bivalve *Teredo navalis*
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Teredo navalis LINNAEUS, 1758 is the dominant wood-boring mussel at the coasts of the Baltic Sea. In contrast to other shipworms, this invasive species is able to live at remarkably low salinities. A community of closely related nitrogen-fixing endosymbiotic bacteria colonizes specialized cells (bacteriocytes) in the gills of *T. navalis*. Although the general features of this symbiosis are well established, our knowledge on the physiology of the bacterial symbionts of shipworms and their role in this marine symbiosis, which causes dramatic damage to wooden sea structures, is very limited to date.
We investigate the bacterial community of *T. navalis* and used a proteogenomic approach to characterize the physiological potential of one of the bacteria, a culturable isolate, in detail. The strain TN10130 was isolated from the gill tissue of a *T. navalis* specimen that had infested a pine board artificially exposed in the Western Baltic Sea (Eckernförde Harbor) for four weeks in summer 2009.
The isolate TN10130 is a diazotrophic, cellulose-degrading γ-Proteobacterium that shares the same basic features as the previously described strains of the cultivable endosymbiont *Teredinibacter turnerae* isolated from the shipworm species *Lyrodus pedicellatus*. Comparative sequence analysis indicates that TN10130 is taxonomically related to this previously sequenced cultivable strain but seems to constitute a separate taxonomic branch. Our genome analysis revealed a remarkably high number of putative secondary metabolite gene clusters (SMCs): approximately 8.4 % (~ 490 kb) of the bacterial genome seem to be dedicated to secondary metabolism. It is interesting to note that only 3 of the identified 14 SMCs of TN10130 show remote resemblance to SMCs of *T. turnerae* T7901. Thus, 11 of these clusters are specific for TN10130. Furthermore, our proteome analyses reveal a versatile potential of this strain for the degradation of complex terrestrial but also marine polysaccharide structures.
Our study gives first insights into the protein inventory and the physiology of a bacterial endosymbiont of the *T. navalis* symbiosis.

MCP78
Ectomycorrhizal metal stress response
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Soil contamination is one reason of increasing land degradation. One problem is the rise in anthropogenically elevated metal concentrations. For optimization of reforestation, a better understanding of the ectomycorrhizal partners' responses facing various metals may be helpful.
The study aims to compare the fungal and the plant responses to different metal chloride salts at the transcriptomic level. For this purpose, an axenic co-culture system with the coniferous tree *Picea abies* (spruce) and the ectomycorrhizal symbiont, the basidiomycete *Tricholoma vaccinum*, is used. Treatments with organic redox cycling compounds such as paraquat and menadione are used to separate metal specific response from that towards reactive oxygen species and formed *via* the Fenton reaction. Developmental parameters are investigated to define minimal inhibitory concentrations.
For Al, Cs, Cu, Fe, Ni and Zn, comparatively high concentrations were tolerated by *T. vaccinum* in the lower millimolar range, whereas lower

tolerance levels were observed towards Cd and Mn. These were found at micromolar levels. Based on previous studies, a similar minimal inhibitory concentration is expected for Cs in *Picea abies* as shown in *T. vaccinum*. The organic redox cycling compounds acted at different concentration ranges on *T. vaccinum*.
These results offer the possibility to obtain a transcriptomic analysis based on growth under relevant stress conditions.

MCP79
Colonization of indigenous gut bacterium *Enterococcus mundtii* in the intestinal tract of *Spodoptera littoralis*
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The cotton leafworm (*Spodoptera littoralis*) is a highly successful polyphagous insect pest that causes economic loss in agricultural industry. Although pesticides are widely used to combat this pest, yet it is biologically unsafe since its toxic chemical components can accumulate in the plants, and remain dangerous for human consumption. In recent years, many researchers have focused in studying the insect gut microbiota. The gut microbial communities of *Spodoptera* are well catalogued and characterized, and emerged as a popular model insect in studying insect-microbe and microbe-microbe interactions, which are still poorly understood. It is known that the gut of *Spodoptera* is dominated by indigenous bacteria of *Enterococcus mundtii* and *Clostridium* sp. towards the late-instar larval stage [1]. These bacteria are constantly present in the digestive tract of the insect. In this study, *E. mundtii* was selected as a model bacterium because of the ability to form biofilm-like protecting layers on the gut epithelium of *S. littoralis* and has the potential of killing other pathogenic bacteria by the release of antimicrobial peptide as shown by *in vitro* studies (Shao et al, unpublished). We know that the intestinal pH of *Spodoptera* differs between gut regions, from highly alkaline in the foregut and midgut, and toward neutral in the hindgut. The mechanism of bacterial survival in harsh gut environment is unknown. To gain better understanding of the strategies used by *Enterococcus* for survival, we have constructed GFP-tagged bacteria to track its colonization in the intestine throughout different stages of development (larvae, pupae, adults and eggs). We showed that the engineered fluorescent bacteria survived and proliferated in the intestinal tract of insect of all life stages for up to second generation offspring following ingestion. We are interested to further explore the underlying factors that drive the survival by analyzing the bacterial and insect gut membrane transcriptomes. We expect that the transcriptome data will significantly expand our understanding of the functional roles of indigenous bacteria toward the development of the insect.

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MCP80
The melting pot ectomycorrhizosphere – communication between *Tricholoma vaccinum* and its tree host spruce affected by soil microorganisms
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Ectomycorrhizal interactions between a fungus and the roots of a host plant support nutrient supply of both partners and increase plant vitality. Development of the symbiosis includes bi-directional signaling. The potential of the widespread basidiomycete *Tricholoma vaccinum* to recognize and respond to its host tree Norway spruce (*Picea abies*) and the influence of the ambient microbiota and the impact of D'orenone, an apocarotenoid intermediate of trisporic acids produced in Mucoromycotina, were investigated.
We sequenced the genome of *T. vaccinum*, predicted its secretome and verified it experimentally. Moreover, the excretion of phytohormones and volatile organic compounds was analyzed. Microbial community structure was studied in an ectomycorrhizospheric habitat with 454 pyrosequencing and via isolation. Furthermore, we investigated the influence of *T. vaccinum* on spruce and the microbial community in microcosms.
T. vaccinum synthesizes phytohormones and plant volatile organic compounds and has the potential to secrete 206 proteins over the classical secretion pathway, e.g. cellulolytic enzymes and mycorrhiza relevant hydrophobins. Supplementation with spruce exudates reduced cellulolytic enzyme abundance and activity in *T. vaccinum* culture supernatants. Dead host material, unable to exudation forces plant degradation and increased fungal abundance and diversity in the microcosm. D'orenone application increased hyphal branching and mitochondrial activity as well as auxin biosynthesis through aldehyde dehydrogenase Ald5 and excretion by the transporter Mte1. D'orenone increased lateral root elongation in *P. abies*, mostly correlated with changed auxin fluxes. D'orenone stimulated mantle thickness but decreased Hartig net formation most probably correlated with plant defense reactions. Most of the effects were comparable with the synthetic strigolactone GR24.
Conclusively, ectomycorrhizal signaling involves multiple signals and can be modulated by D'orenone through changed auxin fluxes. To our knowledge, this is the first report of a fungal hormone-like substance with apocarotenoid character affecting ectomycorrhizal signaling.

MCP81
the effects of diet and caste on the termite hindgut microbiota as seen through 16S rRNA sequencing and artificial neural networks
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The eastern subterranean termite *Reticulitermes flavipes* harbors a tri-partite microbial community in the hindgut consisting of *Archaea*, *Bacteria*, and protozoa which aid in the digestion of lignocellulose. The hindgut microbiota has been studied extensively and is known to be similar between termites of the same colony and stable over time. Termite colonies are made up of multiple castes: workers, soldiers, alates (pre-reproductives), and reproductives (king/queen), each with different tasks and feeding habits. Differences in the composition of the protist community have been shown in different castes, but differences in the bacterial community have not been addressed. Diet is an important facet in symbiotic systems as changes in diet can dictate or alter the microbiota of the host. We hypothesize that the composition of termite hindgut microbiota is partially dependent on diet and that the microbiota will differ between termite castes. A single colony was separated into sub-colonies differentiated by diet (starved, original mulch, cardboard, birch, maple, oak, and spruce). Colonies were also sampled for the caste study when there were members of multiple castes present within the same colony. Hindgut contents were sampled in triplicate from each termite colony over the course of 56 days and the V4 hypervariable region of the 16S rRNA genes were sequenced on an Illumina MiSeq. The merged read pairs were processed using Qiime. An artificial neural network was used to determine the most connected taxa and how each diet affects the microbiota. A qPCR assay for protist 18S rRNA was used to correlate protist and bacterial abundances in termite castes. Each diet showed a shift in the bacterial community over time, with the starved and oak colonies having the most drastic changes. Only 16 out of 130 taxa increased or decreased by more than 5 % among the 7 diets, including *Treponema* and *Endomicrobia*, the two most abundant taxa in the hindgut, as shown by sequencing and the neural network analysis. *Treponema* exist in the hindgut as free-living or ectosymbionts of protists and are the main producers of acetate and nitrogen compounds with acetate being the primary source of energy for the termite. *Endomicrobia* are protist endosymbionts and it has been suggested that they provide amino acids to the host protist and the termite. *Treponema* and *Endomicrobia* showed the highest variability depending on termite caste, with the lowest abundance existing in the alate caste. The overall stability of the microbiota when fed multiple diets suggests the ability of the core microbiota to adapt as the termite diet changes. Interestingly in castes that do not feed on wood (alates) the major bacterial symbionts in the hindgut dramatically decreased in abundance. These findings reinforce the interdependency of the microbial symbionts, the host and their food source.

MCP82
Bacterial virulence depends on host growth phase
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Despite an unambiguous definition, in many biological systems we do not find a neat delineation between mutualism and parasitism. Biotic and abiotic factors influence symbiotic interactions, thus under certain circumstances parasites can provide benefits and mutualists can harm their host. Additionally, the genetic background of both interaction partners can play a significant role. Here we addressed the question which intrinsic

biotic factors are pivotal for the outcome of an intimate host-symbiont interaction and might even tip the scale towards a stronger exploitation of host resources.
As model system we used the obligate intranuclear symbiont *Holospora caryophila* (*Alphaproteobacteria; Rickettsiales*) and its unicellular eukaryotic host *Paramecium biaurelia* (Alveolata; Ciliophora). The impact on host fitness of the supposed energy parasite was determined in presence and absence of *H. caryophila* via growth assays with several genetically identical *P. biaurelia* lines. Maintenance of the intranuclear bacteria was confirmed at the beginning and end of the experiment using fluorescence *in situ* hybridisation and microscopy. Following biotic factors were considered as potentially involved in shaping the outcome of the interaction: (1) the host genotype, (2) the parasite genotype, and (3) the growth phase of the host.
All three factors revealed a strong influence on the outcome of the host-symbiont interaction. In presence of *H. caryophila*, the *Paramecium* density in the stationary growth phase decreased. Conversely, a positive effect of the bacteria during the exponential phase was observed for several host × parasite combinations resulting in an increased growth rate of infected *P. biaurelia*.
The fitness impact of the tested endosymbionts on different *P. biaurelia* lines were not only dependent on either genotype but were specific for the genotype × genotype combination. Interestingly, a typical parasite such as *H. caryophila* has not only negative effects on host fitness. Depending on the actual host growth phase, the presence of these obligate endosymbionts can even be advantageous for *P. biaurelia.*
Thus, under the here tested experimental conditions, the harmful parasite can be a beneficial mutualist changing from one kind of interaction to the next within the same host and a time-span of less than six days.

MCP83 – withdrawn

MCP84
Zero waste and no known diseases in fungus-farming termites
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Thirty million years ago, the ancestors of the termite sub-family Macrotermitinae and the basidiomycete *Termitomyces* joined forces in what became one of the most sophisticated plant biomass decomposition symbioses on Earth. Through intricate interactions between an external fungus garden and the termite host and its accompanying gut microbes, this symbiosis manages to fully decompose nearly any plant polysaccharides while having no apparent problems with infectious diseases. We employed 16S rRNA 454 pyro-sequencing to portray community compositions of Macrotermitinae core gut microbiotas and Illumina sequencing to obtain metagenome insight into the functional gut community roles. The innovation of fungiculture induced a compositional and functional shift in the Macrotermitinae gut microbiota. Gut bacteria contribute fungilytic enzymes and enzymes needed for final plant decomposition after *Termitomyces* degrades the complex carbohydrates. This implies that the shift in gut microbial capacity after *Termitomyces* domestication has involved the evolution of complementary division of symbiont labour. A second gut passage of the fungus comb thereby ensures that all polysaccharides are utilized. Obligate gut passage of the plant substrate may also assure that potential antagonists are efficiently suppressed before entering termite nests, and I will end with a discussion of evidence for this hypothesis.

MCP85
Acclimatization of arbuscular mycorrhizal fungi leads to increased stress tolerance of their host plants
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Arbuscular mycorrhizal (AM) fungal strains differ in their abilities to provide their host plants with mineral nutrients and to confer resistance to root pathogens and tolerance to abiotic stress. For example, mycorrhizal plants perform much better on sites contaminated with heavy metals (HMs) compared to their non-mycorrhizal counterparts. Numerous studies showed that taxonomically-related AM fungal strains differ in their capacity to confer HM tolerance to plants and can even loose this character, if propagated under HM-free conditions. The current study compiles the following hypotheses: (1) AM fungi can acclimatize to HM stress over a few generations. (2) This acclimatization process can be monitored on molecular level. (3) Acclimatized strains show increased abilities to confer heavy metal tolerance to plants.
Rhizophagus irregularis DAOM-197198 was chosen as model AM fungal strain and propagated in root organ cultures in the presence of moderate Zn or Pb concentrations. After five generations, cultures were tested at increased HM concentration for hyphal and spore development and for the expression of a set of HM-regulated genes. The results confirmed the first hypothesis: AM fungi can acclimatize to HM stress. Hyphal density or the number of spores was higher, if the strains grow in the presence of HMs compared to the non-acclimatized control strains. The AM fungal gene expression patterns reflect also the process of acclimatization. RNA of particular genes accumulated earlier or to increased amounts in the acclimatized strain confirming the second hypothesis.
In order to test the third hypothesis, the final generation of newly developing spores from the acclimatized and from the non-acclimatized strain was used for inoculating maize plants in pot cultures, treated with high amounts of Zn and Pb. At harvest, plants inoculated with an acclimatized strain formed higher biomasses and showed increased uptake of phosphate and lower amounts of HMs in their shoots compared to plants colonized by the non-acclimatized strain. We propose that this process of acclimatization accompanied by monitoring RNA accumulation of particular genes can be transferred to a commercial directed inoculum production process with quality control adapted to particular customer needs.

MCP86
Metagenomic potential for and diversity of N-cycle driving microorganisms in the Bothnian Sea sediment
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The biological nitrogen cycle is driven by a plethora of reactions transforming nitrogen compounds between various redox states, which can be performed by a variety of different microorganisms. Whereas some of these processes are fairly widespread (e.g. denitrification), some others are only restricted to narrow phylogenetic guilds (e.g. anammox). Here we investigated the metagenomic potential for Nitrogen cycle of the *in-situ* microbial community in an oligotrophic, low salinity environment of the Bothnian Sea sediment. Total DNA from three depths below the sediment surface was isolated and sequenced with the Ion Torrent technology. The characterization of the total community was performed based on 16S rRNA gene inventory using SILVA database as the reference. The diversity of diagnostic functional genes coding for nitrate reductases (*napA*; *narG*), nitrite reductases (*nirK*; *nirS*; *nrfA*), nitric oxide reductase (*nor*), nitrous oxide reductase (*nosZ*), hydrazine synthase (*hzsA*), ammonia monooxygenase (*amoA*), hydroxylamine oxidoreductase (*hao*) and nitrogenase (*nifH*) were analyzed by blastx analysis against curated reference databases. In addition, PCR-based amplification was performed on the *hzsA* gene of anammox bacteria. Our results reveal high genomic potential for full denitrification to N_2, but minor importance of anaerobic ammonium oxidation (anammox) and dissimilatory nitrite reduction to ammonium. Genomic potential for aerobic ammonia oxidation was dominated by *Thaumarchaeota* while bacterial *amoA* genes were scarce in all sediment depths. In general, phylogenetic composition of core microbial communities correlated well with biogeochemical characteristics of particular depths. Moreover, despite their lower abundance in the Bothnian Sea sediment, we detected a higher diversity of anammox bacteria in metagenomes than with the PCR-based technique. Our metagenome results reveal the quantitative importance of various N-cycle driving processes and highlight the advantage in detection of novel microbial key players which might be overlooked by using traditional PCR-based methods.

MEP01

Evolutionary strategies to efficiently synthesize polyproline containing proteins

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Translation of polyproline proteins leads to ribosome arrest. To overcome this stalling effect, cells depend on a polyproline specific bacterial translation elongation factor P (EF-P), being orthologous to eukaryotic/archaeal initiation factor 5A (e/aIF5A). EF-P consists of three β-barrel domains that form a tRNA-like L-structure. Thus, EF-P can bind to the stalled ribosome between the peptidyl-tRNA binding site and tRNA-exiting site and thereby it stimulates peptidyl-transferase activity. To enhance activity of both EF-P and e/aIF5A a positively charged residue protruding towards the peptidyl-transferase center is post-translationally modified. While IF5A strictly depends on hypusination of a conserved lysine, modification strategies of EF-P are highly diverse: Bacteria such as *Escherichia coli* extend a protruding lysine by β-lysinylation and subsequent hydroxylation by the concerted action of EF-P lysyl-transferase (EpmA), lysine aminomutase (EpmB) and EF-P hydroxylase (EpmC). We have unveiled a markedly different modification strategy present in clinical relevant species such as *Pseudomonas aeruginosa*, *Neisseria gonorrhea* and *Bordetella pertussis*. Here a conserved arginine of EF-P is rhamnosylated by a novel type of glycosyltransferase (EarP) using dTDP-L-rhamnose as a substrate. Inactivation of the EF-P modification system impairs not only bacterial fitness but is also important for development of pathogenicity making EarP and dTDP-L-rhamnose-biosynthesizing enzymes ideal targets for antibiotic development.

MEP02

Genome dynamics in the Roseobacter group

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Question: Alphaproteobacteria of the metabolically versatile Roseobacter group (*Rhodobacteraceae*) are abundant key players of marine ecosystems. Morphological heterogeneity is a common trait and some stains harbor up to a dozen extrachromosomal elements (ECRs; Petersen *et al.* 2013). Plasmid curing in *Dinoroseobacter shibae* DSM16493^T and *Phaeobacter inhibens* DSM17395 exemplified their essential role e.g., for anaerobic growth (Ebert *et al.* 2013), survival under starvation (Soora *et al.* 2015), motility and biofilm formation (Frank *et al.* 2015). The "Jekyll and Hyde" interaction of *D. shibae* with its dinophycean host is moreover mediated by a "killer plasmid" (Wang *et al.* 2015). In the current study we investigated the genomes of more than a dozen mutants of these model organisms to retrace the genetic basis of their phenotypic variability.
Methods: We sequenced genomes of the wild type, plasmid curing and transposon mutants as well as transconjugants of *D. shibae* and *P. inhibens*. Size selected large DNA fragments (>10kb) were used for PacBio sequencing and Illumina HiSeq sequences were established for error correction. Data analysis was performed with the bioinformatics pipeline of the DSMZ. The authenticity of single nucleotide polymorphisms (SNPs) or gaps was checked with *de novo* assembled Illumina data and sequencing of PCR products. Plasmid profiles were determined via pulsed field gel electrophoresis (PFGE).
Results: A combination of second and third generation sequencing (PacBio, Illumina) was used to establish finished genomes of highest quality. Resequencing of the *D. shibae* and *P. inhibens* wild type strains revealed the accuracy of the Sanger reference genomes. Our mutants (curing, transposon) and transconjugants accumulated only a few SNPs, but we observed frequent genomic rearrangements. Plasmid recombination in *D. shibae* was independently validated by altered PFGE profiles. A notable finding was the identification of a chimeric *D. shibae* plasmid that was horizontally transferred into *P. inhibens*, but is lacking a type IV secretion system. A chromosomal inversion in the plasmid-free curing mutant of *P. inhibens* might largely influence its gene expression. Furthermore, we observed spontaneous plasmid loss and native transposons with sizes between 1.5 to 6.3 kb that jumped into protein encoding genes thus disrupting their function.
Conclusion: The technical progress in genome sequencing paved the way to retrace and understand the phenotypic spectrum of a single bacterium. Comparative genomics of various mutants of our model organisms revealed a conspicuous degree of recombination that might be characteristic for *Rhodobacteraceae*. Genome dynamics in combination with the wealth of (conjugative) plasmids provides a plausible explanation for the evolutionary adaptability of roseobacters and their rapid colonization of novel marine habitats.

MEP03

Modeling of evolutionary changes of oligonucleotide usage patterns of whole bacterial genomes and in horizontally transferred loci

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Nowadays, complete genome sequences of multiple bacteria became readily available for analysis. One of the most prospective way for genome comparison, identification of evolutionary meaningful events of horizontal gene transfer (HGT) and phylogenomic inferences is based on annotation-and-alignment free genome linguistic approaches, i.e. comparison of oligonucleotide usage patterns (OUP) of genome-scale DNA fragments. Until now this approach still lacks a reliable evolutionary model to explain the mechanisms and dynamics of changes in OUP that hinders application of this approach. The aim of the current work was to design a mathematical model of evolutionary changes in OUP to explain the following two general processes: i) amelioration of horizontally acquired genomic islands (GIs) in bacterial genomes; ii) estimation of phylogenomic distances between microorganisms by comparison of whole genome OUP. HGT has been thought to be a driving force of bacterial evolution. For decades, majority of studies was done on creation of tools for identification of HGT while little is known about the evolution of the transferred genes. Our focus is on amelioration where the base DNA composition of transferred genes undergoes a mutational pressure to represent similarly to the recipient genome. The model will allow estimation of time of acquisition and identification of possible donors of GIs. We also attempted on using compositional methods to analyze phylogenetic relations between sequenced Corynebacteria. To perform these tasks, a stand-alone Python program was developed. The difference in OUP were analyzed in terms of logistic probability equation and best model fitting approaches to simulate the evolutionary processes. The best fit was obtained as the Verhulst equation. The program predicts a graduate merging of a foreign insert's OUP towards the target genomes that would stabilize at some level of pattern similarity. The dynamics of this process and the level of stabilization depend on the rate of mutations in the target organism as well as the level of compositional dissimilarity between foreign inserts and target sequences. These parameters can be estimated from simulated data and could relate to biologically meaningful characteristics such as selection rate and similarity gap between GIs and host OUP. The results also show significant differences between evolution process of coding and non-coding regions where coding regions showed higher initial substitution rate and reaches equilibrium faster while non-coding had larger fluctuations around equilibrium exhibiting increased random mutations. Comparison of different Corynebacteria also showed fitting genome evolution processes to this model.

MEP04

Bacterial species delineation in the era of whole genome sequence data: application to the clade of rapidly growing thermotolerant mycobacteria

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Question: Rapidly growing thermotolerant mycobacteria as *Mycobacterium smegmatis* serve as model systems and surrogates for pathogenic species of the genus. According to main databases, strains JS623 and mc^{2}155 are both considered members of the species *M. smegmatis*, and are among the few strains of that species with an accessible whole genome sequence (WGS). This is why strains JS623 and

mc²155 are becoming increasingly popular for comparative genome-based studies. We found unexpected differences between both genomes and reasoned that strain JS623 might have been classified erroneously. This work investigates the membership of both strains to the same species, describing at the same time a roadmap based on *in silico* methods for species delineation that can be useful in other similar cases.

Methods: We combined traditional molecular taxonomic procedures– inference of single and concatenated gene trees –on the one hand, and modern whole genome-based *in silico* methods –calculation of Average Nucleotide Identity (ANI), digital DNA-DNA hybridisation (dDDH) and analysis of gene synteny –on the other hand, to address the relationship of strain JS623 to other rapidly growing thermotolerant mycobacteria, especially to independent isolates of *M. smegmatis*.

Results: A single gene tree based on the almost full-length 16S rDNA sequence confirmed classification of strain JS623 in the clade of thermotolerant rapid growers, but revealed that it is clearly distant from *M. smegmatis*. A concatenated gene tree based on partial sequences from 16S rRNA, *rpoB*, *hsp65*, and *tuf* genes supported this result. Only strain mc²155 and another independently isolated strain, MKD8, clustered with the *M. smegmatis* type strain. These molecular phylogenetic analyses further suggested that strain JS623 is more related to *M. moriokaense* than to *M. smegmatis*.

Three online available tools that deliver species identification-relevant parameters from WGS data were applied: the ANI calculator (1), the Genome-to-Genome Distance Calculator (2) and MAUVE (3). According to the standard values accepted for species delimitation, all parameters of JS623 clearly separate this strain from the other two strains of *M. smegmatis* at the genome level.

Conclusion: Our data reveal that strain JS623 is not *M. smegmatis* and underline the strength of genome comparisons to identify erroneously classified genome entries. Systematic application of the novel *in silico* tools helps to avoid confusions as well as misleading conclusions, as, for example, those derived from comparative genome-based analyses of mycobacteria, when a genome wrongly attributed to a certain species in the databases was included in the analysis.

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MEP05

Defeating non-producer exclusion in biofilms via general adaptation rather than by specific adjustment

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During biofilm formation, the secreted matrix provides benefit to the microbial population i.e. protection, attachment to substratum or surface spreading; its production provides high cost to individual cells. In Gram-positive bacterium *Bacillus subtilis*, mutant strains that are matrix deficient (non-producers) have higher fitness gain under well mixed planktonic conditions. Moreover, matrix producers (i.e. cooperators), have an advantage in spatially structured conditions [1]. The density of cells at the onset of biofilm growth on a solid surface is affected by pattern formation and high assortment during biofilm growth facilitates cooperation [2]. However, biofilms on air-liquid interface called pellicles seem to benefit public good producers by utilizing a different strategy. While flagellum-based motility, chemotaxis, and oxygen sensing are not absolutely essential for biofilm development in single strain cultures, as these mentioned traits are important for successful competition during pellicle formation [3].

Non-producer strains deficient in matrix cannot form pellicles alone and are excluded in the community when cultured together with producer strains in pellicles; a developmental mechanism that positively selects for cooperators. While this mechanism offers a strategy to restrict non-producers from the population, experimental evolution revealed that non-producers regain their incorporation ability in pellicles after prolonged repeated co-cultivation in the presence of the producer population. This study was conducted in order to understand if the increased pellicle inclusion of the evolved non-producer strains is due to specific mutations or due to general adaptation. Genome resequencing revealed high amount of synonymous mutations both in the evolved producer and non-producer populations. Additionally, matrix production was subsequently disrupted in the evolved producer strains (i.e. non-producer that were initially evolved as producers) and their fitness gains were compared with that of the evolved non-producer strains. Fitness assay revealed increased fitness behavior similar to the evolved non-producers. These results illustrate how general adaption to certain growth conditions can benefit a non-producer population by regaining its fitness at the expense of the cooperator population frequency.

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MEP06

Whole genome sequencing of *Vibrio parahaemolyticus* strains reveals pathways of adaptation under different evolutionary conditions

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Question: *Vibrio parahaemolyticus* (VP) is an important foodborne pathogen that causes explosive outbreaks with patients suffering severe vomiting and diarrhea.

Methods: Whole genomic sequences is a new and powerful molecular tool that provides new and useful information for data analysis such as multi-locus sequence typing (MLST) and detection genetic variants as in single nucleotide polymorphisms (SNPs). This can certainly enhance our knowledge in understanding the pathogenicity and underlying mechanisms of virulence of VP in epidemic spread of disease, and evolution of the organisms during different historical events including outbreaks.

Results: We studied VP isolates obtained from various sources, including: clinical, environmental as well as from different geographic areas in Asia. A new gene cluster which belonged to biofilm associated proteins of VP had been found, as well as a novel small genomic island. Further analysis is still in progress to investigate different virulence genes and degradation pathways. Phylogenetic analysis revealed close relationships with *Vibrio alginolyticus* as well as *Vibrio vulnificus*. Pathways of evolution might well be related to stress conditions (including climatic changes in sea water temperature) that VP had undergone, and which the organism had struggled to survive and proliferate.

Conclusions: Whole genomic analysis of VP is a critical tool for getting useful information on the phylogenetic diversity and evolutionary pathways that enable the organism fit into different environmental niches during course of adaptation.

MEP07

Role of mannitol dehydrogenases in osmoprotection of *Gluconobacter oxydans*

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Question: Mannitol is a polyol that has been recognised as an osmoprotective compound in different organisms. Recently we discovered that *Gluconobacter oxydans* produces and accumulates mannitol under osmotic stress conditions, which functions as compatible solute[1]. However, the pathways and the enzymes responsible for mannitol synthesis were unknown. Here, we identified these mannitol dehydrogenase encoding genes and their role in osmo-protection of *G. oxydans*.

Methods: *G. oxydans* possesses two cytoplasmic mannitol dehydrogenases (MDH) that vary for co-factor specificity. While Gox1432 is NADP-dependent (EC 1.1.1.138), Gox0849 prefers NAD as cofactor (EC 1.1.1.67). Both MDH encoding genes were deleted in *G. oxydans*, separately using the codAB markerless in-frame deletion method[2]. The effect of these deletions was analysed on growth of the cell and intracellular mannitol formation under osmotic stress.

Results: The deletion of the NADP- dependent MDH encoding gene (*gox1432*) resulted in reduction of cell growth under osmotic stress, while the deletion of the NAD-dependent MDH encoding gene (*gox0849*) had no effect on growth of the cells. In addition, the intracellular mannitol content was reduced in the knockout mutants lacking the NADP-dependent enzyme in comparison to the control strain under stress conditions. These results indicated the role of the NADP-dependent MDH as a major mannitol synthesizing protein, responsible to protect *G. oxydans* from osmotic stress. Moreover, the simultaneous deletion of both genes resulted in a more severe growth defect of *G. oxydans* under stress conditions. Over-expression of *gox1432* in the corresponding deletion mutant restored growth of the cell under osmotic stress, further strengthening the importance of the NADP-dependent MDH for osmo-tolerance in *G. oxydans*.

Conclusion: In summary, we report for the first time about the correlation of mannitol dehydrogenases and osmo-protection of *G. oxydans* under osmotic stress. These findings will be valuable to understand the detailed molecular mechanism of mannitol-mediated osmo-protection and to improve the catalytic efficiency of *G. oxydans* under osmotic stress.

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MEP08

Comparative genomics of *Clostridium difficile* strains isolated from European, African and Asian patients

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Questions: The anaerobic and spore-forming Gram-positive bacterium *Clostridium difficile* is a facultative nosocomial pathogen responsible for antibiotic-associated diarrhea or pseudomembranous colitis. Many studies focused on the epidemiology of hospital-acquired *C. difficile*-associated diarrhea in Europe and North America [1]. However, little is known about the presence and biology of *C. difficile* in Africa and Asia. Furthermore, the role of mobile elements for the evolution of *C. difficile* in general has remained unclear despite massive whole genome sequencing during the past years.
Methods: *C. difficile* strains were isolated from European, African and Asian patients. Complete *de novo* genome sequencing of strains was carried out using a combination of single molecule real time (SMRT) and Illumina sequencing technology and also included *C. difficile* strain 630 [2, 3] and *C. difficile* strain DSM 1296^T [4, 5].
Results: Comparative genome analysis revealed highly syntenous genomic regions in all chromosomes. Genome assembly and analysis revealed the presence of extrachromosomal elements such as different plasmid-like structures and provide evidence for new types of phages infecting *C. difficile*. Besides single nucleotide polymorphism level (SNP) in the core genome, differences between strains were mostly found in the mobilome (e.g., bacteriophages and transposons). Whereas *C. difficile* strains from Europe and Asia contain sequences encoding *C. difficile*-specific toxins, many of the isolates from Africa lack toxins. Whereas the *C. difficile* core genomes fall into six different phylogenetic clades [6] the mobilome showed a markedly different pattern that diverges from that of the core genomes.
Conclusions: Various mobile elements of *C. difficile* are transferred frequently between distantly related clinical strains obtained from different continents. Our results suggest a major role of the mobilome in the evolution of *C. difficile.*

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MEP09

Distribution, domain architecture and phylogeny of proteins with a KdpD domain

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Question: K^+ is the most abundant cation in all living cells and involved in maintaining turgor, metabolism and intercellular communication [(1, 2)]. K^+-transporter systems with various kinetics and expression patterns have been identified. Among them, the inducible high affinity transporter (KdpFABC), the sensor KdpD histidine kinase (HK) and the response regulator (RR) KdpE have been comprehensively investigated in *E. coli* K-12. The HK KdpD has a conserved cytosolic N-terminal domain that is composed of the KdpD and Usp domains. It is the aim of this study to analyze distribution, domain architecture and phylogeny of the KdpD domain.
Methods: A database was created from Uniprot reference proteomes (RF) (2015_10). HMMER 3.0 was utilized to construct Hidden Markov Models (HMMs). KdpD, KdpA and TrkH were fished by querying RF with corresponding HMM models. KdpD protein domain architectures were annotated with CDD v-3.14. Multiple sequences alignment was done with MAFFT v7.221 linsi algorithm, refined with TrimAl v1.2 and submitted to Phyml 3.0 to construct the maximum likelihood tree. Promoter motifs of *kdp* operons were dissected with MEME-ChIP. Proteins with a KdpD domain were grouped into kinase, non-kinase and "others" versions. A reporter strain was constructed in *E. coli kdpDE* $^-$ by replacing P_{lacZ} with a chosen *kdp* promoter.
Results: Distinct distributions and occurrence frequencies of KdpD, KdpA and TrkH super-families were detected. The KdpD domain is highly conserved, and KdpD and KdpA show a high degree of co-occurrence.
KdpD in connection with a kinase domain are prevalent in *Proteobacteria* and *Actinobacteria*, while *Bacteroidetes, Cyanobacteria* and *Deinococcus-Thermus* have dominantly non-kinase KdpD.
Phylogenetic analysis of KdpD-containing proteins suggests that the ancestral form of KdpD domain proteins was a non-kinase and underwent domain rearrangements to evolve into a kinase**.**
Genomic neighborhood analysis of non-kinase KdpD loci identified neighboring ORFs of HKs and RRs.
First results suggest that the *kdp* operons in *Bacteroides* are regulated by σ-54 dependent RRs.
Conclusion: Kdp systems and KdpD proteins are more diverse than we knew from *E. coli* and *Anabaena*.

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MEP10

Ongoing research into ancient pre- and post-contact tuberculosis in the New World

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Question: Today, tuberculosis (TB) infections in the Americas are dominated by human adapted European lineage 4 strains, likely introduced to the New World starting in the 16th century during European colonization. However, archaeological evidence indicates the presence of TB as early as ca. 700AD. A recent study recovered ancient tuberculosis genomes from human remains from three distinct archaeological sites in southern Peru that pre-date European contact (1). These ancient Peruvian strains are closely related to those circulating in seals and other marine mammals today, which are rarely known to cause human infection. This study seeks to further investigate the genetic diversity of TB strains circulating in the Americas pre and post European contact.
Methods & Results: After screening skeletal remains from 23 individuals three TB positive ancient samples were identified using a combination of array DNA-hybridization capture and metagenomic analyses.
Conclusion: Future research will concentrate on isolating more ancient TB DNA from these samples in order to reconstruct full genomes. Genomic data will help to address questions related to the geographic and temporal extent of the ancient Peruvian strains in the pre-contact New World, in addition to providing a better overview of the genetic diversity of TB strains infecting humans in the past.

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MEP11
Rapid evolution of cooperative cross-feeding in auxotrophic bacterial consortia
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Metabolic cross-feeding is very common in natural bacterial communities. However, the evolution of such cooperative interactions poses a conundrum to evolutionary biology: why should bacteria direct costly benefits to other individuals and not use these resources to enhance their own fitness? Here we address this question in a long-term coevolution experiment to identify the factors that facilitate the evolution of cooperative cross-feeding from an obligate by-product interaction. This experiment was initiated with a synthetically designed consortium consisting of two mutants of *Escherichia coli* each auxotrophic for a different amino acid. Populations of prototrophic wild type (WT) cells served as control, in which cell growth was not contingent on amino acid cross-feeding. Serial propagation of both the auxotrophic consortia and prototrophic WT for 80 days in a shaken, liquid environment revealed that the cross- feeding consortium gained significantly increased population densities during this time that were statistically indistinguishable from the population densities achieved by the derived WT populations.
Moreover, while the fitness of the derived auxotrophic consortia showed a two-fold increase relative to their evolutionary ancestors, WT fitness increased only marginally in the course of the experiment. Quantifying the growth of cocultured auxotrophs that were utilised as biosensors indicated significantly increased amino acid production levels in the coevolved auxotrophic mutants, yet not in the WT controls. These findings evidenced that starting from a by-product interaction, auxotrophic consortia evolved cross-feeding interactions, in which they started to invest costly resources into their respective counterpart. Moreover, separating both derived partners with a filter membrane that allows passage of free amino acids in the growth medium but prevents a direct interaction between cells showed that the exchange of amino acid was to a substantial part contact-dependent but also based on diffusion. Direct contact might reduce the loss of amino acids to the external environment and thus minimizes the production costs of amino acids, while diffusion might operate permanent due to leakiness of cells.
Our results show that cooperative cross-feeding interactions, in which costly metabolites are reciprocally exchanged between bacterial cells in a dual strategy, can rapidly evolve from obligate by-product interactions and may thus explain the widespread distribution of these interactions in nature.

MEP12
Molecular profiling of bacterial populations from vaginal samples of calves and health adult Arabian Camels (Dromedary camelus)
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The author does not wish to publish the abstract.

MEP13
Analysis of a high-coverage *Yersinia pestis* Genome from a 6th century Justinian Plague Victim
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Question: The Justinian Plague, which started in the 6th century and lasted to the mid 8th century, is thought to be the first out of at least three historically documented plague pandemics. It was suggested to be one of the main factors that contributed to the decline of the Eastern Roman Empire and marks the transition from the Antique to the Middle ages. Historical accounts as well as molecular data suggest the gram-negative bacterium *Yersinia pestis* (*Y. pestis*) as the etiological agent of this pandemic. Here we attempted to genetically characterize the Justinian strain.
Methods: We used hybridization capture techniques combined with next generation sequencing (NGS) to obtain a high coverage *Y. pestis* genome from a 6th century skeleton recovered from a Southern German graveyard.
Results: The reconstructed ancient *Y. pestis* genome is characterized by substitutions that are unique to this lineage, and structural differences in regions of the genome that have been previously suggested to be plague virulence factors. Since these motifs have not been identified in extant *Y. pestis* strains, this lineage is likely to be extinct.
Conclusion: We confirm that a terminal branch of *Y. pestis* was circulating in mainland Europe during the Justinian pandemic. These results may be influential for functional investigations that could explore the role of these newly discovered genomic characteristics in terms of physiology, virulence and host adaptation.

MEP14
Designing a transitional microcosm for experimental bacterial evolution studies.
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Introduction: Bacterial adaptive radiation has been successfully investigated in the past using *Pseudomonas fluorescens* SBW25 in static liquid microcosms. In these, radiating populations give rise to the novel biofilm-forming adaptive genotype known as the Wrinkly Spreaders (WS). Although the WS has a fitness advantage over the ancestral wild-type strain and other non-biofilm-forming competitors in static microcosms, it is genetically unstable and has a low fitness on agar plates where the cost of expressing biofilm-associated components is costly. We are interested in examining the differences in WS fitness in these two environments and in the transition zone between the two, and are now developing novel static microcosms by incorporating a dry agar surface which we refer to as a 'transitional environment.'
Methods: Standard King's B static microcosms have been developed containing wedges of 0.8-1.2 % (w/v) agar, providing both an air-liquid interface for biofilm-formation, as well as an agar slope in which 'dry' colony development can occur. Replicate microcosms have been tested with wild-type *Pf.* SBW25 (WT) and the archetypal Wrinkly Spreader, as well as with motility (chemotaxis) (MD) and surfactant (SD)-deficient strains over 3-10 days to assess radiation, fitness, and the colonisation of the dry agar surface.
Results: Preliminary experiments confirm that WT populations will radiate in the reduced liquid volume of the transitional microcosm, and produce Wrinkly Spreaders with 3-5 days. These WS isolates had a fitness advantage over the ancestral strain (WT) when tested in standard static microcosms, but differed in wrinkleality (microcosm growth, biofilm strength and attachment levels, and colony expansion) compared to isolates recovered from static microcosms. WT, WS, MD and SD strains all produced colonies on the agar, and over a period of several days the colonisation of the agar surface expanded to 1.5-2 cm from the liquid-agar intersection. Some behavioural differences were noted in the colonisation of the agar, but this has yet to be quantified and analysed.
Conclusion: Although some technical issues needed to be overcome, the transitional microcosm has been shown to have the potential to enable future investigation of the adaptation of bacteria to different environments as well as the transition zone linking the two.

MEP15
Long-term evolution of a fluorinated bacterial proteome
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Fluorinated amino acids are valuable tools for protein engineering as they can dramatically change the properties of target proteins.[1] The potencial of fluorine moieties is well exploited by material science and pharmaceutical industry. On the opposite, fluorine is not found in any class of natural biological macromolecules, most likely because of its low availability and unreactivity in aqueous environments. The only exception known so far is the naturally-occurring fluorinated amino acid 4-fluoro-threonine, produced as precursor of a toxic metabolite.[2] We are interested in expanding the amino acids repertoire beyond the 20 canonical amino acids and to understand the mechanisms that guided the evolution of the current genetic code. We have performed a Lenski-type Long-Term Evolution Experiment [3] to replace the latest acquisition of the genetic code, tryptophan, with two monofluorinated analogues. A *Escherichia coli* tryptophan-auxotrophic strain was grown in presence of fluorinated

precursors and eventually adapted to synthesize the fluorinated amino acids by metabolic reactions while retaining viability. Evidence suggests that all UGG tryptophan codons have been fully reassigned to the fluorinated tryptophan analogues. Corresponding results will be shown.

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NPP01

Mass spectrometry-based identification of siderophores produced by *Streptomyces chartreusis*

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Question: Bacterial secondary metabolites are widely used in medicine and industry. The discovery of novel metabolites is tedious as they normally need to be isolated from complex mixtures prior to their identification. In this study a workflow for the mass spectrometry-based, global analysis of secondary metabolites in complex samples is established. As a proof of concept the siderophores in the culture supernatant of *Streptomyces chartreusis* were identified. Siderophores can chelate and mobilize iron, making them essential for the utilization of Fe^{3+}, which is almost insoluble under oxygenic conditions. As iron is often a growth-limiting factor, siderophores are also important for the microbial competition for nutrients **[1]**.
Methods: The secretion of siderophores upon different growth conditions was detected qualitatively and quantitatively in chrome azurol S (CAS) dye-based assays **[2]**. Culture supernatants as well as fractions of anion exchange chromatography were subjected to UPLC-coupled tandem mass spectrometry. For dereplication and sorting of the obtained fragment spectra, as well as for the identification of siderophores, a molecular network based on cosine similarity scores was created **[3, 4]**. Elemental compositions and structures were predicted based on parent masses and fragmentation spectra in conjunction with *in silico* fragmentation **[5]**. The produced siderophores were quantified by continuous MS^E measurements.
Results: *S. chartreusis* produced siderophores when cultured on solid or in liquid media to which no iron was added. Hydroxamate siderophores of the desferrioxamine, bisucaberin, and coelichelin-families were detected when *S. chartreusis* was cultivated in liquid medium. Aside the already known variants of these siderophores, a variety of previously undescribed derivatives could be identified. Overall, more than twenty siderophores, which were all produced simultaneously in a time-course experiment, were identified and their molecular structures predicted.
Conclusion: We established a workflow for time-efficient and resource-saving analysis of global metabolites in complex samples without the need for compound purification. The high sensitivity of UPLC-MS/MS combined with data evaluation by molecular networking allowed the identification and structure prediction of numerous siderophores produced by *S. chartreusis* when cultivated under iron-limited conditions. It remains to be studied if the simultaneous production of a diverse set of siderophores is advantageous in the competition for scarce iron in natural bacterial habitats.

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NPP02

Antimicrobial activity of crude extracts *of Punica granatum* L. and their liquid fractions

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Punica granatum L. (pomegranate) is an ancient fruit that is widely consumed as fresh fruit and juice. Both *in vitro* and *in vivo* studies have demonstrated that this fruit possess an antioxidant, antidiabetic, hypolipidemic, antibacterial, anti-inflammatory, and antiviral activities. In this study, antimicrobial activity of two cultivars (sour and sweet) was tested. Different fruit parts (carp, peel and seed) of *Punica granatum* L. were evaluated against four Gram-negative bacterial species, three Gram-positive bacteria species, and three fungal species using agar diffusion method as well as broth dilution method. Maximum value of inhibition zone (40 mm) was obtained by ethanolic peel crude extract of *P. granatum* against the Gram-positive *Micrococcus luteus*. The minimum inhibition zone (10 mm) was obtained, however, by ethanolic crude extract of *P. granatum* against *Salmonella typhimurium.* The minimal inhibitory concentrations (MIC) of *P. granatum* crude rxtracts showed that methanolic peel crude of sour *P. granatum* had the lowest MIC value (10 µg/ml) against *Serratia marcescens,* whereas the highest MIC value (350 µg/ml) was in case of methanolic seed crude of *P. granatum* against *Micrococcus luteus.*

NPP03

Evolutionary insight to central carbon metabolism in *Streptomyces* for specialised metabolite production

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Question: The rise of antibiotic resistance represents a significant threat to humankind and there is a need to discover new antibiotics, enhance production of existing antibiotics and gain a greater understanding of specialised metabolite production.
Streptomyces species are prolific producers of specialised metabolites with large genomes. These Gram-positive soil bacteria show a complex life cycle with the formation of vegetative and aerial hyphae, as well as spores. Here we studied the expansion of primary metabolism in *Streptomyces* in comparison to other actinobacteria and in relation to specialised metabolite production.
Method: A database of 614 actinobacterial genomes was created. Each genome was re-annotated in RAST and the total metabolic function of each genome was used to create a table of central carbon metabolic enzymes by counting how many times each reaction occurs per genome to highlight over-represented metabolic reactions per genus. The identified genes were further analysed phylogenetically to characterise their origin and possible recruitment.
Streptomyces coelicolor was used as model organism to create pyruvate kinase deletion mutants, which were phenotypically and biochemically analysed. Gene expression analysis was carried grown in either tween or glucose as sole carbon sources using qPCR. Pyk1 and Pyk2 were heterologously overexpressed in *E. coli* and characterised for their kinetics.
Results: Among the primary metabolic enzymes, pyruvate kinase was detected as a metabolic expansion event in the Streptomycineae family. Phylogenetic analysis revealed a conserved family specific gene duplication. The phenotype of the mutants also differs, with a *pyk1* mutant positively affected in specialised metabolite production on complex culture medium whereas the growth of a *pyk2* mutant is retarded when grown on glucose as the sole carbon source. The expression levels of both genes was found to be the same on a range of carbon sources, suggesting that there were regulated at the post-transcriptional level. Detailed biochemical analysis of both Pyk1 and Pyk2 from *S. coelicolor* revealed that each enzyme has a different affinity for the effector molecule, AMP, indicating diverse physiological roles for these proteins in *Streptomyces*.
Conclusion: It appears that metabolic gene expansion events are predominantly found in genera that are producers of specialised metabolites. These data may be explored in order to investigate the link between primary metabolic scaffolds and specialised metabolites.
The detailed characterisation of pyruvate kinase suggests that Pyk2 is the primary pyruvate kinase in *Streptomyces* and Pyk1 is activated upon accumulation of AMP during slower growth phases. The duplication of this enzyme allows a fine tuning of metabolism and represents an interesting target for metabolic engineering to increase specialised metabolite production.

NPP04

Droplets as active vessels for ultra-high throughput cultivation and screening of microbial cells

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Dried antibiotic pipelines and simultaneously the rapidly increasing occurrence of multiresistant microbial pathogens, escalate to a perfect storm as experts describe it[1]. To find urgently needed new substance classes the return to cell-based screenings that deliver molecules, which

proved already their functionality in complex whole cell settings, has been proposed. But those approaches come with low throughput and high costs, which hinder extensive investigation of microbial diversity.Droplet microfluidics, however, could be the game-changer in classic cell-based screens, presenting a 1,000,000 fold miniaturization of a MTP-well and allowing throughput rates of 1000 Hz[2]. With its fast and stable aqueous droplets, that serve as picoliter bioreactors for highly parallelized cultivation of microbial cells, droplet microfluidics will bring new inspiration to the field of natural product discovery.

Our efforts are aimed at further optimizing droplets as a comprehensive platform for microbial experimentation, since limitations like inappropriate oxygen supply during droplet incubation and restriction to fluorescence based read-outs still exist. We developed a dynamic droplet incubation system that provides sufficient and homogeneous oxygen supply for millions of droplets simultaneously, resulting in successful cultivation of a diverse set of microbial species, including obligate aerobes like *Pseudomonas fluorescens* and fastidious actinobacteria[3]. Additionally, we established imaged-based, label-free detection and analysis of microbial growth in droplets, which facilitates qualitative and quantitative characterization of cell replication. Since the image analysis is performed in real-time, sorting of droplets according to different selection criteria can be implemented[4].

Having optimized this ultra-high throughput method for cultivation of microbial cells allows us to exploit the diversity of natural organisms and their metabolites derived from various natural habitats. By compartmentalizing single cells in droplets, commonly underrepresented species like slow growing organisms, can be targeted, since competition for nutrients is prevented. After obtaining monocultures, which is supported by improved incubation conditions, reporter strains are added to the droplets to screen for antibiotic metabolites. First experiments revealed a production of various antimicrobial substances in significant amounts. Furthermore we achieved a reliable differentiation between strains producing antibiotics and those who do not, which paves the way to soon fill the dried pipelines with new substances found in droplets.

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NPP05

Exploiting and elucidation of a new glycosylated polyketide from fungus *Myrothecium* sp

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Fungi are kinds of eukaryotic organism that can be as am important source of structurally unique and biologically active secondary metabolites[1]. During our efforts to isolate diverse secondary metabolites from fungi, we found a halotolerant fungus, *Myrothecium* sp. GS-17. The internal transcribed spacer (ITS) sequence analysis of *Myrothecium* sp. GS-17 was performed. The crude extract of this fungus was shown significantly growth inhibitory activity against human leukemia (HL-60) cell line. In our previous research, many different bioactive secondary metabolites such as trichothecenes, amides, polyketides, sterols, and lactones, were isolated from this strain[2]. During the process of following research, a new glycosylated polyketide, named myrothecoside, was isolated and its structure was elucidated by 1D-, 2D-NMR and HR-MS experiments. The relative configuration of this new compound was assigned by NOESY and coupling constants. It represents a novel glycosylated polyketide with some chemical structure similarities to lovastatin, tanzawaic acids, and phomopsidin [3]. This compound exhibited weak cytotoxicity against human leukemia(HL-60)cancer cell with an IG_{50}value of 63.61 μM, and also antifungal activities against plant pathogenic fungi *Rhizoctonia solani* and *Fusarium oxysporum* using standard agar diffusion tests at 20μg/disk [4].

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NPP06

Novel siderophores from *Variovorax boronicumulans* discovered by genome mining

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Siderophores are small molecules that are secreted by microorganisms in order to acquire iron. Photoreactive siderophores are unique in that they rapidly undergo photoinduced oxidative cleavage when complexed to Fe(III)[1]. As a result, free Fe(II) is released into the environment, available to uptake by surrounding organisms[2]. Photoreactive lipopetide siderophores thus have the potential to influence the composition of microbial communities[1]. Originally described from marine bacteria[2], photoreactive siderophores have recently also been detected in some freshwater and soil bacteria[3]. Here, we report the discovery of the photoreactive variochelins, novel lipopeptide siderophores from the soil bacterium *Variovorax boronicumulans*. The production of variochelins was initially predicted by genomic analysis and, subsequently, the siderophores were isolated using a bioactivity-guided fractionation. Structure elucidation was performed using NMR, LC-MS and MALDI-MS/MS. Photoreactivity of the compounds was confirmed in two different assays. Variochelins represent another example of photoreactive siderophores from a non-marine bacterium. Their effects on other microbes will be tested in co-cultivation experiments in the future.

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NPP07

Harnessing enzymatic promiscuity in myxochelin biosynthesis for the production of 5-lipoxygenase inhibitors

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Human 5-lipoxygenase (5-LO) catalyzes two steps in the conversion of arachidonic acid to leukotrienes, which are important mediators of inflammatory and allergic reactions [1]. The catechol siderophore myxochelin A from the bacterium *Pyxidicoccus fallax* was recently identified as a potent inhibitor of 5-LO [2]. Since the enzyme harbors a non-heme iron atom acting as redox mediator during catalytic reactions [3], it seemed plausible to ascribe the 5-LO inhibitory effects to the iron chelating properties of myxochelin A.

We applied a precursor-directed biosynthesis approach in order to generate new myxochelin analogues. Preliminary feeding studies were performed with 34 aryl carboxylic acids possessing different aromatic substitution profiles. To upscale the production of incorporated substrates, larger fermentations were carried out in the presence of a polystyrene resin. After purification by reversed-phase HPLC, derivatives were characterized by NMR and MS analyses before testing their activities in a cell free assay against recombinant 5-LO.

We observed that one single feeding experiment led to the production of up to three new analogues due to a randomized incorporation of the precursor at two possible positions [4]. Fluorine and hydroxyl substituted aromatic precursors as well as 2-chlorobenzoic acid were accepted as alternative substrates by the myxochelin biosynthesis enzymes. Derivatives lacking aromatic hydroxyl groups lost their inhibitory activities, whereas analogues harboring one catechol unit still inhibited 5-LO. Interestingly, three new derivatives featuring just one catechol unit showed activities comparable to that of myxochelin A (IC_{50} = 1.72 μM) with IC_{50} values ranging from 1.18 to 1.52 μM. Furthermore, their iron binding affinities were determined as very weak by the chrome azurol S assay, indicating that there is no correlation between the strength of iron binding and the inhibitory activity. Further, it was observed that the presence of one phenolic hydroxyl group seems to be crucial for the inhibition. The myxochelin biosynthetic complex seems to be a promising tool to create new 5-LO inhibitors due to its substrate tolerance.

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NPP08

Functional analysis of microbes commonly associated with *Hydractinia echinata*

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Question: Do associated microbes produce antimicrobial metabolites to fend of predators and pathogens of the cnidarian host? And which bacterial signals are responsible for the larvae settlement and metamorphosis of marine invertebrates commonly known as biofouling?
Recent efforts in natural product research have aimed for more detailed microbial and chemical investigations of specific microbe-host interactions to find new natural products in a targeted way. We selected a cnidarian model system, the marine polyp *H. echinata*,[1] to study the function of the associated microbes as well as their biosynthetic potential.
Methods: We isolated culturable associated microbes from *H. echinata* and prioritized the culture extracts using antimicrobial activity and larvae settlement assays. In addition, we sequenced the genomes of several bacterial isolates to investigate the biosynthetic potential.[2] The production of the respective antimicrobial compounds was optimized, and we are currently aiming for the isolation and structure elucidation of the respective compounds. In addition, we are investigating those bacteria strains which show high activity in our settlement assay to elucidate the structure of the bacterial signal, which induces larvae settlement and morphogenesis in *H. echinata*.
Results: Sequenced strains were analysed using AntiSMASH revealing in particular indole and NRPS biosynthesis gene clusters in our selected strains and are currently investigated in detail for the production of the predicted compounds. Activity assay-guided analysis of antimicrobial strains allowed us to identify *N*-acylamino acids as the active components. And by using a specific metamorphosis-assay, we prioritized the isolated strains and are currently characterizing the microbial signal responsible for the morphogenic activity.
Conclusion: *H. echinata* associated microbes induce larval settlement and metamorphosis. Preliminary data showed that the bacterial signal is part of the microbial biofilm and stable to a broad range of physical and enzymatic treatment. In addition, the inducing strains showed high antimicrobial activity against a broad range of microbial strains indicating a defensive function in this ecological system. The discovery of novel antimicrobial compounds highlights the chemical potential of this unexplored niche.

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NPP09

Screening of cyanobacteria extracts against the cysteine protease rhodesain of *Trypanosoma brucei*

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The trypanosomal cysteine protease rhodesain plays a major role during parasitic infection by *Trypanosoma brucei,* known as human African trypanosomiasis (HAT) [1]. Rhodesain is involved in the parasitic crossover of the blood-brain-barrier, leading to the late-stage of HAT [2] and in the synthesis of variant surface glycoproteins (VSGs) of trypanosomes, enabling *T. brucei* to elude host immune response [3]. Thus it is regarded as a promising target for the development of urgently needed new therapies [1]. Cyanobacteria are known as a rich source of structurally diverse protease inhibitors [4], but no screening for rhodesain inhibitors has been reported yet. Therefore a collection of about 670 cyanobacteria extracts was screened for inhibitory activity against rhodesain according to Breuning, Degel [5].
The screening revealed various hit extracts with inhibitory effects. Hit extracts subsequently were fractionated using chromatographic methods such as flash chromatography and preparative high-performance liquid chromatography (HPLC) in order to isolate the active compounds. Structure elucidation of the active compounds by nuclear magnetic resonance (NMR) spectroscopy and high resolution mass spectrometry (HRMS) as well as characterization of their bioactivity is under way. Our study confirms that cyanobacteria present a valuable source for protease inhibitors. Further investigations concerning activities against other proteases relevant in infectious diseases are planned.

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NPP10

Functional analysis of symbiotic and associated microbes of fungus-growing termites

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Question: Termites of the genus *Macrotermitinae* cultivate a mutualistic food fungus (*Termitomyces spp.*) for nourishment in so called "fungus gardens".[1] The fungus gardens is a nutrient-rich environment, which is prone to exploitation by parasites and pathogens. Symbiotic and associated bacteria are assumed to play a major role in the defense of the fungus garden.[2] Due to the specialized mode of substrate uptake and fungus-farming, we hypothesize that in particular gut bacteria protect the system by secretion of antimicrobial small molecules, which selectively target garden parasites and other invading species.
Methods: To analyze and characterize the secondary metabolites in question, we use culture depend and independent methods. We first isolated a broad range of symbiotic and associated microbes using different cultivation techniques. Then, we performed challenging assays pairing bacterial symbionts and garden parasites to investigate the production of specific antifungal compounds. Subsequently, the produced antimicrobial secondary metabolites were analyzed using HPLC/LC-HRMS/NMR. We are now aiming for a culture-independent approach to describe the antimicrobial (gut) environment.
Results: In particular members of the *Actinobacteria* phylum were isolated from different parts of the termites and termite nest.[3] Extracts of the bacteria culture were tested against known human pathogens showing a high antimicrobial activity against most tested pathogens. Pairing challenging assays of isolated *Actinobacteria* showed selective activity against most co-isolated and antagonistic fungi, such as *Trichoderma* sp. We are now in the progress of analyzing the antimicrobial secondary metabolites. We are also developing a culture-independent approach to describe and analyze the antimicrobial environment, which antagonist's meet when entering the nest.
Conclusion: Microbial symbionts and commensals most likely contribute to the fungus garden homeostasis by secretion specific antimicrobial compounds.

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NPP11

Polyketide synthase chimeras reveal key role of ketosynthase domain in chain branching

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Many therapeutically relevant polyketides are produced by modular type I polyketide synthases, where ketosynthase (KS) domains uniformly catalyze head-to-tail Claisen condensations of malonyl and acyl building

blocks to yield linear chains. The biosynthesis of the highly antimitotic agent rhizoxin[1], however, affords an unusual chain-branching module. KS domain, acyl carrier protein and a branching (B) domain install a d-lactone residue that is pivotal for bioactivity. The unique δ-lactone unit results from Michael addition of a malonyl unit to an α, β-unsaturated intermediate followed by lactonization[2]. To investigate the role of the B domain we designed chimeric modules with structurally similar domains like the X domain from a tentative glutarimide-forming module, and a dehydratase (DH) domain. Biochemical and kinetic analyses revealed that catalytic functions and substrate affinities are retained in all constructs, including mutated B, X, and DH domains[3]. Our results provide clear evidence for a structural role of the accessory domains and a multifarious catalytic function of the KS. This finding is important for synthetic biology approaches towards novel polyketide architectures.

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NPP12

Studying the composition of ClpP complexes in *Streptomyces hawaiiensis*

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ClpP is the proteolytic core of the bacterial caseinolytic protease. In concert with Clp ATPases, ClpP is responsible for degradation of aberrant and regulatory proteins. ClpP is the target of a new class of acyldepsipeptide antibiotics (ADEPs), naturally produced by *Streptomyces hawaiiensis* [1]. ADEPs have potent antibacterial activity against Gram-positive bacteria, including multi-drug resistant clinical isolates [1]. In streptomycetes, ClpP is essential for viability, in contrast to most bacteria [2].
While *Bacillus subtilis* contains only one and *Mycobacterium tuberculosis* two *clpP* genes, most *Streptomyces* species contain five different *clpP* homologues, e.g. *Streptomyces lividans* [2, 3, 4]. There, the *clpP* genes are organised in two bicistronic and one monocistronic operon [2]. Here, we analyzed the *clpP* genes of *S. hawaiiensis* and observed high sequence homology to *S. lividans* as well as the same gene organisation.
In *S. lividans*, the expression of ClpP1/2 and ClpP3/4 is regulated by distinct transcription factors and either ClpP1/2 or ClpP3/4 have to be expressed for viability [2]. This raises the question of interaction and functional cross talk between the ClpP proteins in *Streptomyces*. So far, none of the *Streptomyces* ClpP proteins was studied *in vitro*. By recombinant expression of *Sh*ClpP proteins, we aim to determine the composition of the proteolytically active complex(es) *in vitro*. Peptidase and protein degradation assays will be performed in the absence and presence of ADEP. Constructs containing affinity tags will be used for pull-down experiments and interaction studies. Functional interplay with the ClpP ATPases ClpX or ClpC1 will also be investigated.

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NPP13

Heterologous expression of a biosynthetic gene cluster from *Amycolatopsis sp.*

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Antibiotic resistances are an emerging problem in today's healthcare. There is a great demand for new therapeutic agents with new modes of action that do not develop cross resistances. Secondary metabolites produced by bacteria and their derivatives are commonly used as antibiotics and also find other important applications in medicine. Genome sequencing projects have revealed an immense reservoir of secondary metabolite biosynthetic gene clusters in the order Actinomycetes, most of which have not been connected to known compounds and potentially code for novel molecules. The best investigated Actinomycete genus is the soil dwelling *Streptomyces*. However, recent bioinformatic data show an equal potential for the production of secondary metabolites in other Actinomycete genera.

Here, we focus on *Amycolatopsis*, a genus with known producers of glycopeptides but an otherwise rather unexplored Actinomycete genus. Genome mining efforts identified an unusual NRPS-Lanthipeptide hybrid gene cluster. Here we present the first results on the heterologous expression of the gene cluster using TAR-Cloning (Transformation Associated Recombination) in *Amycolatopsis japonica*, a closely related host strain. The large size of this cluster prompted a dual capture strategy using a standard TAR vector and a new TAR vector with a second integrase site for integration of both halves of the cluster in one heterologous host. Further investigation of the gene expression is envisaged.

NPP14

Local and seasonal distribution of secondary metabolites' biosynthetic pathways in soil bacteria

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The need for new antibiotics has increased in the past years due to the raise of antibiotic resistant genes among pathogenic bacteria. New biotechnology tools allow for screening of biosynthetic pathways in environmental samples, and its use has revealed that the diversity of natural products in bacteria is higher than previously thought. Specifically, soil bacteria possess many more biosynthetic pathways for production of antibiotics than those they express in culture.
Our goal is to identify the potential of antibiotic gene clusters in the environment. Therefore, we are analyzing geographical and seasonal distribution patterns of bacteria and their secondary metabolites in soil. After extracting and sequencing DNA directly from the environment, we are analyzing domains of two major classes of secondary metabolites, polyketide synthases (PKS) and non-ribosomal peptide synthetases (NRPS), in different locations, at various soil horizons, and at several time points. Bioinformatic analysis of these sequence tags in combination with in depth analytics of the soil, weather conditions and bacterial diversity, allows to correlate secondary metabolites to environmental conditions
Here we present first results of the secondary metabolite diversity in different horizons of three types of soils (podzol, cambisol and gleysol) in Tübingen and provide first insights into potential sampling sites for novel antibiotic discovery efforts.

NPP15

Purification of acyldepsipeptide antibiotic ADEP1 from *Streptomyces hawaiiensis* NRRL 15010 culture broth

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Questions: *Streptomyces hawaiiensis* NRRL 15010, a Gram-positive actinomycete, is the producer of the antibacterial complex A54556.[1] The main component, ADEP1, is the precursor of a new class of acyldepsipeptide antibiotics (ADEPs) with an unprecedented mode of action.
ADEP1 and several synthetic congeners were shown to be potent antimicrobials against various Gram-positive bacteria, including multi-resistant nosocomial pathogens.[2]
ADEPs target the proteolytic core of the bacterial caseinolytic protease (ClpP) via a dual mechanism. By using the same binding sites as the associated Clp-ATPases ADEPs inhibit all natural functions of ClpP. Furthermore, ADEP binding to ClpP induces the opening of the entrance pores to the proteolytic chamber of ClpP leading to entry and degradation of essential proteins and finally cell death.[2]
Here, we established a fast purification procedure for ADEP1 from *S. hawaiiensis* culture broth for use in antimicrobial activity tests.
Methods and Results: *S. hawaiiensis* was grown in YM medium, harvested by centrifugation and subsequently filtrated. The supernatant was fractionated by adsorption chromatography using a hydrophobic adsorbent and a water-methanol gradient. While hydrophilic substances were eluted within the water-methanol gradient, the resin retained ADEP1 amongst others until elution with 100 % methanol. After determining the antibiotic containing fraction with bioassays we continued purification of ADEP1 via semi-preparative HPLC, which yielded pure ADEP1.
The antimicrobial potency of ADEP1 was tested in bioassays against different Gram-positive strains. ADEP1 effectively inhibited growth of *Streptomyces lividans*, *Streptomyces coelicolor* and *B. subtilis*.

Conclusions: Under the chosen chromatographic conditions it was possible to improve fractionation of the *S. hawaiiensis* culture supernatant to allow subsequent purification of ADEP1 by semi-preparative HPLC. Easy handling and efficient purification of ADEP1 is a prerequisite for further investigations on this new class of antibiotics that will provide a better understanding of its mechanism of action and insights into the Clp proteolytic machinery.

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[2] Brötz-Oesterhelt *et al.* 2005, Nat. Med. 11: 1082-87*)*.

NPP16

Genome mining reveals the potential for secondary metabolite production in *Amycolatopsis*

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The Actinomycete genus *Amycolatopsis* has already been well recognized as a producer of important antibiotics such as rifamycin and the glycopeptide vancomycin. Besides these notable products, the genus harbors a huge, unexplored reservoir of secondary metabolites. To date 67 different *Amycolatopsis* species from variable origins have been described. They are typically isolated from soil, but also from other environments such as ocean, lake sediment, cave, and clinical environments. For about half of them and for several unclassified *Amycolatopsis* strains complete genome sequences are available for genome mining.
We are interested in studying the potential for secondary metabolite biosynthesis and the evolution of secondary metabolite gene clusters in *Amycolatopsis*, in order to guide the identification of novel secondary metabolites, while minimizing the risk for rediscovery of known compounds, and shedding light on the ecological functions of these molecules.
Newly sequenced strains from the Tübingen Actinomycete collection in addition to the publicly available *Amycolatopsis* genomes were mined for secondary metabolite biosynthesis clusters using antiSMASH 3.0. Furthermore, the phylogenetic relationship between the strains was evaluated based on a multi locus sequence typing (MLST) approach for seven housekeeping genes. As a first target, non-ribosomal peptide (NRPS) biosynthetic clusters were identified, manually trimmed, and different computational methods were evaluated to resolve the relationship between the varying NRPS clusters.
Our initial results show that the genus *Amycolatopsis* has a huge potential to produce different types of secondary metabolites, the most abundant being NRPS, polyketide synthases, lantipeptides and terpenes. Based on a similarity network it was possible to distinguish gene cluster families of known secondary metabolites (e.g. glycopeptides) from unknown NRPS gene cluster families and novel, unique gene clusters. The phylogenetic relationship of the different *Amycolatopsis* strains is further reflected in the abundance and relation of secondary metabolite gene clusters. However, it could be seen that strains isolated from environments with extreme conditions, such as high temperatures, high salinity or high concentrations of methanol, differed from the strains derived from a moderate environment regarding a smaller genome size and a reduced potential for secondary metabolite production. This diversity makes the *Amycolatopsis* species an excellent resource to study the genetics and evolution of secondary metabolite gene clusters.

NPP17

Analysis of two secondary metabolite gene clusters in *Alternaria alternata*

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The production of secondary metabolites is a characteristic of microorganisms such as bacteria and fungi. One of the most common metabolites in fungi are polyketides. Although produced through the same simple mechanism by polyketide synthases (Pks), a huge diversity of small molecules with different functions exists. Besides different pigments and toxins, polyketides can also act as antibiotics and substances with various pharmacological effects.
The filamentous fungus *Alternaria alternata* is an ascomycete that colonizes many different organic substrates and is also able to infect different plant species such as cereals and thus causes a tremendous economic damage every year. *A. alternata* produces a variety of different secondary metabolites but only little is known about their biosynthesis pathways and the genetics of the corresponding genes. One of the most prominent toxins and polyketides is alternariol. Although the toxin itself is well studied, the way of synthesis and the corresponding gene cluster remained unknown. Recent knockdown experiments with RNAi constructs targeting Pks encoding genes resulted in transformants with significantly reduced production of alternariol when *pksI* was silenced. Real time PCR also confirmed the reduced expression, strongly suggesting that alternariol is produced by the genes of the *pksI* cluster. To further understand the mechanisms of alternariol biosynthesis we expressed *pksI* in *Aspergillus nidulans* as a heterologous host. Structural analyses of the newly synthesized compound are on the way.
The same approaches were used for the analysis of a second gene cluster, characterized by *pksJ*. We present evidence that this gene cluster is required for the production of hortein a polyketide so far only known from marine fungi. Hortein has antibacterial activities which makes it a potential antibiotic for various applications. Its unique structure is rarely found among natural products, thus making it a good candidate for investigation of its biosynthesis pathway. Through heterologous expression of *pksJ* in *A. nidulans* synthesis of a new compound could be observed giving insight of the precursor molecule of hortein.

NPP18

Genomic analysis of the biosynthetic potential of *Pyxidicoccus fallax* HKI 727

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The predatory myxobacterium *Pyxidicoccus fallax* HKI 727 was recently reported as a producer of novel polyketide antibiotics [1] and the nonribosomal peptide synthetase (NRPS)-derived siderophore myxochelin A [2, 3]. Preliminary genetic analyses suggested that *P. fallax* might harbor an even larger biosynthetic potential for the production of bioactive secondary metabolites, raising questions concerning the structural features of these compounds.
Sequencing of the *P. fallax* HKI 727 genome with Illumina HiSeq 2000 and PacBio techniques followed by similarity searches against TIGRFAMs, PRIAM, Pfam, SMART, COG, SWISS-PROT/TrEMBL and KEGG revealed the gene cluster responsible for gulmirecin biosynthesis. In addition, a diverse range of undesignated polyketide synthase (PKS), NRPS and PKS-NRPS clusters were annotated. Subsequent analysis of these gene clusters using AntiSMASH [4] allowed structural predictions of the encoded natural products.
The obtained results illustrate the diverse and versatile genomic potential of *P. fallax* for natural product biosynthesis and build a valuable data basis for further drug discovery studies.

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NPP19

Analysis of protein degradation by ADEP-activated ClpP peptidase

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A novel class of antibiotic acyldepsipeptides (ADEPs) exerts prominent antibacterial activity against Gram-positive bacteria incl. *Staphylococcus aureus in vitro* and *in vivo* [1]. ADEPs act via a yet unprecedented mechanism by dysregulating the caseinolytic protease ClpP. Usually, the activity of ClpP is tightly controlled by ATP-dependent Clp-ATPases and accessory proteins. ADEPs overcome these control mechanisms, switching ClpP from a regulated to an uncontrolled protease that targets unstructured proteins as well as nascent polypeptides at the ribosome in the absence of Clp-ATPases [1, 2, 3]. Dissecting the cascade of events that leads to bacterial death, we noticed that at inhibitory concentrations of ADEP close to the MIC, rod-shaped cells formed long filaments and cocci developed into enlarged spheres indicating considerable remaining biosynthetic capacity [4].

The reason for this filamentation phenotype at lower ADEP concentrations was revealed by high-resolution fluorescence microscopy of ADEP-treated *Bacillus subtilis*, which showed the delocalization of central cell division proteins leading to the inhibition of septum formation and cell division [4]. In cytoplasmic extracts from treated cells we observed a significantly reduced amount of intact FtsZ protein, an important cell division mediator that is essential in most bacteria including important human pathogens, and when purified FtsZ was exposed to ADEP-activated ClpP *in vitro*, proteolytic degradation of FtsZ into small fragments took place.
As FtsZ is a well-characterised protein and has not been discussed as particularly unstructured or unstable, we investigated the reason why folded FtsZ is such a preferential target for ADEP-ClpP, while several other stably folded proteins had been resistant to degradation in the course of our studies. To this end, we characterized the role of specific structural features of FtsZ in ADEP-ClpP dependent degradation. Our results allow first insights into the way, how the ADEP-activated ClpP complex attacks proteins to finally lead to suicide-like bacterial killing.

[1] Brötz *et al.* 2005, Nat. Med. 11: 1082-87
[2] Kirstein *et al.* 2009, EMBO Mol. Med. 1: 37-49
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NPP20
Discovery and characterization of novel bioactive peptides from Cyanobacteria
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The group of ribosomally synthesized and posttranslationally modified peptides (RiPPs) has revealed an astonishing diversity of structures with a large variety of posttranslational modifications. Products of such pathways include antimicrobial peptides such as nisin and thiostrepton as well as potent toxins of snails and fungi such as conotoxins and α-amanitin [1].
In previous studies, our group discovered the RiPP pathway for the cyanobacterial microviridin, a depsipeptide which acts as a potent serin-protease inhibitor. Due to the action of two ATP grasp ligases, which catalyze the incorporation of intramolecular lacton- and lactam rings, microviridin shows a unique cage-like structure [2,3]. Microviridins could be successfully manipulated *in vivo* and *in vitro*, however, the size of the three rings is inflexible.
Genome mining of selected bacterial and cyanobacterial strains has unraveled the presence of microviridin-related gene clusters encoding enzymes of the ATP grasp ligase family in direct neighborhood of putative RiPP precursor genes. Based on these bioinformatic studies we anticipate various lacton ring sizes of the novel peptides as well as different processing models. The overarching aim is to generate a cyanobacterial depsipeptide production platform with diverse ring sizes that could be screened against a variety of targets.
Therefore we generated a minimal expression platform for the heterologous production of promising novel peptides in *E. coli* and further performed the cyclization *in vitro*. Currently we are working on the structural characterization of the revealed new peptides. First experimental findings will be presented.

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NPP21
Biochemical dissection of the natural diversification of microcystin provides lessons for synthetic biology of NRPS
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Many small molecule natural products with interesting bioactivities are produced by non-ribosomal peptide synthetases (NRPSs). The modular structure of NRPS provides good opportunities for the development of combinatorial biosynthetic approaches (Stachelhaus *et al.*, 1995). But in the majority of cases the synthetic fusion of NRPS domains results in extreme reduction or loss of peptide synthesis. NRPS are, however, subject of natural recombination events that result in the diversification of their products (Welker *et al.*, 2006). A notable example is the assembly line for the production of the potent hepatotoxin microcystin produced by diverse genera of cyanobacteria. In several *Microcystis* strains intragenomic recombination events within McyB1 adenylation domains led to the emergence of multispecific modules that incorporate chemically distinct amino acids such as leucine, arginine, tyrosine and tryptophane in parallel into the same position. Strains carrying the ancestral McyB1 module incorporate predominantly leucine (Mikalsen *et al.*, 2003).
In our study we compared activities of A-PCP di-domains and C-PCP-C tri-domains of ancestral McyB1 and McyC modules and the chimeric McyB1 module *in vitro* to ascertain the impact of condensation domains on amino acid activation. Further, we initiated a number of point mutations in the adenylation and condensation domains to identify determinants of their multispecificity. The *in vitro* data were compared with *in vivo* data from selected natural *Microcystis* strains. We provide evidence that the production of chemically diverse microcystin variants broadens the functional repertoire of microcystins. The results led to new insights in the function of NRPS and might contribute to a more effective engineering of NRPS.

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NPP22
Metallophore mapping in *Frankia* sp. using metal isotope coded profiling
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Metal chelators are essential to support the acquisition of trace elements in plants, microorganisms and animals. Approximately 500 siderophores (i.e. iron carriers) are known for their unique importance in bacterial iron acquisition and metal cycling. Aside from iron, microorganisms also need to acquire other trace metals to synthesize metalloenzymes such as nitrogenase. Owing to the potential multiple functions of these chelators, the more general term "metallophores" was proposed to define multi-purpose complexing ligands involved in the management of a broad spectrum of metal ions (e.g. Fe, Cu, Zn, Mo, V) [1].
This study was meant to characterize the metal ligandosphere of the filamentous actinomycete *Frankia* spp. in an effort to shed light on its molecular interactions with the environment, emphasizing nitrogen fixing processes. In particular, we are interested in the symbioses between *Frankia* spp., its host plant Alder (*Alnus* sp., *Betulaceae*), and ectomycorrhizal fungi [2]. Plants and bacteria exude a cocktail of compounds which could have a combined impact on the rhizosphere in regards to metal uptake, detoxification, and inhibitory of microbial activity. Because of this, we aimed to decipher the plant-microbe chemosphere in its entirety (metabolites in the community released by the mutualistic organisms), keeping the main focus on detecting the existence of metal complexes. The characterization of *Frankia´s* ligandosphere required particular analytical techniques based on hyphenated approaches in mass spectroscopy. We therefore developed a novel, unbiased metal stable isotope based technique using ultrahigh performance liquid chromatography coupled to mass spectrometry (UHPLC-MS) to identify new metallophores in complex biological matrices[3,4]. Extracts of bacterial cultures were spiked with the isotope-pairs of $^{54}Fe/^{58}Fe$ and $^{95}Mo/^{98}Mo$ creating unique isotopic signatures of the metal complexes. UHPLC-MS mass spectra were scanned for mass differences of Δ4 (siderophores) and of Δ3 (molybdophores) using search algorithms in specialized software (targeted analysis) or chemometric data analysis (multivariate comparative analysis) that combined high sensitivity and robustness. We examined the metallophore production of 15 *Frankia* strains to provide a basis for subsequent modelizing of metal acquisition and homeostasis within the tripartite associations of *Frankia* spp., Alder, and ectomycorrhizal fungi. Hereby, the profile of metallophores in *Frankia* turned out to be very strain specific. We have discovered several

novel metallophores candidates which will be now purified for further characterization and structure elucidation.

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NPP23

Bioactive compounds in the secondary metabolome of a russuloid basidiomycete

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Question: A taxonomically undescribed basidiomycete (BY1) that very likely belongs to the order Russulales was investigated and its secondary metabolome was of interest because i) the fungus showed antifungal activity and ii) as its mycelium showed yellow pigmentation following injury. The objective of the study was to identify the compounds underlying these observed effects.
Methods: The compounds were isolated with organic solvents from the culture broth and injured mycelia, respectively. Subsequent purification was accomplished with normal and reversed phase liquid chromatography. Structure elucidation was based on high-resolution mass spectrometry and 1D and 2D nuclear magnetic resonance spectroscopy.
Results: Two distinct injury-induced yellow pigments were identified as piptoporic acid-related polyenes [1]. The structures were determined as 18-methyl-19-oxoicosa-octaenoic acid and 20-methyl-21-oxodocosa-nonaenoic acid [2]. Subsequent stable-isotope labeling with [1-^{13}C]acetate proved a polyketide origin, most likely mediated by a highly-reducing polyketide synthase (HR-PKS). Candidate PKS genes have been identified in the genomic sequence of BY1. Both polyenes showed a dose-dependent inhibition of *Drosophila melanogaster* larval development in concentrations between 12.5 µM and 100 µM. Further compounds isolated from BY1 include fomannoxin- and vibralactone-related natural products, which were structurally elucidated and identified as antifungally active against various filamentous fungi.
Conclusion: The injury-induced and sustained presence and antilarval activity of the BY1 polyenes suggests an ecological role in long-term defense. The production of antifungal fomannoxin and vibralactone derivatives by BY1 may confer a selective advantage for substrate colonization. Further, it underscores the evolutionary relationship of BY1 with the Russulales and the Stereaceae in particular, as these compounds are known from species in the genera *Heterobasidion*, *Boreostereum* and *Stereum*, respectively.

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NPP24

Characterisation of the adenylation domains of *Coprinopsis cinerea* reductases induced through inter-kingdom interaction

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Question: It is intriguing that basidiomycetes possess far more genes linked to natural products than originally anticipated. In diverse environments, these fungi evince abundant interactions with other microorganisms, and the observation of these behaviours is an important tool to help understand the biosynthesis of such molecules and how they affect other biological systems. A transcriptomic study (Kombrink *et al.*, manuscript in preparation) identified a set of genes of *Coprinopsis cinerea* found to be up-regulated after its co-cultivation with *Escherichia coli*, among which two sequences encoding adenylating multidomain reductases (NRPS_3009 and NRPS_06235). Here we aim to characterise the adenylation (A) domains of both enzymes, elucidating their substrate predilection and optimal functional conditions.
Methods: The sequences encoding the A domain of NRPS_03009 and the AT di-domain of NRPS_06235 were heterologously expressed in *E. coli* BL21 in the form of *N*-histidine tagged fusion proteins. The substrate specificity of both domains was determined by the substrate-dependent ATP-[^{32}P] pyrophosphate exchange assay. Once the substrates were identified, the pH and temperature optima were determined using the aforementioned method.
Results: The A domains of the analysed NRPS-like reductases showed equal preference for l-alanine, l-serine, and l-valine as substrates. The reaction using l-threonine, biochemically similar to the accepted amino acids, was only modestly recognised and, thus, not considered a substrate. Moreover, both domains demonstrated maximum substrate turnover at pH 7.5, albeit NRPS_03009 was more active at 25 °C, whereas the optimal temperature for NRPS_06235 was 37 °C.
Conclusion: The first step of the natural product biosynthesis by adenylate-forming reductases from *C. cinerea* was elucidated by the characterisation of their A domains. The final products of the pathway initiated by these reductases, as well as their role in the basidiomycete *vs*. bacterium confrontation, are still elusive.

NPP25

A novel allelopathic molecule from *Synechococcus elongatus* PCC 7942

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The cyanobacterial production of bioactive metabolites is a poorly explored and understood field in the Natural Product Research. Although many bioactive metabolites from Cyanobacteria were characterized in the past years, the majority remains to be explored. Within these metabolites, allelochemicals play an important role in cyanobacterial survival. Allelopathy is defined as the chemical inhibition of related organisms by the release of selective substances. In Cyanobacteria, allelochemicals are mainly known from section III-V strains, however numerous chemical structures are not elucidated. Investigation of section I strains indicated the presence of an allelopathic compound also in *Synechococcus elongatus* PCC 7942. The isolated compound reveals growth inhibition in filamentous cyanobacteria.

NPP26

Complex dynamics of zwitterionic sulfur species from phytoplankton in the marine sulfur cycle

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Question: The zwitterionic microalgal metabolite dimethylsulfoniopropionate (DMSP) plays a key role in marine sulfur cycling.[1] In addition to the functions as osmolyte and cryoprotectant it is discussed to act as an antioxidant. But also oxidized organic sulfur species might have an important impact in this context. Resulting from the antioxidant activity of reduced sulfur species especially dimethylsulfoxide (DMSO) is considered to be a potential key player. We are looking now for new metabolites from phytoplankton that pave the way for a mechanistic understanding of the antioxidative properties of DMSP and related compounds.
Methods: We recently developed LC/MS based methods to quantify the zwitterionic DMSP as well as to detect related highly polar metabolites from plankton.[2] Guided by their LC/MS properties we identify new metabolites using MS/MS techniques and spectroscopic methods like NMR for structure elucidation as well as synthetic approaches. Zwitterionic metabolites are investigated in the context of growth curve experiments in stationary cultures, but we also investigate the fate of these metabolites by monitor their degradation by microalgae and bacteria.
Results: Using our new LC/MS based method an entire family of structurally related dimethylsulfonio-metabolites as well as N-containing zwitterionic metabolites is detected in phytoplankton with species-specific signatures.[3] By extending our analytical concept we were also able to detect novel zwitterionic compounds including the S-oxidized sulfoxonium analog of DMSP. This metabolite is to our knowledge only the second natural product reported with a sulfoxonium group.[4]
Conclusion: Based on the prevalence in different stressed phytoplankton cultures we suggest novel models for the regulation of highly polar zwitterionic metabolites in phytoplankton and identify a novel candidate for a biomarker for oxidative stress.

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NPP27

A systematic analysis platform to elucidate antibiotic mechanisms

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Novel antibiotics with unprecedented mechanisms of action are urgently needed to overcome rising resistance. Analysis of the mode of action of an antibiotic and identification of the molecular target are integral components of the drug development process. Without this detailed knowledge, rational drug design is strongly hampered.
We built up a comprehensive mode of action (MoA) platform, combining whole cell screenings and a biochemical platform that allows rapid identification of antibiotic mechanisms and targets on all cellular levels. Initial screenings of compound and extract libraries in whole-cell based assays identify biologically active compounds and provide first indications of the metabolic pathway affected. "SmartScreens", such as the antisense (AS) RNA technology, provide intrinsic information on the target structure. AS RNA mediated gene depletion confers selective antibiotic hypersensitivity by lowering the cellular pool of the cognate protein target. A biochemical analysis platform comprising more than 60 individual *in vitro* assays further allows for the identification and characterization of a specific target on the molecular level.

NPP28

Integrated biosurfactant production using *P. putida* – high cell density cultivation and purification

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Question: Applications of surfactants are widely spread throughout different aspects of life. They are applied particularly in washing agents and domestic detergents but are also of importance in pharmaceutical and cosmetic industry. The surfactant producing industry uses petrochemical and oleochemical raw material. A reasonable alternative for an environmentally conscious society are bacterially produced surfactants from renewable resources, which feature high biocompatibility and degradability. Rhamnolipids are– in terms of industrial utilization – a relatively new surfactant. Being surfactants (surface active agents) rhamnolipids are amphiphilic molecules, consisting of a hydrophobic and a hydrophilic part. We successfully created a non-pathogenic bacterium able to produce rhamnolipids [1]. To that end, we used *P. putida* KT2440 as host strain and introduced the two genes responsible for rhamnolipid production from *P. aeruginosa*.
The goals of this work are to establish rhamnolipid production by high cell density cultivation enabling high product titers and the development of a suitable downstream process with focus on capture and purification.
Methods: The first step in establishing high cell density fermentation is the identification of an optimal medium as well as an optimal feeding strategy. Rhamnolipids accumulate in the foam, which is formed during cultivation time. Subsequently downstream processing is focused, using an adsorption/desorption procedure to capture the desired product established previously [2]. To obtain high purity, selection of an optimal adsorbent as well as a suitable elution profile is crucial.
Results: With the developed fermentation process, we are able to reach cell densities of about 50 g CDW/L. The application of a *P. putida* strain with altered cell wall hydrophobicity hampers the foaming out of the cells. An improved elution profile yielded a purity of >90 %. The adjustment of the pH value provides the possibility to determine the product appearance.
Conclusion: We here established a holistic approach for the sustainable production of an industrially interesting biosurfactant including fermentation and downstreaming.

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NPP29

Function of the acyltransferase XrdE in the production of NRPS derived metabolites and cytotoxic amides in *Xenorhabdus doucetiae* DSM 17909

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The entomopathogenic Gram-negative bacterium *Xenorhabdus doucetiae* lives in symbiosis with its nematode *Steinernema diaprepesi*. After entering the insect prey, the infecting nematode releases the bacteria into the insect hemolymph were they proliferate and produce metabolites to kill the insect and food competitors. Furthermore the bacteria support the nematode development into a new infective stage and stabilizing the symbiosis. This complex lifestyle requires a balanced metabolism of compounds for signaling, defense and development. In the biosynthesis pathway for the production of the antimicrobial compound xenorhabdin in *X. doucetiae* we detected the acyltransferase XrdE, that acylates the xenorhabdin precursor and thus diversifies it into different bioactive xenorhabdin derivatives. In *X. doucetiae* XrdE is also responsible for the production of cytotoxic tryptamides by acylating tryptamine with different acyl moieties. The decarboxylase involved in tryptamine biosynthesis was found to be additionally involved in the biosynthesis of cytotoxic phenylethylamindes. Interestingly, the deletion of *xrdE* resulted not only in the loss of xenorhabdine and tryptamine derivatives but also influences the production of other secondary metabolites like xenocoumacins and xenoamicins. From our findings, we conclude, that XrdE might be an important "key enzyme" in the metabolism of *X. doucetiae*, that shows unique activity compared to our investigations in other *Xenorhabdus* strains.

NPP30

Structure prediction, biosynthesis and purification of a siderophore from a thermophilic bacterium

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Siderophores are low-molecular weight compounds produced by microorganisms to assimilate vital Fe^{3+} out of iron-deficient environments. These molecules are heterogenic according to there structure, metal-selectivity and binding affinity [1]. Their selectivity for other elements makes metallophores interesting for bioremediation of heavy metals and the extraction of high value elements like Au, Ag, Ga, V, I and others. Further they have a high potential in medical applications as they might be used as a carrier for antibiotics to resistant bacteria, for iron overload therapy, as an antimalarial drug, removal of radioactive elements and for cancer therapy [2]. Thus it is essential to understand the synthesis of these molecules to further increase the set of available metallophores that are stable and suited for the respective application.
We have chosen the thermophilic actinobacterium *Thermocrispum agreste* DSM 44070 as template and applied bioinformatic tools to predict the biosynthetic pathway and the probable structure of the metallophore. An N-hydroxylating monooxygenase (NMO: TheA) of *T. agreste* DSM 44070 that catalyzes an initial step was synthesized and characterized in detail. The respective metallophore was synthesized, purified and studied.
The siderophore prediction of *T. agreste* DSM 44070 suggested a hydroxamate-type siderophore (Erythrochelin-like) that contains L-N5-hydroxyornithine. This precursor is provided by TheA which was approved by activity determination. As known for other NMOs the substrate specificity is restricted, in this case to L-ornithine. The siderophore "Thermochelin" was synthesized under iron limitation, extracted and purified successfully. Iron complexation was confirmed by means of the CAS-assay. Thermochelin is also able to bind other metals like Vanadium.
Here we expanded the scope of siderophores and the knowledge towards there biosynthetic pathways. Thermochelin is the second siderophore which was purified from a thermophilic organism [3] and TheA is the first NMO which was characterized from an extremophile.

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NPP31

The role of carbon catabolite repression in the emission of ‚sodorifen' by *Serratia plymuthica* 4Rx13

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During the past years we were trying to elucidate the biological function of the unusual and unique volatile organic compound sodorifen. This volatile was first discovered in 2009 as the dominant compound (≈ 45 %) in the volatile spectrum of the rhizobacterium *Serratia plymuthica* 4Rx13 [1]. By the use of NMR & GC/HRMS the structure of sodorifen could be identified as a polymethylated bicyclus with the molecular formula $C_{16}H_{26}$ [2]. We expect by unraveling its biosynthesis and the regulation of the sodorifen emission to get insight into the biological role of sodorifen.
With this strategy we were able to find a cluster of four genes that is involved in the biosynthesis of sodorifen since knockout mutants showed a sodorifen negative phenotype [3]. Furthermore, we observed that glucose as the sole carbon source reduced the sodorifen emission in *S.p.* 4Rx13 to very low levels. In contrast, adding succinate to the growth medium lead to a 200-fold increase in sodorifen emission relative to complex medium [4]. In our study we want to prove that the sodorifen emission is regulated by the carbon catabolite repression (CCR) mechanism.
Therefore we constructed knockout mutants of the central CCR genes *cya* & *crp* and deleted a potential activator binding site of the CCR (CRE) from the upstream sequence of the sodorifen cluster in *S.p.* 4Rx13. Following this, we analyzed the sodorifen emission of the mutant strains in comparison to the wild type using a VOC collection system (modified according to [1]) and investigated the expression of the sodorifen cluster genes by RT-PCR and Northern blot.
These tests revealed that in the knockout/deletion mutants Δ*cya*, Δ*crp* & ΔCRE i) the sodorifen emission was significantly reduced by 30 - 90 % and ii) the transcription of the sodorifen cluster was severely inhibited. Surprisingly, the mutants Δ*cya* & Δ*crp* were no longer capable of growing in minimal medium supplemented with succinate whereas ΔCRE was still able to do so.
In this study we obtained comprehensive data proving that the regulation of the sodorifen emission takes place at a transcriptional level. Also, we could show that this regulation is specific for the sodorifen cluster and not due to a pleiotropic effect. Taken together, these results clearly suggest that the CCR mechanism plays a central role in the regulation of the sodorifen emission.

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NPP32

PHB and PHA granules have no phospholipids

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Polyhydroxybutyrate (PHB) granules are supra-molecular complexes in many prokaryotes consisting of a PHB polymer core and a surface layer of various structural and functional proteins. The finding of so many proteins with different functions on the PHB granule surface has led to the classification of PHB granules as multifunctional units and the designation as carbonosomes has been proposed for these organelle-like structures (1, 2). While it is generally accepted that several proteins are *in vivo* part of the PHB granule surface layer, the presence or absence of phospholipids on the PHB granule surface is controversially discussed in literature.
We addressed this topic by expression of DsRed2 and other fluorescent proteins (mTurquoise2 and sfGFP) that were fused to the LactC2-domain of lactadherin in *Ralstonia eutropha*. Lactadherin is a protein of milk fat that specifically binds to phospholipids *in vivo* via its C2 domain. The fusion proteins specifically labeled the cell membranes of *R. eutropha* but PHB granules were not labeled *in vivo*. The same result was obtained for *Pseudomonas putida*, a species that accumulates another type of polyhydroxyalkanate (PHA) granules related to PHB. Remarkably, expression of the Dsred2-LactC2 fusion in *Magnetospirillum gryphiswaldense* resulted in labeling of the magnetosome chains thus validating the ability of the fusion to detect phospholipids in subcellular organelles.
To find an explanation for the *in vitro* detection of phospholipids in isolated PHB granules as described 50 years ago we isolated native PHB granules from *R. eutropha* Purified PHB granules from DsRed2-LactC2- but not from DsRed2-expressing *R. eutropha* occasionally were fluorescent indicating that PHB granules *in vitro* can be contaminated by cell membrane debris to which DsRed2-LactC2 can bind.
In conclusion, native carbonosomes have no phospholipids *in vivo* and we postulate that the PHB/PHA granule surface layers in natural producers generally consist of proteins only.

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NPP33

Function and biosynthesis of aryl polyene pigments, one of the most widespread class of bacterial natural products

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Pigments of the aryl polyene (APE) type are structurally related to the well known carotenoids and were previously only found in bacteria of the *Cytophaga-Flavobacteria-Bacteroides group (APE/dialkylresorcinol hybrid) and the xanthomonadaceae (APE). Recent research showed* that the APE biosynthetic gene cluster (BGC) is actually one of the most common BGCs found within sequenced bacteria (around 1000 APE BGCs detected) and that they are widespread throughout taxonomically distant Gram-negative bacteria including bacterial pathogens of animals, humans and plants but also plant symbionts, human commensals and bacteria found in heavy metal and crude oil contaminated soils.[1,2] However, the biological function and biosynthesis of these APEs is mostly unknown.
Here we report our current progress on the biosynthesis of APEs and APE/dialkylresorcinol hybrids and the structure of arcuflavin, which has finally been solved by NMR.[1] Furthermore, the biological function of the APE/dialkylresorcinol pigment arcuflavin was elucidated.[1] An analysis of the distribution of BGCs for APE pigments and biosynthetic genes for carotenoids in bacterial genomes is presented that shows a complementary distribution of these pigments in bacteria.[1]

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NPP34

Heterologous *in vivo* and *in vitro* reconstitution of the pigment synthesis from *Aspergillus terreus* conidia

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Aspergillus species produce coloured asexual conidia. The pigment in these conidia generally derives from a polyketide synthase that produces a naphthopyrone, which eventually ends in the formation of a dihydroxynaphtalene melanin polymer. Unexpectedly, *Aspergillus terreus* lacks such a polyketide synthase despite producing a cinnamon-brown pigment. Therefore, we addressed the question which enzymes and pathways might be involved.
cDNA analyses identified several genes encoding NRPS-like enzymes that were highly induced during conidiation. Subsequent deletion of promising upregulated genes in *A. terreus* and their heterologous expression in *A. niger* led to identification of genes and precursors involved in pigment synthesis. Isolation and characterisation of natural products included HPLC analyses and NMR.
Deletion of the NRPS-like gene *melA* resulted in white conidia. Furthermore, deletion of the neighbouring tyrosinase gene *tyrP* produced conidia with an intermediate brightly fluorescent yellow colour, indicating

that *melA* and *tyrP* are jointly involved in pigment formation. The yellow fluorescent intermediate was isolated from *tyrP* mutants and from an *A. niger* strain expressing the *melA* gene and was identified as aspulvinone E, which is formed from the condensation of two *p*-hydroxyphenylpyruvate residues. Solving the function of TyrP in the pigment formation process turned out difficult due to incorrect reading frame predictions. Elucidation of the correct open reading frame showed that TyrP codes for a tyrosinase like enzyme, which could be involved in hydroxylation and oxidation with subsequent auto-polymerisation of aspulvinone E. This was confirmed by co-expressing the *tyrP* gene in *melA* expressing *A. niger* strains, which resulted in cinnamon-brown mycelium. While MelA appears to localise to the cytoplasm, TyrP contains an export signal sequence. A TyrP fusion with a red fluorescent protein implied accumulation of the protein in vesicles without subsequent secretion. This may ensure the correct reaction environment for TyrP, which is sensitive to reducing agents.
A. terreus follows an unprecedented pathway for production of its conidial pigment. Currently, we are elucidating the structure of TyrP reaction intermediates in order to draw a complete picture on the formation of this new pigment. However, from our research it can already be concluded that the *A. terreus* pigment is produced from the tyrosine derived aspulvinone E rather than a polyketide.

NPP35
The CASSIS suite – promoter-based prediction of secondary metabolite gene clusters and their anchor genes in eukaryotic genomes
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Introduction: Secondary metabolites (SM) are structurally diverse natural products of high pharmaceutical importance. Genes involved in their biosynthesis are often organized in clusters, i.e., they are co-localized and co-expressed.
Objectives: *In silico* cluster prediction in eukaryotic genomes remains problematic mainly due to the high variability of the clusters' content and lack of other distinguishing sequence features. Our aim is to develop a novel method and tool which accurately predicts the borders of SM gene clusters based on motifs, i.e. transcription factor binding sites, in promoter sequences.
Methods: We present the CASSIS suite, which consists of two novel software tools: (i) CASSIS, a method for the prediction of SM gene clusters in eukaryotic genomes. Unlike other tools based on protein similarity, e.g. SMURF [1] and antiSMASH [2], CASSIS exploits the idea of co-regulation of the cluster genes [3], which assumes the existence of common regulatory patterns in the cluster promoters. The method searches for "islands" of enriched cluster-specific motifs in the vicinity of anchor genes. (ii) SMIPS, a tool for the genome-wide detection of SM key enzymes ("anchor" genes): polyketide synthases, non-ribosomal peptide synthetases, and dimethylallyl tryptophan synthases. The anchor gene predictions are based on protein domain annotations by InterProScan [4].
Results: CASSIS was validated in a series of cross-validation experiments. In comparision to the similarity-based tools, CASSIS showed a much higher precision und comparable sensitivity. SMIPS recognized all given anchor genes, whereas SMURF and antiSMASH did not. Online versions of CASSIS and SMIPS, as well as downloads for both tools, are freely available athttps://sbi.hki-jena.de/cassis.
Conclusion: Most of the cluster prediction approaches developed so far rely on protein domain similarity. They ignore the co-expression (or co-regulation), which is an elementary feature of SM gene clusters. Conversely, CASSIS considers the promoter information and achieves better prediction results.

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NPP36
The hidden world of non-canonical aliphatic amino acids in fungi and bacteria
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Introduction: Amino acids are the essential building blocks of proteins and, therefore, living organisms. While the focus often lies on the canonical or proteinogenic amino acids, there is also a large number of non-canonical amino acids to explore. Some of them are part of toxins or antibiotics in fungi or bacteria. Here we give an overview of aliphatic amino acids up to a chain length of five carbons occurring in micro-organisms and have a closer look on each of them.
Objectives: A literature review about non-proteinogenic aliphatic amino acids, that are really synthesized in at least one organism. This is likely to be of interest for numerous medical applications: the discovery of new antibiotics, support in designing synthetic antibiotics, improvement of protein and peptide pharmaceuticals by avoiding incorportation of non-canonical amino acids, study of toxic cyanobacteria and other applications.
Methods: A literature overview is provided and the functions of non-canonical aliphatic amino acids are discussed. Moreover, we outline mathematical methods for enumerating the complete list of all potential aliphatic amino acids of a given chain length.
Results: 32 non-canonical aliphatic amino acids up to a chain length of five carbons have been found so far in fungi and bacteria. Examples are (i) dehydroalanine, which is involved in lantibiotics and microcystins, (ii) propargylglycine (2-amino-pent-4-ynoic acid), which is produced by Amanita pseudoporphyria and involves a triple bond, (iii) norvaline, which deteriorates the quality of several pharmaceuticals and (iv) homoleucine, being a constituent of longicatenamycines.
Conclusion: Most non-proteinogenic amino acids are found within fungi, with particularly many produced by Amanita species as defense chemicals. Several are incorporated into peptide antibiotics. Some of the amino acids occur due to broad substrate specificity of the branched-chain amino acid synthesis pathways.

NPP37
Comparative genomics of Moorea, a marine filamentous cyanobacterial genus with unprecedented natural products potential
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During the last three decades, tropical marine cyanobacteria have emerged as an extraordinarily prolific source of promising biomedical natural products (NPs). One aspect of our research has focused on providing a better understanding of how NPs are distributed among different cyanobacterial groups so as to improve the efficiency of future investigations. As a result, we have found that bioactive secondary metabolites are unequally distributed among different taxonomic groups with a few groups being responsible for the majority of the isolated NPs. For example, collections of the genus Lyngbya (reclassified as Moorea) have yielded more than 40 % of the nearly 800 secondary metabolites isolated to date from marine cyanobacteria. We have sequenced four Moorea genomes, two of the organisms collected in the Caribbean and two in the Pacific. The sequencing effort resulted in one closed genome that serves as our reference genome and the other three consists of around 10 scaffolds. Careful comparative genomic and a through examination of the secondary metabolite potential will be presented.

OTP01
Omics meets aesthetics – requirements for data visualization in functional genomics
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Modern gene expression analysis is faced with a variety of differently structured data. One can find data on gene sequences and genome structure, transcripts, gene regulatory interrelations, proteins, their modified forms and complexes as well as metabolites; from single cells, from cellular and sub-cellular compartments or from more diverse and heterogeneous (meta)samples.
How an efficient interpretation of data from OMICS based experiments can be supported by powerful and sophisticated data visualization tools and how these tools can be brought to the public?

At first we will demonstrate aspects of graphical perception and visual channels and highlight their role in efficient data visualization. Secondly we will introduce new tailor-made tools which have been adapted for data visualization tasks in OMICS driven research such as Voronoi treemaps and streamgraphs. They have been used to analyze data sets from a variety of real life experiments. Concerns regarded to the display of simple but also complex data, of clearly defined but also uncertain data, of nominal, ordinal but also quantitative data will be demonstrated and discussed
Finally we will introduce the web ressource http://bionic-vis.biologie.uni-greifswald.de that makes tools introduced in our presentation available for the public.

OTP02

Microbiological endocrinology – the fate of catecholamines during cultivation with *Vibrio cholerae*

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Question: Catecholamines such as adrenaline and noradrenaline are known to stimulate growth and swarming of some enterobacteria like *EHEC* [1] or *Salmonella* enterica Typhimurium [2] and also, recently shown by our group, *Vibrio cholerae* [3], the causative agent of the Cholerae disease. A major drawback in these studies is the prolonged incubation of bacteria with catecholamines [4]. Since catecholamines are known to undergo oxidative degradation [5], we investigated their stability during bacterial cultivation.
Methods: *V. cholerae* was grown in the presence of 0.1 mM adrenaline in different media, at varying temperatures, aerobically or anaerobically. After different incubation times, aliquots of the medium were analyzed by HPLC connected to an UV/VIS detector for detection and quantification of adrenaline at 279 nm or the presumed conversion product adrenochrome at 480 nm.
Results: Adrenochrome was confirmed as an oxidation product of adrenaline in the controls (cell free medium) and in minimal medium or LB medium inoculated with *V. cholerae* [3]. In the presence of *V. cholerae*, degradation of adrenaline was delayed compared to cell-free controls. Aerobic conditions promoted the formation of adrenochrome out of adrenaline both in the presence and in the absence of cells.
Conclusion: Adrenaline undergoes partial oxidation during cultivation of *V. cholerae*. We propose that the availability of O_2 and reactive oxygen species (ROS) in solution determines the amount of adrenochrome formed. In the presence of respiring bacteria, O_2 partial pressure decreases, concomitant with diminished adrenochrome formation compared to the cell-free control. ROS like superoxide enhance adrenochrome formation [5]. In the presence of *V. cholerae* adrenochrome formation decreases due to the periplasmic superoxide dismutase [3]. Hence, the concentration of a signaling molecule like adrenaline is affected by the O_2 partial pressure during growth of *V. cholerae*.

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OTP03

Electron transfer, voltage generation and sodium binding characteristics of the Na^+-translocating NADH–quinone oxidoreductase (Na^+-NQR) from *Vibrio cholerae*

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Question: The Na^+-translocating NADH:quinone oxidoreductase (NQR) is the respiratory complex of *Vibrio cholerae*, which couples the electron transfer from NADH to ubiquinone to the translocation of sodium ions across the membrane [1]. Until now it is unknown which electron transfer step is essential for ion translocation. Here we characterize the effect of Rb^+ on NQR and analyze its binding of Na^+ in a quantitative manner. Since the quinone substrate undergoes protonation after reduction, the influence of pH (neutral-alkaline) on electron transfer by the NQR was also studied.
Methods: To determine the electron transfer activity of NQR *in vitro*, the reduction of quinone was followed by UV-VIS spectroscopy using purified NQR [2]. The Na^+ transport was monitored with NQR reconstituted into liposomes using the voltage-sensitive dye oxonol VI, supported by fluorophore-dependent Na^+ transport measurements [2]. The amount of sodium bound to the NQR in the reduced or oxidized state was determined by atomic absorption spectroscopy using enzyme purified by gel filtration in Na^+-free buffers.
Results: Electron transfer was stimulated by Rb^+, but this cation was not translocated. Rb^+ did not diminish Na^+-dependent voltage formation of NQR in proteoliposomes. We could show that Na^+ -translocation activities of NQR are most pronounced around pH 7.5 and 8.0. Occlusion experiments show that Na^+ binds in a substoichiometric manner to the reduced or the oxidized NQR.
Conclusion: Although Rb^+ does not act as a coupling cation for NQR, it does not inhibit the Na^+-stimulated electron transfer activity of the NQR. Voltage generation by NQR is more efficient in slightly acidic pH. We found no evidence for the occlusion of Na^+ within the purified NQR which binds Na^+ in a clearly substoichiometric manner.

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OTP04

Analyzing the RNA-seq-based transcriptome of the acetogenic bacterium *Clostridium aceticum*

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Question: *Clostridium aceticum* was the first isolated autotrophic acetogen (1936). It is able to use gases such as syngas and H_2+CO_2 via the Wood-Ljungdahl pathway and forms acetate. Therefore, *C. aceticum* is an interesting biocatalyst for production of biofuels and biochemicals. Like *C. ljungdahlii* and *Acetobacterium woodii* it uses an Rnf complex to pump cations for energy conservation. Unlike those two organisms, *C. aceticum* also contains a cytochrome. The role of cytochromes during autotrophic growth is unclear. Therefore, a transcriptome study was performed using autotrophic and heterotrophic conditions to analyse the differentially expressed genes (DEGs).
Methods: After sequencing the whole genome of *C. aceticum*, the respective transcriptome was investigated. RNA-seq-based global transcriptome analysis was performed and compared for autotrophic growth on H_2+CO_2 and heterotrophic growth on fructose. Cells for RNA preparation and cDNA synthesis were harvested at mid exponential phase under both conditions. Sequencing was performed using the Illumina MiSeq system and the identification of DEGs was achieved by the baySeq algorithm.
Results: In total, 847 genes were differentially expressed under the two conditions applying restrictive evaluation parameters. Comparing autotrophic with heterotrophic growth, 228 genes were upregulated and 619 genes were downregulated. Interestingly, a *hem* cluster coding for cytochrome synthesis was also upregulated. These DEGs were classified into 20 functional categories of COGs.
Conclusion: Comparing autotrophic and heterotrophic growth of *C. aceticum*, significant changes in gene expression levels were observed. The transcriptome data shows an upregulation of a *hem* cluster which indicates its involvement in autotrophic growth. In sum, the analysis of DEGs involved in the utilization of different carbon sources allows the identification of relevant genes to target for metabolic engineering.

OTP05

SMC condensin entraps chromosomal DNA by an ATP hydrolysis dependent loading mechanism in *Bacillus subtilis*

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Every living organism relies on cell divisions for survival. In this essential process the complete genetic information has to be precisely replicated and segregated to opposite cell halves before cell division. To ensure faithful segregation of chromosomes, the genome of a cell has to be highly condensed and well-organized in the form of chromosomes. Amongst the various types of proteins implicated in structuring the chromosome, the SMC family of proteins, which is highly conserved in all branches of life, is known to play an important role in many organisms. In eukaryotes, several SMC complexes are present within a cell such as cohesin, condensin and the Smc5/6 complex, in prokaryotes, however, usually one

of two types, Smc-ScpAB or MukBEF, is present. In *Bacillus subtilis*, the deletion of the Smc protein is detrimental and leads to severe defects in chromosome segregation. Presumably, Smc-ScpAB mediates the compaction and resolution of sister DNA molecules during cell division, however, the molecular mechanism for this action is only poorly understood. We have developed a novel biochemical assay to determine whether the binding of Smc-ScpAB to native chromosomes in *B. subtilis* involves entrapment of DNA by the Smc-ScpA ring. The assay is based on chemical cross-linking, with which the three interfaces of the Smc-ScpA ring can be linked together covalently in living cells. During the isolation of whole intact chromosomes under protein denaturing conditions, exclusively the cross-linked ring species of Smc-ScpA remains associated with the chromosomes. The chromosome entrapment by Smc-ScpAB is abolished by mutations interfering with the Smc ATPase cycle of the complex and the ParB protein. Taken together, we show that the prokaryotic Smc-ScpAB complex in *B. subtilis* entraps the bacterial chromosome, in an ATP-hydrolysis dependent manner. The entrapment of chromosomal DNA by SMC-complexes is an evolutionary conserved feature of different SMC complexes. Our entrapment assay presents a new method to determine the physical association of ring-shaped protein complexes with whole bacterial chromosomes.

OTP06

Interplay between SMC head dimerization and coiled-coil conformation in Smc-ScpAB

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Smc-ScpAB is the most widely distributed SMC complex in prokaryotes, and promotes proper chromosome segregation in many species. Its structural core is formed by a homodimer of Smc that is asymmetrically bridged by the kleisin ScpA, a configuration which has probably served as the evolutionary blueprint for all eukaryotic SMC-kleisin complexes.
Smc-ScpAB adopts a rod-like shape, in which the ~40 nm long intramolecular coiled-coil domains of the Smc dimer are closely annealed. The rod contains the constitutively dimeric Smc hinge domains at one end, whereas the other end terminates in the Smc head domains, whose dimerization is regulated by ATP binding. Here we monitor Smc head-domain dimerization and coiled-coild alignment *in vivo* using site-specific cross-linking, and propose that ATP-induced head engagement drives coiled-coil disengagement. The resulting conformational change might open up the complex for the entrapment of DNA fibres, and ultimately allow Smc-ScpAB to organize the bacterial chromosome.

OTP07

Structure and function of phycobiliprotein lyases of *Guillardia theta*

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Cyanobacteria, red algae, glaucophytes and cryptophytes employ phycobiliproteins (PBP) for light-harvesting. Therefore, the apo-proteins carry covalently attached open-chain tetrapyrrole chromophores called phycobilins. These pigments are connected with the apo-protein via thioether bonds to conserved cysteine residues. In contrast to the majority of organisms in which the individual PBPs are organized in larger aggregates, known as phycobilisomes, cryptophytes employ a single type of PBP which is localized in the thylakoid lumen (1). The cryptophyte *Guillardia theta* utilizes the PBP phycoerythrin PE545 with 15,16-dihydrobiliverdin (DHBV) in addition to phycoerythrobilin (PEB) as chromophore.
Thus far, the attachment of bilins to cryptophyte apo-PBPs is not yet completely understood. Here we present the structural and functional characterization of eukaryotic S-type PBP lyase *Gt*CPES from *G. theta*. *Gt*CPES is specific for binding phycobilins containing reduced C15-C16 double bonds (DHBV, PEB).
The X-ray structure of *Gt*CPES revealed a 10 stranded β-barrel with a lipocalin fold (1). PEB is the substrate of *Gt*CPES which mediates the specific attachment of PEB to Cys82 of the PBP β-subunit (CpeB) (2). Transfer can be monitored due to fluorescent product formation (CpeB:PEB). With aid of site-directed mutagenesis the binding pocket was investigated and amino acid residues involved in phycobilin binding were identified. Based on our data, a model for the assembly of PBPs will be presented.

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OTP08

Metabolic channeling during phycoerythrobilin biosynthesis in cyanobacteria

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Phycoerythrobilin (PEB) is a linear tetrapyrrole molecule found in cyanobacteria, red algae and cryptomonads. It serves as a light-harvesting pigment in the phycobiliproteins of these organisms. The biosynthesis of PEB requires two subsequent reduction steps which are catalyzed by ferredoxin-dependent bilin reductases (FDBR). The first step of PEB biosynthesis starts with a specific reduction of biliverdin IXα (BV) by dihydrobiliverdin:ferredoxin oxidoreductase (PebA) to 15,16-dihydrobiliverdin (15,16-DHBV) which then serves as a substrate for the second reduction catalyzed by phycoerythrobilin:ferredoxin oxidoreductase (PebB) (1). During PEB biosynthesis, the intermediate 15,16-DHBV is suggested to be transferred in a transient interaction from PebA to PebB via metabolic channeling. The knowledge of the new FDBR member phycoerythrobilin synthase (PebS) revealed a direct reduction from BV to PEB (2). Originated in cyanophages, PebS shows a high structural homology to PebA but is more efficient than the dual enzyme system (PebA & PebB) of cyanobacteria (2,4). Here we describe the construction of a translational fusion between *pebA* and *pebB* and its heterologoues expression in *E. coli*. We were able to show a functional fusion-enzyme with a PebS-like activity which converts BV to PEB. This fusion will be used for crystallization studies for a better understanding of the proposed protein-protein interaction. In a second approach, the transient interaction of PebA and PebB will be trapped employing different types of cross-linking reagents in combination with the SPINE method for a rapid analysis of protein interactions during *in vivo* experiments (5).

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OTP09

Visceral leishmaniasis and chronic granulomatous disease in an infant

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Objective: to report a case of an infant with disseminated leishmania Donovani (LD) and Chronic Granulomatous Disease (CGD).
Method: A report of the clinical and laboratory data on a six months old infant who presented with multiple skin lesions and fever since second week of life.
Result: The infant had indolent fever since the second week of life associated with skin lesions on the face, neck and the limbs. He came from an area where both visceral and cutaneous leishmaniasis is endemic. Skin biopsy and the bone marrow aspirate showed leishmania Donovani bodies and the culture revealed Staphylococcus aurous and serratia marssisinse on two different occasions. His immune work up confirmed CGD and living related bone marrow transplantation was successful but complicated with cerebro vascular accident.
Conclusion: Although few case reports had been reported regarding this subject but up to my knowledge this is the first case to be reported in infant with CGD and disseminated VL. As the prognosis of CGD is poor, with high morbidity and mortality and infantile leishmaniasis also adds on high rate of morbidity and mortality if not treated early. Establishing an early diagnosis has important practical implications in the successful treatment of these patients. The description of this case and a brief review

of the current literature are provided to familiarize physicians mainly in the endemic areas with the relatively rare presentations of these tow conditions together.

OTP10

Characterization of *Escherichia coli* K12 mutants hypersensitive to photodynamic inactivation with Chlorin e6

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Question: Photodynamic inactivation (PDI) using the photosensitizer Chlorin e6 is a potential alternative to antibiotic therapy. *Escherichia coli* K12 is relatively insensitive to such a treatment and it has been suggested that the killing is due to surface binding of photosensitizer rather than uptake by the cells. This study aimed to investigate this question by isolating hypersensitive mutants.
Methods: A genome-wide screening system for PDI-sensitivity was developed using 96 well microtiter plates and high power LEDs (wavelength 670 nm, power density 31 mW/cm^2). Cells from liquid cultures in mid-log phase were incubated in PBS buffer (pH 6.7) containing 128 µM Chlorin e6 for two hours at 37 °C. The bacterial suspensions were diluted 600-fold with PBS and irradiated for two minutes. The survival rate was determined by plating on LB agar.
Results: Screening of the 3985 single-gene knock-out mutants of the Keio collection isolated 16 hypersensitive mutants with a survival rate under 30 %. The genes affected included *tolC* (encodes an outer membrane channel protein) and the *mdtABC* genes (encode an inner membrane export system, which interacts with TolC). Three genes encoding enzymes of lipopolysaccharide synthesis also yielded hypersensitive knock-out mutants. The screening also showed a role for the genes *recA* and *rdgC*, which are involved in the SOS response. Catalase and superoxide dismutase mutants did not have a hypersensitive phenotype.
Conclusion: The hypersensitive phenotypes of the transport system mutants suggest that Chlorin e6 enters the periplasm and cytoplasm and the low sensitivity of *E. coli* K12 is due to the efficient export of the photosensitizer. The deletion of genes involved in the lipopolysaccharide synthesis shows that a modified outer membrane structure has also an influence on the sensitivity. The sensitivity of *recA* and *rdgC* mutants suggests that DNA damage is responsible for part of the killing by photodynamic inactivation.

OTP11

New RNA thermometers from *Yersinia pseudotuberculosis*

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The three dimensional architecture plays a critical role in the function of protein coding and non-coding RNAs. Dynamic changes in regulatory RNAs structure enable a rapid modulation of gene expression as demonstrated for RNA thermometers (RNATs) or small RNAs (sRNAs).
RNA thermometers (RNATs) are regulatory RNA-elements that respond rapidly to shifting temperature. These are mainly localized in the 5' untranslated region of temperature controlled mRNAs and regulate gene expression at translational level. At low temperatures, the mRNA forms a secondary structure that occludes the Shine-Dalgarno sequence and inhibits ribosome binding. Increasing temperature leads to a zipper-like opening of the structured mRNA and enables translation [1].
The foodborne pathogen *Yersinia pseudotuberculosis*, causing a variety of gut associated diseases, rapidly adjusts its lifestyle and pathogenesis upon entry from external reservoirs into the warm-blooded host (37 °C) [2]. It has been shown that a RNA thermometer controls translation of lcrF, coding for the transcriptional activator of virulence factors in Y. *pseudotuberculosis* [3].
The goal of this study is the identification of new RNAT from *Y. pseudotuberculosis*. The RNAT candidates were chosen from a dataset of a global structure probing approach of the whole *Y. pseudotuberculosis* transcriptome at different temperatures. Further characterization included reporter gene studies, western blot analysis and site-directed mutagenesis.
8 out of 18 tested candidates showed temperature-dependent expression during reporter gene studies and western blot analysis. The potential RNATs control expression of genes that influence virulence processes like defense against oxidative stress or a host-adapted metabolism. Additionally, one RNAT candidate probably regulates the expression of a major virulence factor. These findings suggest that a variety of cellular processes is translationally regulated by RNAT in *Y. pseudotuberculosis* during infection.

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OTP12

The influence of high temperature and water stress on *in vitro* growth in isolates of the nematode-trapping fungus *Arthrobotrys*

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Nematodes one of abundant and successful organisms which are located in the very nest of ecological. They are parasites of plants and animals on earth. Nematode effects on humans are by reducing of crops, direct infection of humans and incapable of domestic animals. Nematophagous fungi have been seen as potential biological control agents against nematodes for a long time. Among the nematophagous fungi, the nematode-trapping fungi are the most studied. Nematode-trapping fungi are ubiquitous in soil environments. These fungi capture nematodes by three-dimensional networks, adhesive columnar branches, adhesive knobs, constricting rings, and non-constricting rings. The genus *Arthrobotrys* belongs to the group of nematode-trappings fungi. These fungi are relatively easy to culture on artificial media and numerous isolates are currently maintained in various laboratories worldwide.
The objectives of this study were to determine the influence of high temperature, low water availability, and their combination on *in vitro* growth of strains of nematode-trapping fungus *Arthrobotrys*. All isolates (CEA-1, CEA-2, CEA-3, CEA-4 and CEA-5) were obtained from alfalfa fields in Isparta province (Turkey). Growth assays were done at 25, 30, 35, and 40 °C and in media (potato dextrose broth) with water stress created by 10, 20, 30, and 40 % polyethylene glycol (PEG 6000).
There were significant interspecies variations in growth rates on media modified with PEG 6000. There is no growth at 35 and 40 °C *Arthrobotrys* isolates, only isolate CEA-5 growth at 35 °C and low concentration (10 and 20 %) of PEG 600.
These findings will aid the selection of isolates nematode-trapping fungus *Arthrobotrys* for use in field trials in hot or dry agricultural climates.
The work was supported by the Ministry of Food, Agriculture and Livestock, the General Directorate of Agricultural Research and Policies (TAGEM), Project No. TAGEM/ 14 / AR-GE / 07

OTP13

Advantages and disadvantages of 16S-rRNA gene and ITS/5,8S-rRNA gene region sequencing for Identification of unknown cultures

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Question: A fast, reliable and cost effective method to Identify unknown microorganisms is very important in many areas of research and also for commercial organisations. Since more than 20 years a PCR based method is established to differentiate bacterial cultures: 16S/ITS-rRNA gene sequencing.
Is 16S/ITS-rRNA gene analysis sufficient to identify unknown cultures?
Method: The 16S/ITS-rRNA gene is located on the ribosomal DNA which allows, due to small genome size, the reproducible amplification of genes starting from very low amounts of template DNA. Since the 16S/ITS-rRNA genes are flanked by highly conserved regions, universal PCR primer sets can be used for broad range amplification.
The first step is the extraction of the genomic DNA. Afterwards the target region is amplified by PCR: 16S-rRNA gene for bacterial or ITS/5,8S-rRNA gene region for fungi and yeasts, followed by a gel electrophoresis as quality check and purification to clean out the PCR primers and nucleotides prior sequencing reaction. In case of pure cultures Sanger sequencing of amplicons is performed and the sequences were compared against internal and external databases to identify the sample by homology. Most databases are based on NCBI (National Center for Biotechnology Information) entries. If a mixed-culture is object of inquiry the partial sequencing of 16S/ITS rRNA gene by Next Generation Sequencing is recommend, e.g. MiSeq.
Results: 16S/ITS-rRNA gene sequencing can characterize a strain **rapid** (within 24 hours). It can be done in 96 well plates which reduces cost

significant: This method represents a **cost-effective high throughput analysis**. In contrast to classical taxonomic methods like morphological analysis the molecular biological based 16S/ITS-rRNA gene sequencing method can also **differentiate non-cultivable isolates** and **pathogen strains** without risks for lab staff. The database is often a limiting factor since NCBI is a huge and actual database, but not in all cases all entries are validated.
For an exact identification of strains a significant difference in genotype is necessary. If the genotype of 16S/ITS-rRNA gene shows no significant differences the strains were summarized in **taxonomic groups**.
Also a qualitative and semi-quantitative analysis of mixed cultures can be done which allows details analysis of biodiversity studies of microbioms.
Conclusion: Since the 16S/ITS-rRNA gene does not show significant differences in genotype for all species this method cannot be used as stand-alone method for differentiation of **subspecies**. Additional methods were required (as like MALDI-TOF, API, RAPD-PCR and MLST) to differentiate the isolates exactly. The best method differs by genus: The optimal combination of these methods is currently the main focus in the development activities of selekt-ID BIOLABS GmbH.
However, for routine diagnostics the 16S/ITS-rRNA gene sequencing is a very attractive method since it can identify/analyse an unknown culture up to species level rapid and cost-effective.

OTP14

The two-component regulatory system CiaRH of *Streptococcus pneumoniae* R6 is subject to feed-back regulation by several members of its regulon.

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The two-component regulatory system CiaRH of *Streptococcus pneumoniae* is a pleiotropic control device influencing processes such as competence, maintenance of cell integrity, ß-lactam resistance, bacteriocin production, host colonization, and virulence. The response regulator CiaR controls directly 15 promoters and thereby 25 genes, 19 of which code for proteins, while five specify highly similar small non-coding base-pairing RNAs, designated csRNAs (cia-dependentsmall RNAs) (1). The csRNAs control competence development by interfering with quorum sensing (2, 3). The CiaRH system is active under a variety of conditions and a signal for its activation is still missing. In experiments to explore conditions altering the activity of CiaRH, evidence for feed-back regulation by at least one regulon member was obtained. Therefore, we tested systematically, if members of the CiaR regulon could influence CiaR activity.
Results: Genes controlled by CiaR that could be inactivated were disrupted by resistance cassettes and the activity of promoters known to be strongly activated by CiaR were measured by means of ß-galactosidase reporter fusions. These experiments indicated that CiaR-dependent promoter activities raised upon inactivation of *htrA*, encoding a cell surface exposed protease, of the five csRNA genes, and of *manLMN*, encoding the major glucose phosphotransferase system of *S. pneumoniae.* Inactivation of *htrA* had the strongest effect on promoter activities, while deletion of the csRNA genes or *manLMN* were less effective. In a strain without HtrA and the csRNAs, CiaR-dependent promoters were most active. More than one csRNA gene must be inactivated to achieve full promoter activation, but which csRNA combination is most effective remains to be determined.
Conclusions: The two-component regulatory system CiaRH of *S. pneumoniae* is subject to extensive feed-back control by several members of the CiaR regulon. The extent of this negative regulation suggests that the system has evolved to counteract strong activation by negative feed-back mechanisms to maintain a rather constant expression level.

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OTP15

How are polyphosphate biosynthesis and PHB biosynthesis related in *Ralstonia eutropha*?

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A physiological connection between polyhydroxybutyrate (PHB) metabolism and the formation of polyphosphate (polyp) granules is known in form of the "enhanced biological phosphate removal process" (EBPR) and is used to remove phosphate from waste-water. The coupling of PHB synthesis in the first phase with the accumulation of polyP in the second phase on the expense of accumulated PHB during EBPR is indicative for a metabolic link between synthesis of PHB and synthesis of polyP. Unfortunately, EBPR bacteria such as *candidatus Accumulibacter phosphatis* cannot be cultivated in pure cultures. Here, the question of a potential link between PHB and polyP metabolism in *Ralstonia eutropha* H16, an easy to cultivate β-proteobacterium with documented ability to synthesize PHB and polyP and with taxonomical relationship to *Candidatus Accumulibacter phosphatis* was addressed., *R. eutropha* was used to simulate the EBPR process as a model organism and the adaptation to EBPR conditions will be screened on genomic, transcriptomic, proteomic and metabolomic levels in order to find the key players of the EBPR process. First data will be presented.

OTP16

Regulation by five related small non-coding RNAs in *Streptococcus pneumoniae*

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The two-component regulatory system CiaRH of *Streptococcus pneumoniae* is implicated in ß-lactam resistance, maintenance of cell integrity, bacteriocin production, host colonization, virulence, and competence. The response regulator CiaR controls directly 25 genes, five of which specify highly similar small non-coding RNAs, designated csRNAs (cia-dependentsmall RNAs). The csRNAs were predicted to act as regulatory base-pairing sRNAs. Therefore, we tested their implication in CiaR-dependent phenotypes.

Results: Inactivation of the csRNA genes showed that at least some of the CiaRH-related phenotypes, enhanced ß-lactam resistance, autolysis, and prevention of genetic competence, are dependent on the csRNAs. Computational target predictions and micro-array experiments followed by evaluations identified eight genes to be under negative csRNA control. Six of them encoded transport proteins, while the remaining two are involved in regulatory processes. Measuring the effect of single csRNAs on three of these targets indicated that individual csRNAs were not as effective in downregulation as all csRNAs together. Thus, csRNAs acted additively, at least for these targets (1).
Remarkably, one of the csRNA-controlled genes is *comC* encoding the precursor of the competence stimulating pheromone CSP. Production of CSP is absolutely essential for the quorum sensing process leading to competence development. Suppression of its synthesis by the csRNAs will therefore interfere with competence induction. In the presence of five csRNAs, competence did not develop, but several combinations of three csRNAs were also effective. Partially disrupting predicted csRNA-*comC* complementarity by mutating *comC* (*comC8*) led to strongly diminished repression of a *comC8'-'lacZ* translational fusion by the csRNAs and to transformability in a *comC8* strain with all csRNAs. Reconstitution of csRNA complementarity to *comC8* restored competence suppression. Again, more than one csRNA was needed. In this case, even two mutated csRNAs complementary to *comC8*, csRNA1-8 and csRNA2-8, were suppressive (2).
Conclusions: The csRNAs greatly expand the regulatory potential of CiaRH by targeting a number of genes. One of the targets, *comC*, is additively controlled by the csRNAs resulting in downregulation of genetic competence.

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OTP17

Expansion of the substrate range of the gentisate 1,2-dioxygenase from *Corynebacterium glutamicum* ATCC 13032 for the conversion of (substituted) salicylate(s)

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Question: The "conventional" gentisate-1,2-dioxygenase (GDO) from *Corynebacterium glutamicum* ATCC 13032 converts gentisate (2,5-dihydroxybenzoate) but no monohydroxylated benzoates. In contrast, the GDO from the α-proteobacterium *Pseudaminobacter salicylatoxidans* BN12 oxidatively cleaves in addition to gentisate also salicylate, various amino-, chloro-, fluoro-, hydroxy-, and methylsalicylates, and 1-hydroxy-2-naphthoate [1,2]. Therefore, it was attempted to modify the GDO from *C. glutamicum* in order to enable the conversion of (substituted) salicylate(s).
Methods: Multiple sequence alignments in combination with structural analyses were performed in order to identify specific amino acid residues which distinguish the GDO from *P. salicylatoxidans* from "conventional" GDOs. Accordingly, the gene encoding the GDO from *C. glutamicum* was mutated at different positions and the mutated genes expressed in *E. coli* cells. A colony screening system was established which allowed to detect the salicylate cleavage product after derivatisation with 2,4-dinitrophenylhydrazine by the formation of a deep red color. Cell extracts were prepared from positive clones and further analyzed by UV/Vis-spectroscopy and HPLC.
Results: The GDO from *C. glutamicum* was modified by saturation mutagenesis at three amino acid positions. The obtained clones were screened for the conversion of salicylate by the newly established color assay. It became evident that only the exchange at position Ala112 gave rise to salicylate converting clones. The variants were tested by enzyme assays for the conversion of salicylate and clustered into 4 groups. The genes coding for the salicylate converting enzyme variants were sequenced and found to correspond to the amino acid exchanges Ala112Gly, Ala112Ser, Ala112Ile and Ala112Asp. The Ala112Gly variant was further studied and shown to oxidatively cleave salicylate and 1-hydroxy-2-naphthoate, also various substituted salicylates.
Conclusion: Our results demonstrate that simple point mutations are sufficient to allow "conventional" gentisate 1,2-dioxygenases to convert monohydroxylated aromatics.

[1] Hintner *et al.*, J. Biol.Chem. 279 (2004), p.37250
[2] Eppinger *et al.*, FEMS Microbiol. Lett. (2015)

OTP18

Contribution of lateral gene transfers from bacteria to the genome evolution of the nematode *Deladenus siricidicola*

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Question: The asexual movement of genetic material between different species, collectively termed Lateral Gene Transfer (or LGT), has long been known to play a significant role in the genome evolution of prokaryotes. Whether LGT occurs at appreciable levels in eukaryotic organisms is still under debate. In order to aid in answering principle questions regarding LGT in eukaryotes, the genome of *Deladenus siricidicola* (nematode) was subjected to a study to identify LGTs from bacterial origin, as well as the functions associated with these genes. We also consider the possible mechanisms of transfer from this data.
Methods: The utility and accuracy of SeqWord Genomic Island Sniffer, SigHunt and AlienHunter as genomic island (GI) prediction tools were compared. All three these tools exploit the compositional bias of lateral transferred sequences to infer GIs. The combined results of these tools were used to annotate GIs using InterProScan, Blast2GO and other eukaryotic annotation tools. Compositional and sequence similarity comparisons were done against the Pre_GI database (http://pregi.bi.up.ac.za/index.php), which contains known bacterial GIs. This was done to determine whether bacterial functions are similar to identified GIs and also to assign possible donor species. Phylogenetic validation of a subset of genes was performed using MEGA6.
Results: The study revealed that approximately 2 % of the genome showed variance in oligonucleotide usage pattern and were most likely acquired from foreign origins. These regions were associated with a diverse range of functions, but were found to be mostly involved in transmembrane transport, metabolism and immunity. Genes commonly associated with GIs, such as integrases, ABC-transporters and transposable elements were also identified. Searches against the Pre_GI database showed that the majority of foreign genes were closely related to members of the *Bacillus*, *Staphylococcus* and *Clostridium* genera. Phylogenetic analyses clustered many of the identified genes with bacteria instead of other eukaryotes, and confirmed them as LGT events.
Conclusions: A significant proportion of the *D. siricidicola* genome appears to consist of bacterial genetic material. The study lays the foundation to explore the potentially significant influence that these genes have had on the evolution of the symbioses of this nematode with insects and fungi, as well as mechanisms of LGT in eukaryotes. The unique pipeline used here can increase the rate of GI identification in other eukaryotic species.

Slippers, B., B. P. Hurley and M. J. Wingfield, 2015 *Sirex* Woodwasp: A Model for Evolving Management Paradigms of Invasive Forest Pests. Annual Review of Entomology 60: 601-619.

OTP19

Adaptation of *S. aureus* Δ*mvaS* to mevalonate depletion

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Isoprenoids are a very large and diverse group of organic compounds with a wide range of biochemical functions [1]. They take part in the synthesis of the cell wall as the lipid carrier undecaprenol, in the transport of electrons as ubiquinones, in protein biosynthesis as prenylated tRNAs, and many more. The universal precursor for all isoprenoids is isopentenyl pyrophosphate (IPP), which is synthesized in *Staphylococcus aureus* exclusively by the mevalonate (MVA) pathway. The synthesis of IPP starts with the acetylation of acetoacetyl-CoA to Hydroxy-3-methylglutaryl-CoA (HMG-CoA) catalyzed by the HMG-CoA synthase (MvaS) [1]. HMG-CoA is then converted via mevalonate to IPP, which is essential for all living organisms. An *mvaS* deletion mutant in *S. aureus* grows normal in the presence of mevalonate but not in its absence. However, when the mutant was cultivated in the absence of mevalonate for several days it was able to adapt and started to grow. CFU values dropped drastically during the first 24 h but afterwards the culture recovers and the CFU increased again [2]. Once adapted the *mvaS* mutant (Δ*mvaS*-ad) is permanently able to grow without mevalonate.
The main task of this project is to find out how it was possible for the mutant to grow without *mvaS* and to elucidate the underlying mechanism. The phenotype of Δ*mvaS*-ad shows a severe growth defect, which is mainly caused by the missing menaquinone in the respiratory chain. The cell and colony morphology are instead similar to that of the wild type and the cell wall surprisingly shows no differences in the peptidoglycan structure as well as the amount of wall teichoic acids, which suggests the presence of undecaprenol as a lipid carrier for the precursors. However, the MIC of bacitracin, which is tremendously higher for Δ*mvaS*-ad than for the wild type, suggests otherwise. So far it is known that Δ*mvaS*-ad is able to translocate the PGN and WTA precursors across the membrane but not if undecaprenol is involved in this process.
To elucidate the underlying mechanism of the adaptation the genomes of the wildtype, the not adapted mutant and Δ*mvaS*-ad were sequenced. Two interesting SNPs, which were only present in Δ*mvaS*-ad could be found. One SNP is located in the promoter region of *drp35*, which encodes for a lactonase with yet unknown function. It could be shown that the mutation leads to an upregulation of *drp35* and that it is involved but not alone responsible for the adaptation. The role of the other SNP has to be elucidated. So the mechanism of the adaptation is not fully understood yet.

OTP20

Occurrence and function of DNA methylation in the cyanobacterium *Synechocystis* sp. PCC 6803

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Introduction: Methylation of the prokaryotic genome is a crucial epigenetic signal for various mechanisms, including DNA repair, recognition of foreign DNA or chromosome portioning. This ubiquitous mechanism of DNA modification is catalysed by DNA-methyltransferases, utilizing S-adenosyl methionine as methyl group donor. Recent studies suggested a regulative role of DNA methylation within gene expression (Lluch-Senar *et al.*, 2013). However, the underlying mechanism of epigenetic control is entirely unknown.
Objectives: The aim of this project is to unravel the casual links between DNA methylation, control of gene expression and physiological effects within the cyanobacterial model strain *Synechocystis* sp. strain PCC 6803.

Methods: Single molecule real-time DNA sequencing (SMRT-Sequencing) was performed to analyse base specific DNA modification in *Synechocystis* wild type. Interposon mutagenesis was used to knockout genes for putative DNA methyltransferases. Subsequently, microarrays were applied to search genome-wide transcriptional changes.
Results: Initial studies showed that the genome of *Synechocystis* harbors genes for three methyltransferases, but no restrictase. The gene *slr1803* codes for MSsp68033II which was shown to methylate the motif 5´-GA^{m}TC-3´. This *dam*-like activity is essential for the viability of *Synechocystis*. The genes *slr0214* (Scharnagl *et al.*, 1998) and *sll0729* code for non-essential, orphan methyltransferases MSsp6803I and MSsp6083III. Analysis of mutants lacking *slr0214* or *sll0729* reveal differences in gene expression and phenotypes compared to wild type. Moreover, SMRT-Sequencing identified an additional DNA modification within the palindromic sequence 5`-GA^{m}AGGC-3´. *In silico* analysis detected three genes that potentially code for the missing methyltransferase. These genes were found to code for non-essential proteins, because completely segregated knockout mutants were identified. Further research will be conducted regarding changed methylation pattern and gene expression.
Conclusion: We identified up to five methyltransferases and started analysing their physiological functions.

M. Lluch-Senar, K. Luon, V. Lloréns-Rico, J. Delgado, G. Fang, K. Spittle, T. A. Clark, E. Schadt, S. W. Turner, J. Korlach, L. Serrano (2013). PLoS Genet 9(1): e1003191.
M. Scharnagl, S. Richter, M. Hagemann (1998). J Bacteriol 180(24):6794.

OTP21

An integrated platform for high-throughput whole genome mlst and whole genome snp analysis

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Objectives: With the increasing availability of next generation sequencing technology, whole genome sequencing (WGS) methods are increasingly being used for bacterial typing. Key challenge is the ability to rapidly extract the relevant information from large sequence data files.
We present two pipelines for high resolution WGS-based molecular typing: whole genome multilocus sequence typing (wgMLST) and whole genome single-nucleotide polymorphism analysis (wgSNP). Both strategies are compared using data from a hospital *Staphylococcus aureus* outbreak.
Methods: Using an in-house developed wgMLST schema that extends the core genome schema from Leopold *et al.* (2014), we apply two independent allele calling approaches, an assembly-free and a BLAST-based allele calling algorithm, to determine locus presence and detect allelic variants in a quality-controlled manner. The wgSNP pipeline, tuned to reduce false positives while maximizing resolution, detects SNP variants by mapping the WGS reads to a reference sequence internal or external to the data set.
For both methods, all calculation-intensive data processing steps are performed on the BioNumerics® Calculation Engine, deployed locally or in the cloud.
Results and Conclusions: In the data set at hand, wgMLST detected two distinct outbreak clusters, clearly separating the outbreak from non-outbreak isolates. Among the used methods (cgMLST, wgMLST and wgSNP) to resolve the largest outbreak cluster, wgSNP proved to offer the highest resolution.
wgMLST holds the advantage that the data is highly portable and enables to make functional predictions as the loci are based on annotated genomic features. In contrast to cgMLST, wgMLST is not limited to a core subset of alleles but represents the whole available pan genome of the organism.
The BioNumerics® 7.6 software and its integrated Calculation Engine offer a powerful platform where both wgMLST and wgSNP can be performed to provide a robust, portable and high resolution picture of molecular typing data. The polyphasic approach allows for validation both between WGS analysis techniques and traditional techniques such as MLST or PFGE.

Leopold S.R., Goering R.V., Witten A., Harmsen D., Mellmann A. (2014) Bacterial whole-genome sequencing revisited: portable, scalable, and standardized analysis for typing and detection of virulence and antibiotic resistance genes. *J Clin Microbiol.* 2014 Jul; 52(7):2365-70.

OTP22

The involvement of the anti-sense RNA RSaspufL in regulated formation of photosynthesis complexes in *Rhodobacter sphaeroides*

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The facultative phototrophic model bacterium *Rhodobacter sphaeroides* is known for its metabolic versatility. Under low oxygen conditions it synthesizes intracytoplasmic membranes harboring the pigment protein complexes needed for anoxygenic photosynthesis. For fast adaption to varying environmental conditions the transcription of genes in the photosynthetic gene clusters is tightly regulated. The *puf* operon comprises genes which encode proteins of the light harvesting complex I (LHI) and of the reaction centre (RC). RNAseq and *Northern blot* analysis of transcripts derived from the *puf* operon revealed that also certain small RNAs (sRNAs) are transcribed. Up to date two different *puf* operon *a*ssociated *s*RNAs (RSspufX and RSaspufL) were identified. One abundant putative sRNA, RSspufX, was detected downstream of the *pufX* gene with transcription in the same direction as the *puf* genes (Berghoff *et al.*, 2009). Another, less abundant sRNA RSaspufL was detected antisense to the 5´region of the *pufL* gene, extending into the *pufA-pufL* intercistronic region and possibly even further. The *pufL* sequence is quite conserved among different *Rhodobacter* species (78 % identity between *R. sphaeroides* 2.4.1 and *R. capsulatus* SB1003). Nevertheless *Rhodobacter capsulatus pufL* contains an RNase E cleavage site which is not conserved in *R. sphaeroides*. This RNase E cleavage site is essential for differential *puf* mRNA processing and degradation which contributes to the stoichiometry of LHI and RC complexes in *R. capsulatus*. RNAseq data also indicated that the abundance of RSspufL was strongly dependent on growth conditions. Northern blot results confirmed the presence of RSspufL (about 140 nt nucleotides), under microaerobic and aerobic conditions. The highest abundance could be observed after 7 min of singlet oxygen stress. Under phototrophic conditions strong bands of larger size (up to 400 nt) were visible indicating the presence of precursor molecules. Under singlet oxygen stress and phototrophic conditions also a shorter band (40 nt), most likely stemming from processing is clearly visible. An artificial increase in the amount of the sRNA by plasmid driven over-expression led to a reduced pigmentation and LHC amount in the cells. We hypothesize that the antisense RNA RSaspufL contributes to the regulated processing and degradation of the *puf* mRNA in *R. sphaeroides*. The influence of RSapufL on the *puf* mRNA should be investigated via Northern blot analysis, Real-Time PCR and reporter gene fusion. We will present data on the effect of RSspufL on *pufL* mRNA levels and stability and on the involvement of different RNases in *puf* processing in *R. sphaeroides*.

Berghoff BA, Glaeser J, Sharma CM, Vogel J, Klug G. (2009) Photooxidative stress-induced and abundant small RNAs in Rhodobacter sphaeroides. *Mol Microbiol*. 74(6):1497-512.

OTP23

Translation termination/re-initiation in prokaryotes

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Many genes in archaea and bacteria are organized in operons and are transcribed into polycistronic mRNAs. This enables translational coupling, meaning translation of a downstream gene depends on translation of an upstream gene. One mechanism of translational coupling is based on the sequestration of the Shine-Dalgarno (SD) motif of the downstream gene in a secondary structure and unmasking of the SD motif by a ribosome translating the upstream gene. Another mechanism of translational coupling known from eukaryotic viruses can operate on closely spaced or overlapping genes, which enables ribosomes that terminate translation of an upstream gene to remain on the transcript (at least the small subunit) and reinitiate translation on a downstream gene ("termination-reinitiation"). Archaea and bacteria contain many gene pairs that overlap by 4 nt (A**TG**A) or by 1 nt (T^{G}/$_{A}$**A**TG). For example, the genome of the haloarchaeon *Haloferax volcanii* contains 4128 genes, 886 of which form 443 overlapping gene pairs[1], and similarly, 820 of the 4288 *Escherichia coli* genes form 410 overlapping gene pairs. It was observed that in *H. volcanii* SD motifs are enriched in the upstream genes with an optimal distance to the start codon of the downstream genes, and postulated that they might be involved in termination-reinitiation[1]. To test experimentally whether termination-reinitiation operates in *H. volcanii* nine gene pairs were selected and the upstream genes were fused with the native overlap to the reporter gene *dhfr* (dihydrofolate reductase). In each case variants were generated that contain a stop codon in the upstream gene, inhibiting

ribosomes from reaching the overlap. The levels of the bicistronic transcripts as well as the DHFR levels were quantified. Strikingly, the DHFR levels were zero when ribosomes terminated prematurely, strongly indicating that termination-reinitiation operates in *H. volcanii* and there is no *de novo* initiation at the downstream gene. To investigate whether termination-reinitation operates in *E. coli*, a dual reporter gene system was established. Translational fusions were constructed of 1) the reporter gene *glpD* with the last 33 codons of selected upstream genes, and 2) of the first ten codons of downstream genes with the reporter gene *gusA*. Again, variants with premature stop codons were generated, and transcript levels and reporter enzyme levels were quantified. The GusA levels were zero in the stop codon-containing variants, indicating that termination-reinitiation operates also in *E. coli*. Investigations aiming at analyzing the role of the SD motif in termination-reinitiation are currently under way. Possible evolutionary advantages of termination-reinitation (compared to independent initiation) will be discussed.

1 Kramer *et al.*, (2014) *Haloferax volcanii*, a species that does not use the SD mechanism for translation initiation at 5'-UTRs. PLoS ONE 9,394979.

OTP24

The regulatory influence of $sRNA_{132}$ on potential target mRNAs in *Haloferax volcanii*

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The investigation of small non-coding RNAs (sRNAs) of the haloarchaeal model species *Haloferax volcanii* led to the identification of approximately 250 sRNA genes (1, 2). By generating deletion mutants of about 30 sRNA genes, haloarchaeal sRNAs were found to be involved in many different biological processes (3). While eukaryotic sRNAs typically bind to the 3´-UTRs of their targets, bacterial sRNAs often bind to the 5´-UTRs. In contrast, only very few targets are known for archaeal sRNAs. Therefore, we aimed at identifying target mRNAs of *Haloferax volcanii* sRNAs and to characterize sRNA-/target mRNA interactions.
Putative target mRNAs were identified by comparing the transcriptomes of the wild-type and sRNA deletion mutants using DNA microarrays. For the $sRNA_{132}$ this led to the identification of an operon encoding an ABC-transporter that had phosphate as an annotated target. *In silico* analysis revealed a potential interaction site of 20 nt between $sRNA_{132}$ and the 3'-UTR of the operon mRNA. Northern blot analysis revealed that both $sRNA_{132}$ and the operon mRNA are highly upregulated under phosphate-limiting conditions. In the sRNA deletion mutant the operon mRNA level is lower and the induction after the start of phosphate starvation is slower, revealing 1) that the sRNA is a positive regulator of the operon mRNA, and 2) that a second regulatory mechanism for the expression of the operon exists.
To identify additional potential targets of $sRNA_{132}$, the transcriptomes of wild-type and deletion mutant were compared under phosphate starvation conditions. It was revealed that the operon mRNA had the highest difference also under this condition. In addition, transcripts encoding several kinases, a glycerol-phosphate transporter, and several zinc-finger proteins were identified as further members of the $sRNA_{132}$ regulon.
In *Halobacterium salinarum* a "P box" could be identified as a motif involved in phosphate-dependent transcriptional regulation (4). A potential P box motive was also found upstream of the $sRNA_{132}$ gene and the ABC transporter operon of *H. volcanii*. Fusion of the respective promotor regions to a reporter gene to characterize its function in phosphate-dependent gene regulation is currently under way.

1) Straub, J., Brenneis, M., Jellen-Ritter, A., Heyer, R., Soppa, J. and Marchfelder, A. RNA Biol 6: 281-292 (2009)
2) Babski, J., Tjaden, B., Voss, B., Jellen-Ritter, A., Marchfelder, A., Hess, WR., and Soppa, J. RNA Biol 5, 806-816 (2011)
3) Jaschinski, K., Babski, J., Lehr, M., Burmester, A., Benz, J., Heyer, R., Dörr, M., Marchfelder, A. and Soppa, J PLOS ONE 9, e90763 (2014)
4) Furtwängler, K., Tarasov, V., Wende, A., Schwarz, C. and Oesterhelt, D. Mol Microbiol 76, 378-392 (2010)

OTP25

Game-based learning in a virtual microbiology laboratory

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Game-based learning is gaining increasing attention in different areas of school and university teaching and industrial training. We apply game-based learning to laboratory exercises in microbiology. These are composed as a complex ensemble of interconnected parallel processes. Using a game-based simulation, students should get the opportunity for independent, realistic preparation of the experiments. This asks for special requirements in the graphical user interface of the game-based learning application. It should promote independent learning and render the virtual operations in the game transferable to he real laboratory. To do this the user interface must provide a context-sensitive help, give immediate feedback in the form of praise and censure, and contain an evaluation and reward system. We demonstrate the realization of the user interface of the game-based learning application as a virtual lab including an assistant as avatar. The application has been evaluated quantitatively in a field trial with students in a real laboratory internship. The analysis of the results shows the added value of the application for the participants. It demonstrates in particular the ease of learning and the usability of the interface, as well as an increase of effectively for participants using the game-based application.

Bonde M., Makransky G, Wandall, Larsen M.., Morsing M., Jarmer H, Sommer M. (2014). Improving biotech education through gamified laboratory simulations. Nature Biotechnology 2014 Jul, 32, 694-697.
Bowling, K. G., Klisch, Y., Wang, S., Beier, M. (2013). Examining an Online Microbiology Game as an Effective Tool for Teaching the Scientific Process. J MICROBIOL BIOL EDU 14, 2013.
Gers, F.A., Prowe, S. (2014). Game Based Learning im virtuellen Mikrobiologie-Labor. In Seidl, M., Schmiedl, G. (Hrsg.), Forum Medientechnik – Next Generation, New Ideas. Glückstadt: Verlag Werner Hülsbusch VWH.
Gers, F.A., Prowe, S. (2015). Logik zur Simulation von Laborversuchen für eine Game Based Learning Anwendung. In Gross, M., von Klinski, S. (Hrsg.), Research Day 2015 Stadt der Zukunft. Berlin: Mensch und Buch Verlag.

OTP26

Impact of genetics and parental microbiota on the gut microbiome of offspring

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The gut microbiome has great impact on the physical condition of its host. It is involved in the development of innate and adaptive immunity, defends its host against pathogens and influences the degradation and uptake of nutrients. Therefor the gut microbiome is strongly linked to phenotypic features of the host. Consistently, several studies showed that the transfer of gut microbiota from obese mice to germ-free mice increases total body fat compared to the transfer of a "lean microbiome", which might be explained by the fact that the obese microbiome has an increased capacity to harvest energy from nutrition.
Moreover, several studies suggest a genetic contribution to the composition of the gut microbiome: The gut microbiomes of one family are more similar to each other than to unrelated individuals and more similar within monozygotic twin pairs compared to dizygotic twin pairs (Goodrich 2014).
To further investigate the impact of the genetic make-up and the contribution of the microbiota of the mother on the gut microbiome of the offspring, we selected two mouse lines, that were derived from a long term divergent selection on body fat and weight (Bünger 1999), resulting in one mouse line that is prone to be obese (FLI), while the other is lean (FHI).
To discern the genetic contribution on the gut microbiome from the maternal one a cross fostering experiment was conducted. Half of a litter was exchanged between a FLI and a FHI mother. The other half of the litter was not exchanged and served as a control. The mice were dissected at 3 weeks age, and the DNA was extracted from the colon with content and the cecum with content. Investigation of the microbiome was performed by paired-end next generation sequencing. The sequences were taxonomically affiliated and analyzed at 97 % sequence identity with QIIME using the Greengenes database.
β-diversity analysis shows a distinct influence of the mother as well as clustering of the samples according to genotype. This indicates that the genetics as well as the inherited communities have an impact on the individual's microbiome. The two dominating phyla are, as expected *Firmicutes* and Bacteroidetes. On order level there is a noticeable difference in the abundance of *Clostridiales* and *Bacteroidales*. While *Clostridiales* are dominating in the gut microbiome of the lean FHI mice with 52 % abundance of all bacteria found, *Bacteroidales* take over the majority in the obese FLI mice. The abundances of these two orders in the mice that were cross fostered are lying in between the ones of the controls.
In future data will be analyzed on family and species level, and an experimental set up with more time points, to investigate the further development of the microbiome, will be set up.

Lutz Bünger, William G. Hill; "Inbred lines of mice derived from long-term divergent selection on fat content and body weight" Mammalian Genome 10, 645-648 (1999).

OTP27

The conserved *dcw* gene cluster of *R. sphaeroides* encodes an uncommonly extended 5'UTR featuring sRNA and sORF

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Cell division and cell wall synthesis mechanisms are similar among bacteria. Therefore some bacterial species encode comparable sets of genes organized in the *dcw* (division and cell wall) gene cluster. *dcw* genes, their regulation and their relative order within the cluster are outstandingly conserved among rod shaped and Gram-negative bacteria to ensure an efficient coordination of growth and division. A well described example is the *dcw* gene cluster of *E. coli*. The first promotor of the gene cluster (mraZ1p) gives rise to polycistronic transcripts containing a 38nt long 5'UTR followed by the first gene *mraZ*. Despite reported conservation we can present evidence for a much longer 5'UTR in the Gram-negative and rod shaped bacterium *Rhodobacter sphaeroides* and probably in the whole order of *Rhodobacterales*. This extended 268nt long 5'UTR encodes a small open reading frame (sORF) and features a *rho* independent terminator, which in case of transcription attenuation gives rise to a non-coding sRNA (UpsM). The sRNA contains an endonucleolytic RNase E cleavage site. However cleavage is Hfq-dependent and only occurs conditionally during stress conditions in presence of a target mRNA, which is controlled by the alternative sigma factors RpoHI/II. Phenotypically, overexpression of UpsM and sORF puts *R. sphaeroides* cells into a dormant state under phototrophic growth conditions. These results raise the question for the regulatory function of this extended 5'UTR and its exclusive presence in the *Rhodobacterales* clade.

OTP28

Glycine betaine influences the cytoplasmic volume of *Bacillus subtilis* cells under high-salinity conditions

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Question: The soil bacterium *B. subtilis* uses compatible solutes such as glycine betaine and L-proline to cope with hyperosmotic stress. Besides upregulation of genes involved in the uptake and synthesis of osmoprotectants, *B. subtilis* cells grown under sustained high-salinity conditions show a number of additional gene expression changes, particularly concerning genes involved in peptidoglycan synthesis and remodeling of the peptidoglycan sacculus [1]. During the onset of growth of *B. subtilis* in high-salinity medium (1.2 M NaCl), strong changes in cell morphology occur which imply alterations in the peptidoglycan structure [2]. Other studies reported that an increase in medium osmolarity leads to a decrease of the cell volume [3]. We therefore sought to establish a fluorescence microscopy method in order to determine the cytoplasmic volume of *B. subtilis* cells grown with increasing concentrations of NaCl and accumulating either L-proline or externally provided glycine betaine [4].
Methods: A tryptophan-prototrophic derivative of the *B. subtilis* strain 168 was cultivated to mid-exponential phase in Spizizen's minimal medium containing various concentrations of NaCl (0 to 1.2 M) in the presence or absence of 1 mM glycine betaine. For visualization of cytoplasmic membranes by fluorescence microscopy, membrane staining was performed with the fluorescent dye Nile Red and cells were immobilized on agarose slides.
Results: The average cytoplasmic volume of *B. subtilis* cells decreases with increasing NaCl concentrations in the growth medium, most probably as a result of the lower growth rates. In contrast, in the presence of glycine betaine, we observed a remarkable increase in the cytoplasmic volume with increasing medium salinity: 1.5-fold higher average values were measured in medium with 1.2 M NaCl compared to medium without additional NaCl. The changes caused by glycine betaine are related to both cell length and cell width.
Conclusion: A method for cytoplasmic volume determination applicable to different medium osmolarities was established. The data thus obtained revealed that in the presence of the osmoprotectant glycine betaine, increasing NaCl concentrations lead to a significant volume increase of *B. subtilis* cells suggesting an impact of the intracellular glycine betaine pool on cell volume regulation. In order to shed light on the underlying mechanisms we are currently investigating transcriptomic and proteomic changes in response to glycine betaine under control and high salinity conditions.

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OTP29

Morphogenesis of the stalked budding bacterium *Hyphomonas neptunium*

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The spatial and temporal regulation of peptidoglycan biosynthesis and its role in cell morphology has been studied intensively in well-characterized model organisms such as *Escherichia coli*, *Bacillus subtilis*, and *Caulobacter crescentus*, which divide either by symmetric or asymmetric binary fission. To broaden our knowledge of the mechanisms governing bacterial morphogenesis, we have started to investigate the dimorphic marine alphaproteobacterium *Hyphomonas neptunium* as a new model organism.[1] *H. neptunium* is characterized by a unique mode of proliferation whereby new offspring is generated by the formation of buds at the tip of a stalk that emanates from the mother cell body.
The main focus of our studies was the identification of cell wall biosynthetic enzymes and regulatory factors enzymes that are critically involved in stalk and bud biogenesis. To this end, we performed a comprehensive analysis of the localization patterns of all proteins with cell wall-related functions encoded in the *H. neptunium* genome. In addition, we probed the essentiality of these candidate proteins by deletion analysis and investigated the phenotypes of the resulting strains. These studies revealed that peptidoglycan biosynthesis in *H. neptunium* is a complex process based on an intricate interplay between many different factors. Using a fluorophore-conjugated D-amino acid to visualize nascent peptidoglycan, we found that the stalk is growing from the basis of the stalked pole through insertion of new peptidoglycan. In accordance to that homologs of peptidoglycan synthases and hydrolases localize to the stalked pole in a cell cycle-dependent manner. Those results are the basis for future studies in *H. neptunium* to reveal the regulatory mechanisms of cell differentiation and budding.

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OTP30

Bioleaching of metals and rare earth elements from waste incineration bed ashes using acidophilic bacteria and fungi

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The criticality of raw materials increases due to a growing market for consumer goods and a thereby initiated higher demand for these metals. In consequence, a recycling of process waste and end of life products will be in future an essential step in order to meet the demand. Recycling of incineration slags/bed ashes could be one step to meet the demands. Whereas the recycling of the bigger particle size fractions of the ashes were already performed the small particle size fractions below 4 mm are not recycled. One solution for these fractions could be bioleaching. Therefore small particle size fractions of dry discharged ashes were bioleached with cultures of *Acidithiobacillus thiooxidans*, *Acidithiobacillus ferrooxidans,* a co-culture of both *Acidithiobacillus* species, *Leptospirillum ferrooxidans* and *Aspergillus niger*. Fractions of the size 0 to 2 mm, 2 to 4 mm and these fractions after high energy grinding were used in bioleaching approaches performed in batch flask experiments with and without bacteria. Extracted metals and in some experiments also non extracted residues were analyzed by ICP-OES directly and after aqua regia microwave dissolution, respectively. For Al, Cr, Cu, Ni and Pb significant more metal content was extracted in the experiments with bacteria compared to the abiotic controls. The same was true for the rare earth elements Ce, La and Er. Extraction rates for Al, Cu, Mn, Cr and Er with bacteria were between 70 to 100 % and for Ce, Ni and La around 50 %. A smaller particle size seems to diminish the differences

between biotic and abiotic treatments. No differences of metal extraction rates have been found between the treatment with *Aspergillus niger* and the abiotic treatment.

OTP31

Characterization of DivIVA-ParB complexes dynamics in *Corynebacterium glutamicum*

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Question: The partitioning protein ParB is known to organize the origin region of the bacterial chromosome. In *Corynebacterium glutamicum* ParB interacts with the polar localized protein DivIVA, thereby tethering the chromosome to the cell pole. Characterization of the ParB-DiviVA interaction, of their complex formation and their dynamics are of fundamental importance to understand the mechanisms underlying chromosome segregation in *Corynebacterium glutamicum*.
Methods: Dynamic protein localization, complex formation and protein interactions are analysed in strains encoding fluorescent fusion proteins at their native loci. Protein dynamics are visualized by means offluorescencerecoveryafterphoto bleaching (FRAP) and subsequent statistical analysis using custom made R scripts. More detailed insights into cluster formation and subcellular structures are gained by usingphotoactivatedlocalizationmicroscopy (PALM).
Results: Complex formation of DivIVA-mCherry at the cell poles is influenced by the presence of ParB in a concentration dependent manner. Compared to DivIVA dynamics in absence of ParB, we observed a decreased half time recovery in presence of increasing ParB levels. ParB and DivIVA complexes form distinct nanostructures at the poles and PALM microscopy with 20 nm spatial resolution allows formulating a model for ParB and DivIVA organization within *C. glutamicum* cells.
Conclusion: Increased dynamics on the cytoplasmic side of DivIVA complexes, due to the increased concentration of an interaction protein (ParB), enhance the cluster subunit exchange rate. While the biological meaning of such a behaviour is still unclear, it can be speculated that increased ParB levels naturally occur in WT cells when extra replication cycle are already undergoing; this usually occur during cell division, a cell cycle phase where fast re-localisation of DivIVA at the new forming septum occur. Analysis of the DivIVA complexes during different cell cycle phases by means of PALM will bring new insight on the matter and increase the knowledge on both ParB and DivIVA clusters development.

OTP32

Microbial adhesion on nanorough titanium – insight into the nanostructure of the microbe-material-interface

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Background: Implant-associated infections are primarily initiated by the adhesion of microorganisms on the implants` surfaces. Conventional approaches for preventing these infections most often comprise the use of antibiotics for coating or incorporation into the biomaterials, e.g. titanium prostheses. In the light of increasing numbers of antibiotic-resistant microorganisms new strategies are needed. Recently, materials with surface roughnesses in the nanometer range gained interest to reduce bacterial adhesion as well. Understanding the fundamental physical mechanisms of the microbial interaction with nanorough material surfaces is the first step for developing new strategies to prevent implant-associated infections.
Aim: The aim of this study was to explore the unknown structure of the microbe-titanium-interface to shed light on microbial adhesion on this material. To gain related understanding of the physical mechanism of microbial adhesion as a function of nanoroughness, we linked it to the interfacial structure.
Methods: Adhesion of *Escherichia coli* and *Staphylococcus aureus* was investigated between 1 h and 11 h using physical vapor deposited titanium thin films as 2D model surfaces with nanoroughnesses (Rq) of 2.0 nm, 2.3 nm, 3.0 nm and 6.1 nm. Titanium surface topography was characterized with atomic force microscopy. Titanium surface coverage with the microbes was analyzed using confocal laser scanning microscopy. Bacterial cells adherent to the nanorough titanium thin films were cross-sectioned after 3 h and 9 h, respectively, using a focused ion beam. The microbe-material-interface was imaged with high resolution scanning electron microscopy (SEM).
Results: Surface coverages of the nanorough titanium thin films with *E. coli* and *S. aureus* were statistically significantly ($p \leq 0.05$) reduced by 40.5 % and 55 %, respectively, after 11 h of adhesion on the roughest surface (6.1 nm) compared to the smoothest (2.0 nm). We found evidence that with decreasing titanium surface peak density and decreasing specific titanium surface area the surface coverage with microbes was reduced. Investigating the structure of the microbe-material-interface indicated that the initial adhesion of the microbes might be controlled by the number of nano contact points between the microbial cell and the material`s surface.
Conclusions: Our results suggest that surface roughnesses even in the very low nanometer range significantly influence microbial adhesion. These findings support the development of new antibiotic-free strategies to prevent implant-associated infections.

OTP33

Identification of antibiotic-tolerant persister cells in the zoonotic pathogen *Streptococcus suis*

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For pathogenic bacteria, phenotypic heterogeneity of clonal populations is discussed to affect the success of antimicrobial therapy. Persister cells constitute such a heterogeneous subpopulation which is genetically identical but phenotypically different to regular cells. In contrast to regular cells, persister cells typically show an elevated tolerance to antimicrobial agents. Here, we studied the ability of the zoonotic pathogen *Streptococcus suis* to form multi-drug tolerant cells. We observed that the initial bacterial growth phase is critical for persister cell formation and detected lower numbers of persisters in exponential phase cultures than in stationary growth phase populations. Biphasic killing, typical for persister cell enrichment, was observed in both growth phases. Notably, *S. suis* persister cells can tolerate a variety of different antimicrobial compounds that were applied at concentrations vastly exceeding the minimal inhibitory concentration (MIC). Furthermore, we could show that phenotypical tolerance is not heritable within a bacterial population, at least at the time points we investigated, and that the majority of persister cells consist of so called type-I-persisters. Testing of additional *S. suis* strains and specific *S. suis* mutants indicate that persister cell formation may be very common in this species and seems to be related to central metabolic processes. Taken together, this is the first study that reports multi-drug tolerant persister cells in the zoonotic pathogen *S. suis*.

OTP34

Adaptation of *Dinoroseobacter shibae* to oxidative stress

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Question*:* The Gram-negative photoheterotophic bacterium *Dinoroseobacter shibae* is a member of the marine *Roseobacter* group. In contrast to the closely related photothrophic purple bacteria, *D. shibae* performs aerobic anoxygenic photosynthesis. Living in the photic zone of marine ecosystems *D. shibae* is frequently exposed to oxygen and an effective oxidative stress defence response is required.
Methods: To define the peroxide stress stimulon, exponentially growing *D. shibae* cells were supplemented with 10 mM H_2O_2 and intracellular as well as surface-associated proteins were identified by 1D-gel based LC-MS analyses.
Results: In this study, we identified 2316 *D. shibae* proteins. 73 of these proteins showed an at least 1.5 fold change in response to hydrogen peroxide when a label-free approach was used for protein quantification. Increasing amounts were detected for 55 proteins and additionally 7 were *de novo* synthesized proteins.
Conclusion: While significantly higher levels of proteins involved in protection and repair of DNA and proteins were found after exposure of *D. shibae* to hydrogen peroxide stress, the protein levels of classical oxidative stress proteins involved in detoxification of oxygen radicals such as

catalases remained constant. Considering the high hydrogen peroxide tolerance of *D. shibae* in contrast to other bacteria, it seems very likely that these proteins are constitutively highly expressed. Furthermore, induction of proteins belonging to the glucose (KDPG pathway) and iron metabolism was detected. Noticeably, the amount of one potential iron regulator has been found to be reduced in response to oxidative stress, which is accompanied by a derepression of iron responsive genes.

OTP35
Creation of an IPTG and tetracycline inducible duet-expression shuttle vector for *Corynebacterium glutamicum* and *Escherichia coli*
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Question: *C. glutamicum* is a Gram-positive, biotechnological relevant organism, which also serves as a model organism for various pathogens like *Mycobacterium tuberculosis*. Vector based protein-expression is a powerful tool for the investigation of various questions in this organism with regard to physiology, biotechnology and biochemistry. However, while duet-expression vectors are well established for *E. coli*, no such system was described so far for *C. glutamicum*. In this work we aimed to create an expression-system with two independently inducible promoters, followed by unique multiple cloning sites which enable the specific introduction of target genes. The creation of such a vector would bypass the two vector solutions used hitherto.
Methods: The vector backbone is a fusion of the pCLton1[1] plasmid with its tetracycline inducible ptet/tetR system, and the ptac/lacI^{Q} derived from the pEKEx2[2] vector. Standard cloning procedures were conducted to obtain the final vector, designated pOGOduet. Genes encoding the fluorescent proteins eYFP and eCFP were introduced in either one of the multiple cloning sites. Measurements of the exhibited fluorescence and fluorescence microscopic pictures of *E. coli* or *C. glutamicum* strains harboring the vector were performed to test the functionality of both sites of expression.
Results: Restriction analysis of the final vector resulted in the expected fragment-pattern. Transformation of the plasmid and derivatives encoding either eYFP, eCFP or both into *E. coli* or *C. glutamicum* was successful. Fluorescence measurements showed expression of both proteins and a tight regulation in correlation with the titration of the two inductor molecules in both *E. coli* as well as in *C. glutamicum*. The ptet/tetR system of the vector was tightly adjustable with anhydrotetracycline, although its expression is weaker compared to the ptac/lacI^{Q}. The IPTG induction was less sensitive, but gave higher levels of expression.
Conclusion: The control experiments with fluorescent proteins proved that the new pOGOduet vector and its duet feature is functional in both *E. coli* and *C. glutamicum*, offering versatile possibilities for its application.

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OTP36
Spore wall biosynthesis in *Streptomyces coelicolor*
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The Gram-positive soil bacterium *Streptomyces coelicolor* undergoes a complex life cycle with differentiation in substrate mycelium, aerial mycelium and spores. In contrast to the early developmental steps, the formation of proper spores is dependent on a protein complex similar to the lateral cell wall synthesizing complex of rod-shaped bacteria[(1)]. Several diverse proteins are involved in this so called "*Streptomyces* Spore Wall Synthesizing Complex", including penicillin binding proteins, cytoskeletal proteins and putative wall teichoic acid biosynthetic enzymes. Unlike in other bacteria the deletion of various of the encoding genes causes only an impaired spore wall but is not lethal[(1,2)]. Therefore *S. coelicolor* is an interesting model organism for studying the synthesis of the bacterial cell envelope. In order to increase the understanding of the function and interplay of the involved proteins we biochemically analyze and compare wildtype and mutant spore walls, focusing on the two major components - peptidoglycan and anionic cell wall polymers.

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OTP37
Subunit composition analysis of respiratory reductive dehalogenase complex in *Dehalococcoides mccartyi*
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Persistent organic pollutants such as halogenated aromatic compounds and alkenes can be transformed by some microorganisms, e.g. the organohalide-respiring bacteria *Dehalococcoides mccartyi* strain CBDB1. Strain CBDB1 utilizes hydrogen as electron donor and chlorinated aromatic compounds or alkenes as electron acceptor. The reductive dehalogenase (RdhA) is the enzyme that catalyses the dechlorination reaction. Investigations showed that RdhA is localised in a larger protein complex which is attached to the extracellular site of the cell membrane. Besides RdhA, the complex also contains a protein annotated as complex iron-sulfur molybdoenzyme (CISM), a hydrogen uptake hydrogenase (Hup) and its three membrane anchoring proteins as well as an iron-sulfur cluster binding protein that is encoded in the Hup operon. In this study, we aimed to describe the composition and stability of this complex in more detail to get insight into how electron transfer takes place. First, we reproduced SDS-PAGE and BN-PAGE analyses combined with nLC-MS/MS measurements to confirm the presence of all subunits and to determine the dehalogenation activity of the complex depending on the presence of different subunits. In the mass spectrometric analyses the outer peripheral proteins of the complex were detected with more unique peptides and higher score values than the integral membrane proteins predicted to bind the peripheral proteins to the membrane. For example, in previous experiments only one putative anchoring protein (RdhB) of RdhA with only one unique peptide was found. To improve the detection of these membrane proteins, membrane preparations were incubated with proteinase K to cleave extra-membrane loops of integral membrane proteins ("Membrane Shaving") and after solubilisation the naked transmembrane helices were objected to mass spectrometry. This resulted in an increased number of hits for RdhB proteins and the number of unique peptides for the membrane subunit of CISM. A further task was the purification of active complex or sub-complexes from crude cell extracts. Different chromatographic techniques, including anion exchange chromatography, gel filtration, hydrophobic interaction chromatography, and a combination of them were established. Refined understanding of the complex composition in terms of stoichiometry and a purified complex will allow further studies to address interaction sites and interaction strength among the subunits as well as experiments on how the subunits function together.

OTP38
***Bacillus subtilis* phages – new isolates and insides**
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Bacteriophages are viruses of bacteria and probably the most abundant biological entities on Earth. It is estimated that each bacterial species is associated with approximately 10 different phages. However, the number of available bacterial genome sequences and strains is more than ten times higher than those of phages. This situation demonstrates the need of research on phages and the huge undiscovered potential.
Bacillus subtilis phages were isolated from sewage. With Transmission Electron Microscopy (TEM) we analyzed the morphology of the isolated phages and classified them. In addition, the phage genome sequences were determined.
Here, we present three phage isolates. The first phage vB_BsuP_Goe1 belongs to the Podoviridae phage family and exhibits properties of the Phi29likevirus genus. As a special feature head fibres could be identified. The genome size is approximately 18 kb and shows high similarity to the Phi29likevirus Nf. The other two isolates vB_BsuM_Goe2 and vB_BsuM_Goe3 could be classified as members of the Myoviridae family, with properties of the Spounalikevirus genus (formerly SPO1-like phages). The broad diversity of this group is mirrored by the morphological differences of our two isolates. Phage vB_BsuM_Goe2 has an approximate genome size of 130 kb and shows high similarity to the phage CampHawk. Phage vB_BsuM_Goe3 has the largest genome of about 150 kb with only partial similarity to known phages at nucleotide level.

OTP39

Clonal diversity of multidrug resistant *Acinetobacter baumannii* strains disseminated among hospitals in Upper Egypt

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Acinetobacter baumannii growing resistance is a worldwide problem. The incurable strains of *A. baumannii* endanger the lives of millions of hospitalized patients every year. Continuous study of its resistance pattern is a must to control its devastating effect on the quality of medical treatment.

From January 2014 to May 2015, a total of 1021 isolates were collected from different wards of Al-Azhar and Assuit University hospitals, Assuit Government, Upper Egypt. A number of 69 (6.76 %) *A. baumannii* isolates were identified using both biochemical and PCR reactions. The PCR detected the presence of bla_{OXA51}gene unique to *A. baumannii* different strains.

The antimicrobial susceptibility pattern of the 69 *A. baumannii* isolates, determined by Kirby Bauer disc diffusion method, showed high resistance rates against Cefpime 94.2 % (65/69), Cefotaxime 92.75 % (64), Pipracillin 91.3 % (63), Ceftazidime 89.85 % (62), Ceftriaxone 88.40 % (61),Ciprofloxacin 84.05 % (58),Tobramycin 82.60 % (57), Tazopactam 81.16 % (56), Sulbactam 79.71 % (55),Imipenem & Meropenem 76.81 % (53),Sulphamethoxazole 75.36 % (52) Amikacin & Gatifloxacin 73.91 % (51/69), Tigecycline 71 % (49). In addition to 2 pan-drug resistant isolates (2.90 %). On the other hand, Colistin was the most effective antibiotic with a susceptibility of 92.75 % followed by Doxycycline with 49.27 %.

The detection of metallo-β -lactamases (MBLs) using combined disk test (CDT) showed its presence in 90.56 % (48/53) of the Imipenem resistant isolates, while Cefotaxime resistant isolates of extended spectrum-β-lactamase (ESBLs), tested by double disk synergy test (DDST), represented 73.84 % (48/64).

Genotypic detection of the ESBLs encoding genes bla_{GES}, bla_{PER} and bla_{VEB} were present in 39.05 % (25/64),71.87 % (46/64) and 25 % (16/64) respectively of the tested Cefotaxime resistant isolates, while the MBLs resistant genes bla_{IMP} and bla_{VIM} were present in 69.81 % (37/53) and 62.626 %(33/53) respectively. For the $bla_{oxa\ 23}$ like gene (intrinsic Carbapenamase), it was detected in 81.13 % (43/53) of Imipenem resistant isolates, while the bla_{SIM} gene was totally undetected. Finally, integrase I genes was present in 82.60 % (57/69) of the total *A. baumannii* isolates giving a clue about their high epidemic potentials.

Enterobacterial repetitive intergenic consensus (ERIC) PCR analysis showed the diversity of the isolates, dividing them into three major clusters representing multiple contamination sources of *A. baumannii*.

This study demonstrated the dissemination of diverse clones of *A. baumannii* carrying MDR among hospitals in Upper Egypt.

OTP40

Hidden treasures – uncovering unrecognized staphylococcal inhibitors by considering metal-mediated innate immune functions

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Question: Despite innovative technologies, advanced antimicrobial screening did not live up to expectations over the last 4 decades. Of the 16 traditional antibiotic classes, all but 2 were discovered prior to the 1970s. A growing chorus advocates returning to nature inspired discovery platforms from which almost all antibiotics previously arose. A new inspiration from a natural source can be taken from metal-mediated innate immunity, by which macrophages use copper ions (Cu) to kill bacteria [1]. This recently described phenomenon has galvanized research exploring the pharmacological potential of molecules that enhance copper's antibacterial properties. Here, we describe the results of a pilot screen for novel inhibitors of *Staphylococcus aureus* displaying potent antibacterial properties in Cu-rich environments.

Methods: The whole-cell based screening assay was previously developed in our laboratory [2, 3]. We used a chemoinformatic approach to identify and expand hit clusters. Activity on *S. aureus* and toxicity on eukaryotic cells were determined using previously developed assays [3]. To evaluate pharmacokinetic properties, we used the ToxGlo mitochondrial toxicity assay and tested for metabolic stability against human and mouse liver derived microsomes. UV/Vis spectroscopy and ^{1}H-NMR titration were used to characterize the interaction between Cu and compounds.

Results: Most prominent was a novel hit family featuring a pyrazole extended thiourea core structure, termed PET motif. This motif resulted in Cu-dependent and Cu-specific *S. aureus* inhibition in the nanomolar range, while simultaneously being well-tolerated by eukaryotic cells (*in vitro* therapeutic index of ~ 30). Mitochondrial toxicity was not observed. In addition, we found that PET-class molecules interact with Cu by a novel mechanism, which in some instances dramatically enhanced their metabolic stability ($T_{1/2}$ = 300 min).

Conclusion: We present the PET compounds as a new class of copper-related antibiotics with favorable medicinal characteristics and discuss their mode of action in the context of a potential synergy to Cu-driven innate immunity.

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OTP41

A small RNA involved in regulation of bacterial photosynthesis genes

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The photosynthetic model bacterium *Rhodobacter sphaeroides* faces photooxidative stress by the bacteriochlorophyll-mediated generation of singlet oxygen ($^1O_2$) in the light. This leads to a need of a tight regulation of the formation of photosynthetic complexes in the presence of oxygen. Our group intensively investigated this regulatory network and could identify a small RNA (sRNA), namely PcrZ [1], which controls a small subset of photosynthetic genes. Therefore we further focus on other potentially involved sRNAs.

Essential for the development of the two light harvesting complexes (LHCI and LHCII), are the genes encoded in the *puf* and the *puc* operons. RNAseq data from these photosynthetic gene clusters indicated the presence of an sRNA, preliminary called RSspufX, directly downstream of the *pufX* gene in the same transcriptional direction as the *puf* genes [2]. The RNAseq data also showed that the abundance of the sRNA is strongly dependent on growth conditions, which could be verified via Northern blot analysis. RSspufX has a size of around 77 nt and is strongly expressed under phototrophic growth and its expression is induced upon a shift from high to low oxygen conditions. The latter induction seems to be dependent on PrrA and FnrL, two major regulatory proteins controlling photosynthesis gene expression. An artificial increase in the amount of the sRNA by plasmid driven over-expression led to a reduced pigmentation and LHC amount in the cells, measured via absorption spectra and bacteriochlorophyll extraction. These findings further underline the sRNAs potential role in regulation of photosynthesis genes. Interestingly RSspufX as well as parts of the *pufX* mRNA were enriched in an Hfq-coimmunoprecipitation [3]. To test whether RSspufX binds and influences the *pufX* mRNA we use a *lacZ*-based *in vivo* reporter system. In addition this system is used to uncover the influence of RSspufX on a second potential target, *bchY* which encodes a subunit of the chlorophyllide reductase and was predicted as a target using the IntaRNA webtool.

Overall RSspufX can be seen as a new RNA player in the control of photosynthesis gene expression, even tough its exact function remains to be elucidated.

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OTP42

H-NS/StpA repression and its interference by transcription in *Escherichia coli*

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H-NS is a global repressor in *E.coli* which controls the expression of 5 % of *E. coli* genome. It binds to AT-rich, curved sequences and forms extended complexes on the DNA. H-NS abrogates transcription initiation by physically blocking the promoter or by trapping RNA polymerase. H-NS repression can be relieved by specific transcription factors, by changes

in the DNA structure or by other mechanisms[1]. Furthermore, single molecule experiments have revealed that a force of about 7pN is sufficient to disrupt H-NS complexes, while the elongating RNA polymerase moves with the force of 25pN[2]. This suggests that an elongating RNA polymerase may be able to dislodge bound H-NS.
The aim of the project is to study the effect of transcription on H-NS repression. In our experimental system we use a module consisting of a constitutive promoter and conditional transcriptional terminator (λtR1), and vary the transcription rate by expressing anti-terminator protein λN. At classical H-NS repressed locus *proU,* the increase in transcription into H-NS binding regions reduces repression by H-NS. Similar results were obtained for other H-NS and H-NS/StpA repressed loci, namely *yahA* and *appY* respectively. Further, we modulated the rate of transcription into the H-NS repressed *proU* locus by using the arabinose inducible P_{BAD} promoter and by varying the concentration of arabinose. An increasing transcription rate could relieve the H-NS repression of *proU* and increased the expression linearly with respect to the arabinose concentration. Similarly, in the native context, read through from the upstream operon reduced the H-NS repression of the *bgl* operon.
Taken together, our data suggest that the transcribing RNA polymerase can dislodge H-NS from the DNA. The model is that at high transcription rates H-NS will not be able to rebind and form a repressing complex due to the engagement of RNA polymerase in the H-NS binding region, whereas at a low transcription rate H-NS is able to re-bind and form a repression complex.

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OTP43

Feedback control of *leuO* encoding a pleiotropic regulator and H-NS antagonist in *Escherichia coli*

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The enterobacterial LeuO protein is as a pleiotropic LysR-type transcriptional regulator and plays an important role in pathogenicity, stress adaptation and the CRISPR/Cas immunity system. Expression of *leuO* is silenced by the master regulator H-NS. Expression of *leuO* can be activated by BglJ-RcsB and involves a double-positive feedback loop regulation. The *leuO* gene is activated by BglJ-RcsB [2], and LeuO activates expression of *bglJ*, encoded within the H-NS repressed *yjjQ-bglJ* operon [1]. Activation of *leuO* by BglJ-RcsB is in addition antagonistically controlled by LeuO [2] suggesting that the double-positive feedback regulation of *leuO* is tightly controlled. The activation dynamics of the *leuO* promoter by the antagonistic action of LeuO and BglJ-RcsB were characterized by a *leuO* promoter fluorescence reporter fusion in dependence of ectopically expressed LeuO and BglJ. The *leuO* promoter activity was analyzed by flow cytometry which suggests that the antagonistic control of the *leuO* promoter activity by LeuO and BglJ is controlled in a concentration-dependent manner. Furthermore, screening for additional activators of *leuO* revealed a further regulator which activates a third and a fourth *leuO* promoter and shows direct DNA-binding suggesting a more complex regulation of *leuO* expression. The obtained data serve as a basis for a theoretical model of the antagonistic regulation of the *leuO* promoter. With this model we wish to describe the regulatory elements of the *leuO* control circuit, which is likely to be important in the response to specific, virulence-related environments.

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OTP44

Cooperative binding of the transcriptional regulator RhaR to its operator sequence

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The ubiquitous gram-positive bacterium *Bacillus subtilis* is mainly found in the soil and in the rhizosphere of plants. For the breakdown of plant cell walls, which consist of complex, heterogeneous polysaccharides, *B. subtilis* secretes several carbohydrate degrading enzymes into its environment. The resulting mono-, di- and oligosaccharides can be taken up by the cells through specific transport systems for further metabolism. An example is the pectin macromolecule of plants which is composed of linear polygalacturonan and branched rhamnogalacturonan chains. The enzymes for rhamnogalacturonan degradation are encoded by two separate gene clusters. Another transcriptional unit encodes enzymes for the degradation of L-rhamnose. Remarkably, no rhamnose-specific transporter is encoded within the L-rhamnose utilization operon. According to this, *B. subtilis* grows poorly in minimal medium when rhamnose is added as the sole carbohydrate source. Up to now, the transcriptional regulatory mechanisms of the genes involved in the utilization of rhamnose mono- and oligosaccharides in *B. subtilis* have been proposed only on the basis of bioinformatical studies. Our focus lies on the regulation of the utilization of the deoxy-hexose sugar L-rhamnose which is a major component of the cell wall of plants and many other organisms. As previously experimentally validated, the second gene of the rhamnose operon, *rhaR*, encodes for a transcriptional repressor. In this study, we characterize the binding site and properties of the RhaR protein. By deletion analysis, the beginning of the operator sequence of the rhamnose operon was located at about position -60 relative to the transcription start site. In footprint experiments, RhaR-Strep-tag protects a region of 38-47 bp followed by a smaller region of about 22 bp. The major protected stretch comprises a direct repeat motif CAAAAA(T/C)AAACA(A/G)AAA. The operator sequence seems to be bound by monomeric RhaR proteins as indicated by molecular weight determination by GPC. Binding of RhaR to the operator sequence was also affirmed by electrophoretic mobility shift assay leading to the presumption of a cooperative manner of the binding reaction. In addition, by this method, L-rhamnulose 1-phosphate has been demonstrated to be the effector molecule of RhaR.

OTP45

First steps towards understanding the plant growth-promoting actions of *Hartmannibacter diazotrophicus*

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The recently described genus and species *Hartmannibacter diazotrophicus* $E19^T$ is part of a research project on halotolerant PGPR isolated from the rhizosphere of *Hordeum secalinum* and *Plantago winteri* plants growing in a natural salt meadow in Hesse (1, 3).
Strain $E19^T$ is able to grow on non-water-soluble PO_4 sources, nitrogen free media and showed acetylene-reduction activity; moreover growth on DF medium supplemented with ACC and its respective ACC-deaminase activity was demonstrated in cell suspensions. To test the interaction of strain $E19^T$ on barley, seeds of *H. vulgare* cv. Propino were surface sterilized and inoculated with the bacterium. Non-sterile salt adjusted soil (NaCl 1.75 %) was used for seed germination and 15 days after germination salt concentration was increased to 4.4 % NaCl. Forty-two days grown barley plants inoculated with strain $E19^T$ showed significantly increased root (308 %) and shoot (189 %) dry weights, and water content in the root system (378 %) compared to control treatment ($E19^T$ dead biomass). Also, root-to-shoot ratio was significantly increased, whereas the root Na^+ concentration and root surface sodium uptake in barley plants decreased. Reduction of ethylene emission measured on barley plantlets under salt stress showed positive stress relieving effect of $E19^T$ due to its ACC deaminase activity. Roots colonization of $E19^T$ under salt stress conditions was revealed with a specifically designed fluorescence *in situ* hybridization (FISH) probe (2).
$E19^T$ draft genome sequence consists of a single circular chromosome of 5.4 Mbp. Its genome relationship based on average nucleotide identity (ANI) mean values with available genome sequences shows as closest relative members *Pleomorphomonas* within unclassified *Alphaproteobacteria*. $E19^T$ genome includes several genes involved in PGP traits including phosphate solubilization, nitrogen fixation, and ACC deaminase production. Improvement in gene annotation is in progress in order to assign gene functional classification, metabolic reconstruction and comparative genome sequence analysis with other PGPR to better understand the bacterium gene content that could be involved in PGP.

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OTP46

Characteristics of antibiotic sensitivity *S. aureus*, extracted from milk of clinically healthy highly productive animals.

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Causes significant economic losses to animal husbandry is mastitis (inflammation of the mammary gland), caused by infection with hemolytic *streptococcus* and *staphylococcus*. They stand out in the treatment of acute and sub-clinical mastitis (Black R. A. *et al.*, 2014, J. Dairy Sci.). The aim is to monitor the herd of cows for the presence of hemolytic streptococci and staphylococci, followed by determination of antibiotic resistance *Staphylococcus aureus* strains isolated from milk. Milk samples of clinically healthy Holsteincows have been investigated from each animal individually, from 1 to 7 of lactation (n = 345, in2015). The animals were divided into 4 groups: I - streptococci and *S. aureus* were not found; II - only streptococci were found; III - only *S. aureus* were found; IV - streptococci and *S. aureus* were detected. 1.0 mL of sample was added to 9.0 ml of saline broth, cultured for 24-48 hours at t =38.0 ± 1.0^{0}C under aerobic conditions, followed transfer by the method debilitating stroke to the surface Baird Parker Agar and Azide Blood Agar «Pronadisa» with defibrinated sheep blood, with the same culture conditions. The species group was determined using set of HiStaph KB004R and coagulation reaction using "rabbit citrate plasma dry". Profile of antibiotic susceptibility of S. aureus isolates was determined by the method disk-diffusion in accordance with diameter of growth inhibition zones (mm) in the medium of AGV type. To determine methicillin resistant *staphylococcus* (MRSA) was used test MRSA Alert w/swabs. As a reference strains were used *Staphylococcus aureus* MRSA ATCC 43300 and *Staphylococcus aureus* ATCC 25923. The studied forms of microorganisms in 22.0 % cows were not detected, whereas in 78.0 % cows the presence of hemolytic streptococci and/or *S. aureus* were determined. Contents of *S. aureus* ranged from 3 in the milk cows of group III, and in group IV - from 1.0 to 7.8 log10 CFU/cm^3. The *S. aureus* isolates were resistance to benzylpenicillin (12.7 %), gentamycin (3,8 %), erythromycin (59.5 %), lincomycin (97.5 %), rifampicin (16.5 %), ciprofloxacin (11.4 %), vancomycin (1.3 %), fusidic acid (50.6 %) and novobiocin (26.6 %). One isolate (1.3 %) was resistant to oxacillin. This requires further investigation their genetic structure by using molecular methods. The research was supported by the Russian Science Foundation, project № 15-16-00020.

OTP47

Gas chromatographic/mass spectrometric approach to the detection of peptidoglycan in Gram-negative bacteria

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Peptidoglycan (PG) layers in cell walls of Gram-negative bacteria are occasionally very thin and hardly detectable. Due to the lack of sensitive detection methods for PG and to the resistance to betalactam antibiotics targeting the peptidoglycan synthesis, it had been assumed that peptidoglycan is absent in certain Gram-negatives like e.g. in planctomycetes, chlamydiae or rickettsiae. Recent bioinformatic evidence of the presence of enzymes essential for PG synthesis in such organisms challenged these hypotheses. Therefore sensitive analytical approaches for detection of peptidoglycan gained in importance.

PG of Gram-negatives is structurally rather uniform with 2 types, A1γ based on *meso*-2,6-diaminopimelic acid (*meso*-Dpm) and A1β with L-ornithine (L-Orn). The non-proteinogenic diamino acids Dpm and Orn may therefore serve as PG indicators in hydrolysates of Gram-negative cells. Gas chromatography/mass spectrometry (GC/MS) allows for highly sensitive detection and quantification of *N*-heptafluorobutyryl isobutyl ester derivatives of PG-relevant amino acids by extracted ion monitoring (EIC) of characteristic fragment-ions [1].

This GC/MS approach was successfully tested by detecting 63 nmol Dpm and 90 nmol Orn per mg lyophilized cells in the model organisms *Escherichia coli* DSM 498 and *Spirochaeta dissipatitropha* DSM 23605^T, respectively. Then the method was applied to the examination of planctomycetes for which the absence of PG was supposed. EIC based on the Dpm-specific fragment-ion set 380, 324, 306 and 278 m/z revealed the presence of PG in *Planctopirus limnophila* DSM 3776^T, *Gemmata obscuriglobus* DSM 5831^T and *Rhodopirellula baltica* DSM 10527^T [2]. Both Dpm (7 and 17 nmol/mg) and Orn (25 and 47 nmol/mg) indicated the presence of PG in the *Verrucomicrobia* strain L21-Fru-AB and in *Victivallis vadensis* DSM 14823^T, respectively [3]. Low amounts of PG based on Orn (19 nmol/mg) as diagnostic diamino acid were found in *Oligosphaera ethanolica* DSM 24202^T.

This GS/MS method demonstrated in agreement with results of bioinformatic and electron microscopic studies the hitherto questioned presence of PG in planctomycetes [2] and contributed to the taxonomic characterization of the first cultured representative of the *Verrucomicrobia* subdivision 5 [3].

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OTP48

Marine metagenomes as a source for novel enzymes involved in pigment biosynthesis.

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All functional light harvesting bilin chromophores in cyanobacteria are derived from heme. The heme oxygenase (HO) catalyzed cleavage of heme at the α-carbon position yields the first open-chain reaction product biliverdin IXα (BV). BV is further reduced by ferredoxin-dependent bilin reductases (FDBRs) to the specific light-harvesting pigments. During a deep screening of the VirMic dataset, a new family of putative FDBRs (designated PcyX) as well as a new family of HOs (designated HemO) was discovered that each group together as a new branch in a phylogenetic tree. The VirMic dataset [1] includes scaffolds from the "global ocean survey" (GOS) [2] that are considered to be of viral origin but contain microbial gene clusters. In order to determine whether PcyX and HemO are functional enzymes, synthetic genes were expressed in *E. coli*. HO and anaerobic FDBR assays with affinity purified protein and the respective substrate established that the enzymes are functional. Furthermore, we identified the specific reaction product of HemO as biliverdin IXα. Also, first results showed that a phage derived PcyX reduces BV to phycoerythrobilin (PEB) with 15,16-dihydrobiliverdin (DHBV) as an intermediate. Moreover, a related PcyX sequence from *Actinobacterium* reduces BV IXα to a pigment with retention times similar to 3(*E*)-phytochromobilin (PΦB) and/or 18^1, 18^2-DHBV. To further characterize the PcyX reactions, we are currently working on time-resolved FDBR assays, mutagenesis experiments as well as crystallization studies.

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STP01 – withdrawn

STP02

Nanotube mediated cross-feeding leads to metabolic coupling in bacteria

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Microbial communities and many host-microbe symbioses display the phenomenon of metabolite exchange between individuals. An exchange of essential metabolite enhances growth and reduces production cost of that metabolite for the involved species. The physiological consequences of such an exchange on the participating cells as well as the mechanism of exchange are still not clearly known. The complexity of interactions and culturing of different species from microbial communities and symbiotic relationships makes it challenging to study. Using a synthetically designed cross-feeding system in *Escherichia coli* involving exchange of amino acids between a donor and recipient strain we aim to gain insight into the phenomenon of cross-feeding. We show an exchange of essential amino acids between the donor and recipient via nanotubes. Furthermore this exchange leads to a coupling of the metabolism of donor and recipient cells in a source-sink-like relationship. Interestingly the exchange does not incur a fitness cost to the donor strain. We do see an increase in production

of amino acids in the donor cell, possibly due to deregulation of feedback control of amino acid biosynthesis pathway. Altogether, our results show a metabolic coupling between cells via nanotubes which suggests that bacteria may function as interconnected entities rather than single, autonomous units

STP03

The multi-functional transcriptional activator ComA from *Bacillus subtilis* has distinct sequence requirements for interacting with DNA

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There are multiple ways how transcription factors regulate gene expression. If a transcription factor is capable of inserting itself into the transcription process in more than one way, and thus effectively acts as a bi- or multi-functional transcription factor, alternative DNA binding sites could have evolved to enable it to carry out each separate function. Here we report that the quorum sensing master regulator ComA from Bacillus subtilis recognizes a set of topologically dinstinct sequence motifs. We provide *in vitro* (EMSAs) and *in vivo* (promoter activity measurements) evidence that the different sites play an important role in facilitating type I and type II promoter activation, respectively, by interacting with different subunits of RNA polymerase. Together our data support a model in which the topology of the ComA binding site serve different functions in activating transcription. We furthermore show that there is a variety of contexts in which these sites can occur and identify new direct target genes of ComA. Moreover, we show that ComA has surprisingly stringent sequence requirements for binding to isolated sites *in vitro*. This is in stark contrast to the sequence degeneracy seen in many natural promoters, to which ComA binds cooperatively *in vitro*. We thus suggest that the DNA sequence evolved and reflects on the constraints imposed by all the interactions that the transcription factor faces in the transcription initiation complex.

D.Wolf, V.Rippa *et al.*, Nucleic Acids Research 2015; doi: 10.1093/nar/gkv1242

STP04

Stress sensing and signal transmission by PspB and PspC in the cytoplasmic membrane of *Escherichia coli*

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In *Escherichia coli* the phage shock protein (Psp) system is up-regulated by manifold stresses that harm the cytoplasmic membrane. The expression of the *pspABCDE* operon and *pspG* is σ^{54}-dependent and up-regulated by PspF, which in turn is negatively regulated by PspA [1]. It is thought that the membrane-integral components PspB and PspC are able to sense membrane stress and transduce the signal to PspA. Despite of various Psp component interaction studies under stress and non-stress conditions, it is still unresolved how exactly the stress signal is sensed and transduced between the Psp components. We used a site-directed mutagenesis approach with a *pspA* promotor activity read-out to determine the domains of the membrane-bound components PspB and PspC that are involved in signal sensing and transduction in *E. coli*. According to our data, a specific C-terminal region of PspC is essential for signal transduction from the membrane-bound components PspB and PspC to PspA, resulting in activation of PspF. Most likely, the C-terminal PspC domain adopts a specific PspA-interacting conformation in response to stress sensing at other regions of PspB or PspC.

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STP05

Pyruvate – the stimulus for the two-component system YehU/YehT in *Escherichia coli*

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Two-component systems represent a prevalent bacterial mechanism to respond to changing environmental conditions. In most cases they consist of a membrane-integrated histidine kinase (HK) and a soluble response regulator (RR) that controls target gene expression. In *E. coli* the HK/RR YehU/YehT system contributes to carbon scavenging before entry into the stationary phase. The system belongs to the LytS/LytTR family and regulates the expression of *yjiY,* encoding a putative peptide transporter belonging to the CstA superfamily (1).

Here we investigated the chemical stimulus perceived by the YehU/YehT system. We found that activation of YehU/YehT and expression of *yjiY* is dependent on nutrient starvation and the extracellular availability of pyruvate. Binding of pyruvate to YehU in right-side-out membrane vesicles was confirmed *in vitro* by using the differential radial capillary action of ligand assay (DRaCALA). This study also demonstrates that the method of DRaCALA can also be applied to binding analysis of radioactive ligands to proteins integrated in membrane vesicles. Furthermore, we determined the extra- and intracellular concentration of pyruvate of a growing culture of *E. coli* using hydrophilic liquid chromatography and observed correlation with *yjiY* expression.

Taken together, we present evidence that the histidine kinase YehU responds to extracellular pyruvate under nutrient limitation.

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STP06

Functional characterization of novel extracytoplasmic function (ECF) sigma factors from *Streptomyces venezuelae*

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Bacteria populate complex habitats in which extracellular conditions can rapidly change. In order to survive in such environments, bacterial cells need to be able to sense and respond to such changes. A common mechanism to control gene expression is the use of alternative sigma factors, which are the subunit of the RNA polymerase that determines promoter specificity. In addition to the primary sigma factor, most bacteria harbor a number of alternative sigma factors of extracytoplasmic function (ECFs) that redirect the RNA polymerase to alternative promoters. Ongoing comparative genomics efforts in our group have identified numerous novel ECF groups with potentially unique regulatory features (1, 2). ECF42 is one such novel group of ECF sigma factors identified in our previous work. This large and phylogenetically diverse group is distributed over ten bacterial phyla. ECF42 sigma factors are longer than traditional ECF sigma factors due to the presence of a C-terminal extension containing tetratricopeptide repeat (TPR) domains, which are usually involved in mediating protein-protein interactions. Additionally, one or more copies of genes encoding DGPF proteins of unknown function are found in the direct vicinity of those encoding ECF42s. So far, none of the sigma factors belonging to this group has been experimentally analyzed. It is our aim to functionally characterize ECF42 sigma factors in *Streptomyces venezuelae*. To investigate the biological function of ECF42 sigma factors we have created deletion and overexpression mutants to be subjected to phenotypic screening. Additionally, putative target promoter sequences were investigated in *S. venezuelae.* ECF42 coding genes were overexpressed from a constitutive promoter and their putative cognate promoters fused to the β-glucuronidase gene. The same sequences were also analyzed in *Bacillus subtilis*. In this case, ECF42 coding genes were codon optimized for expression in the heterologous host and placed under the control of a xylose inducible promoter. Target promoter activity was monitored through bioluminescence production. Moreover, we have demonstrated interaction between *S. venezuelae* ECF42 sigma factors and *B. subtilis* RNA polymerase through pull-down assays. Additionally, bacterial two-hybrid assays performed in *Escherichia coli* suggest that ECF42 sigma factors do not interact with the cognate DGPF proteins. In conclusion, our work provides first insights into the function and mechanism behind ECF42 sigma factors activation in *S. venezuelae*.

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STP07

Physiological importance of the YehUT/YpdAB nutrient-sensing network in *Escherichia coli*

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Varying environmental conditions (e.g., nutrients, pH, ions) force bacteria to constantly sense changes, transmit the information to the interior and

respond accordingly. *Escherichia coli* has 30 two-component systems, and most of them are composed of a membrane-integrated histidine kinase and a cytosolic response regulator. In our laboratory we focus on the two histidine kinase/response regulator systems YehU/YehT and YpdA/YpdB, which are involved in nutrient-sensing. Both systems are activated at the transition to stationary phase, and regulate the expression of *yjiY* and *yhjX*, respectively, each coding for a nutrient transporter of yet unknown function. An interconnected response between both signalling systems is mediated via the transporters, suggesting the existence of a larger signalling unit [1].
On single cell level we observed heterogeneous expression of *yjiY* and *yhjX* in LB medium. In strains lacking one of the transporters the heterogeneous response was converted into homogenous expression. This indicates the importance of feedback regulation to establish heterogeneous behaviour.
Furthermore, we found a significantly higher percentage of persister cells in mutants lacking both systems, providing first evidence of a connection between the YehU/T and YpdA/B systems and persister cell formation. Overall, it is hypothesized that differences in carbon source availability trigger the formation of subpopulations as part of a bet-hedging strategy.

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STP08
GbsR – transcriptional regulator of glycine betaine synthesis in *Bacillus subtilis*
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Question: Confronted with hyper osmotic stress, the soil bacterium *Bacillus subtilis* accumulates compatible solutes to maintain cell turgor and viability. Glycine betaine is such a compatible solute and it can be taken up from the environment by the Opu-transporters or synthesized from the precursor choline. Oxidation of choline is mediated by the dehydrogenases GbsB and GbsA, whose structural genes are transcribed from the same promoter (Boch *et al.*, 1996, 1997). Upstream of the *gbsAB* operon the *gbsR* gene is located which encodes a choline-responsive repressor regulating the *gbsAB* operon as well as the *opuB* operon, encoding a specific transporter for choline (Nau-Wagner *et al.*, 2012). Here we present a characterization of GbsR and its involvement in regulation of both the glycine betaine synthesis pathway and the uptake of the precursor choline by the choline-specific OpuB-transporter in *B. subtilis*.
Methods: We carried out bioinformatic analysis to identify GbsR binding sites upstream of *gbsAB* and *opuB*, followed by mutagenesis of putative binding sites and measurement of transcriptional activity using *treA*-reporter gene fusions. Fluorescence spectroscopy measurements were performed to analyze the choline binding pocket of the GbsR homologue OpuAR from the marine bacterium *Bacillus infantis* NRRL B-14911.
Results: Bioinformatic analysis revealed a palindromic repeat within the *in silico* predicted GbsR binding site by Leyn *et al.* (2012). Mutations of this region abolishe GbsR-mediated repression as shown by *gbsA-treA* reporter fusions. Binding of the ligand choline to the GbsR regulator relieves repression of the *gbsAB* operon. An *in silico* model of the GbsR protein structure showed four phenylalanines, that are supposed to form a choline binding pocket. We constructed mutants of the GbsR homologue OpuAR of *B. infantis* NRRL B-14911 and determined their binding affinity for choline by fluorescence spectroscopy. With this approach we were able to show that each of the phenylalanine residues is involved in the binding of choline.
Conclusion: Through bioinformatics and mutational studies we identified GbsR binding sites in the *gbsA* and *opuB* operons downstream of the transcriptional start site. Hence, the choline-responsive GbsR-repressor genetically acts through a road-block mechanism. Site-directed mutagenesis of the *gbsR*-related repressor protein OpuAR suggests the location of the binding-site for the inducer choline.

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STP09
Screening for effector molecules driving pristinamycin biosynthesis
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The biosynthesis of the streptogramin antibiotic pristinamycin in *Streptomyces pristinaespiralis* is governed by a complex signaling cascade that involves at least seven different transcriptional regulators (SpbR, PapR1, PapR2, PapR3, PapR4, PapR5, and PapR6)[1]. Three of them - SpbR, PapR3 and PapR5 - belong to the TetR-like family. SpbR (*S. pristinaespiralis* butyrolactone-responsive transcriptional repressor) is suggested to be a γ-butyrolactone (GBL) receptor protein[2], whereas the ligand binding activity of PapR3 and PapR5 is not known so far. TetR-like regulators, derived from antibiotic gene clusters, can either act as 'real' GBL receptors or as 'pseudo'-GBL receptors. 'Real' GBL receptors accept GBLs as ligands, whereas 'pseudo'-GBL receptors bind antibiotics or intermediates thereof. To investigate if SpbR, PapR3 and PapR5 act as 'real' or 'pseudo'-GBL receptors, electromobility shift assays (EMSAs) have been performed with SpbR, PapR3 and PapR5 together with their cognate target DNA in the presence of different effectors.
EMSA studies showed that the addition of synthetic GBL (1,4-butyrolactone), as well as the addition of crude extracts from the *S. pristinaespiralis* wildtype or the pristinamycin-deficient mutant Δ*papR2apra* prevents the DNA-binding of the TetR-like regulators, whereas the addition of pure pristinamycins does not, suggesting that GBL-like molecules, but not pristinamycin or its intermediates, serve as effectors for the TetR-like regulators. Thus, SpbR, as well as PapR3 and PapR5 act as 'real' GBL receptors. In accordance with these data, we found that the addition of synthetic GBL to the *S. pristinaespiralis* culture leads to a significant increase of pristinamycin production, which demonstrates that GBL is an inductor of pristinamycin biosynthesis.
So far the GBL biosynthetic gene(s) is/are not known. Recently, a putative pristinamycin effector biosynthesis gene, *snbU*, has been identified within the pristinamycin gene cluster, which codes for a putative cytochrome P450 monooxygenase[1]. Interestingly, inactivation of the *snbU* gene led to an increase of pristinamycin biosynthesis, which indicates that SnbU has a regulatory effect on pristinamycin production. Furthermore, EMSA studies showed that culture extracts from the *snbU* mutant could not prevent the above mentioned regulator-DNA binding, suggesting that SnbU is involved in pristinamycin effector biosynthesis.

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STP10
Unraveling unknown factors for ClpC-dependent proteolysis during sporulation in *Bacillus subtilis*
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Protein degradation is an important physiological process over all three kingdoms of life, ensuring a functional proteome by removal of damaged or misfolded proteins and as an essential aspect of cell regulation. For regulatory purposes, fully functional molecules such as transcription factors are degraded rapidly, setting a complex regulatory circuit in motion. Hsp100/Clp proteins are ATP-dependent chaperones and are part of AAA+ proteases, which drive the protein degradation in low GC, Gram-positive bacteria such as *Bacillus subtilis*. The Hsp100/Clp protein ClpC is part of a protease complex that mediates degradation of different regulatory proteins. All ClpC activities in *B. subtilis* require adaptors that mediate assembly of the protease complex, recognition of the substrate and unfoldase activity. To this point in time, only two adaptor proteins for ClpC have been identified but previous research gives reason to suspect the existence of at least one more adaptor.
For this project we are focusing on investigating the proteolytic events that mediate the activation of specific regulatory processes during the onset and early stages of sporulation in Gram-positive bacteria. This knowledge will provide information that might enable the manipulation of important physiological processes not only in *B. subtilis* but also in other Gram-positive spore formers.

STP11
A well-orchestrated program of cell division and DNA replication upon stringent response induction in *Vibrio cholerae*
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The stringent response is a starvation stress response which is highly conserved in bacteria. Upon induction, the small alarmone (p)ppGpp is produced and regulates multiple cellular functions. In *Escherichia coli*, initiation of DNA replication and cell division are blocked by the stringent response. The aim of this work was to analyze the influence of the stringent response on DNA replication and cell division in *Vibrio cholerae*, the causative agent of the cholera disease. Because of its two chromosomes, *V. cholerae* is commonly used as a model organism for bacteria with multipartite genomes. In great contrast to *E. coli*, *V. cholerae* cells pass through a controlled process with several cell divisions and a re-initiation of DNA replication upon stringent response induction. Flow cytometry analyses of DNA-stained cells revealed that this process consists of two phases. In the first phase, cells finish their ongoing DNA replication and divide. In the second phase, cells initiate the DNA replication again synchronously before dividing into cells with only one complete chromosomal set. The amount of cells entering the second phase and the time point of the second phase start was found to be dependent on the stringent response induction level. These findings might indicate a homeostatic regulation mechanism. Analysis of mutant strains revealed that the stringent response induced division-initiation program was dependent on RelA, one out of three (p)ppGpp synthetases in *V. cholerae.* Using a *Vibrio cholerae* strain in which the two chromosomes are fused we found that the division-initiation program was not dependent on chromosome II replication. Further investigations on the cellular program following stringent response induction might help to understand the general mechanisms governing DNA replication of the multipartite genome in *Vibrio cholerae*.

STP12
Substrate-induced protein acetylation dynamics in *Dehalococcoides mccartyi* CBDB1
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Halogenated organic compounds are among the most widespread groundwater contaminants in industrialized countries. *Dehalococcoides mccartyi* CBDB1 is an obligate organohalide-respiring anaerobic bacterium which can detoxify a wide range of these compounds. For catalyzing this step CBDB1 possesses as many as 32 rdhA genes, encoding confirmed and putative reductive dehalogenases. It is of great interest to understand the substrate specificity of these enzymes and how they are regulated. Protein lysine acetylation is a dynamic and reversible post-translational modification (PTM) for global regulation which has long been underestimated. It is known that acetylation profiles change depending on carbon source and growth phase.
Here we present the proteome and acetylome of CBDB1 grown with 1,2,3-trichlorobenzene as an electron acceptor and harvested at different growth phases. Lysine-acetylated peptides were enriched by affinity purification, followed by nano-HPLC-MS/MS analysis for lysine acetylation characterization. As a result, we identified the metabolic pathways and protein subcellular localizations which are mainly affected by acetylations. In addition, we compared the acetylome results using different search engines to identify proteins from mass spectra.
The results reveal metabolic networks and signal transduction pathways of *Dehalococcoides mccartyi* CBDB1. The magnitude of acetylation sites and broad range of fundamental cellular processes affected by this post-translational modification suggest its pivotal role in regulating this strict organohalide respiration-dependent life-style.

STP13
Adaption to stationary phase and nutrient limitation via the replication initiator DnaA in *C. crescentus* and *E. coli*
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Chromosome replication is a crucial part of the cell cycle of all organisms and is subject to strict regulation. In almost all bacteria, initiation of chromosome replication requires the conserved protein DnaA. Tight regulation of DnaA is important to coordinate DNA replication with growth rate and to block replication initiation under stress conditions. Previous work in the model bacterium *Caulobacter crescentus* has shown that DnaA levels are downregulated under different stress conditions. It was recently shown that DnaA degradation by the protease Lon is upregulated in response to proteotoxic stress, resulting in a drop in DnaA levels and cell cycle arrest. We now show that the synthesis of DnaA is adjusted upon entry to stationary phase and in response to nutrient levels, in both *C. crescentus* and *E. coli*, coupling replication initation to growth rate.
We measured replication status and DnaA levels by flow cytometry and western blotting upon growth into stationary phase in rich medium, and during carbon exhaustion in minimal medium. In *C. crescentus,* we observe an elimination of DnaA under both conditions, accompanied by replication arrest. We show that the constitutive degradation of DnaA by Lon remains unchanged, and that a dramatic downregulation of DnaA synthesis is responsible for the decrease in DnaA levels. We also show that the long 5' untranslated region (UTR) of *dnaA* is required for the downregulation of DnaA synthesis. DnaA levels are higher in rich medium than in minimal medium, indicating a link between nutrient availability and DnaA synthesis via a mechanism involving the 5'-UTR.
In *E. coli*, we also observe the elimination of DnaA upon entry into stationary phase, accompanied by replication arrest. Our preliminary results suggest that regulation of synthesis is responsible for the decrease in DnaA levels, and that the alarmone (p)ppGpp plays a role in this regulation, in contrast to *C. crescentus*, where DnaA levels are regulated independently of (p)ppGpp. In further contrast to *C. crescentus,* in *E. coli* DnaA is not rapidly degraded.
Our results show that precise control of DnaA levels plays an important role in adapting replication and growth to changing environmental conditions, and that in *C. crescentus* this is achieved by adjusting both translation and proteolysis of DnaA. Although the mechanisms employed by *E. coli* may be different, the patterns of DnaA regulation and replication arrest achieved appear to be very similar.

STP14
BceRSAB-like systems from *Lactobacillus casei*: delving into function and regulation.
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Lactobacillus casei is a probiotic lactic acid bacteria exposed to a wide variety of environmental stresses, such as antimicrobial peptides (AMPs). Signal transduction Two Component Systems (TCS) play a major role in bacteria to detect adverse changes in the environmental conditions and to activate specific responses to counteract them. They typically consist of an intramembrane sensor histidine kinase (HK), and a response regulator (RR) which controls the expression of specific genes mediating the specific adaptive response. A special case are BceRS-like TCS since they are associated with BceAB-like ABC transporters. Together BceRSAB-like modules mediate resistance against AMPs in some Firmicutes bacteria. *L. casei* BL23 possesses two paralogous BceRSAB modules involved in AMP resistance, Module09 and Module12 [1]. An orphan BceAB-like ABC transporter is also present in its genome. We previously described that TCS09 induces the expression of the ABC09 in response to nisin whereas module 12 acts as a sensory system without detoxification function. TCS12 regulates the expression of genes involved in controlling cell surface properties (the *dlt* operon and the *mprF* gene) as well as the orphan ABC transporter encoding genes, whose function is still unknown [1]. Maintaining specificity in TCS signaling is often critical for survival

but how specificity is attained in TCS with a high degree of similarity at the sequence and structural levels remains an unanswered question. In the current study, we focus in two main questions: firstly, if cross-regulation is possible between these highly similar signaling pathways; secondly, what is the function of Orphan ABC. To address these questions we created a collection of single and double mutants deficient in different combinations of Bce-like elements. These mutants were phenotypically characterized in response to AMPs and the expression of the genes of interest was monitored by RT-qPCR after nisin or subtilin challenges. We also performed recombinant expression of RR12 and binding assays (EMSAs) to the Bce regulated promoters [1]. Our results show firstly, that BceRS-like TCS are not essential in *L. casei* BL23. Secondly, that Orphan ABC inactivation increased the resistance to nisin. Thirdly, that cross-regulation between Module09 and Module12 is possible at least at the level of RR-promoter interaction, under the conditions tested. Our results highlight the regulatory complexity within BceRSAB-mediated signal transduction.

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STP15

Induction of the heat shock response inhibits proliferative processes in *Caulobacter crescentus*

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In most organisms the highly conserved DnaK chaperone homologs play a key role in thermal stress adaptation by preventing the accumulation of toxic protein aggregates. In addition to these direct damage-preventing functions, these proteins can also act as negative regulators of the heat shock response (HSR). In various bacteria, DnaK is not only required during heat shock conditions but is essential for proliferation at physiological and low temperatures. In the freshwater bacterium *Caulobacter crescentus*, depletion of DnaK results in a block of DNA replication initiation and a growth arrest. While our previous work revealed how DnaK affects DNA replication, it remains unclear how it promotes cellular growth under non-stress conditions.
Here, we use a combination of genetics and cell biology to elucidate how DnaK function is linked to the regulation of the growth rate at different temperatures.
Since DnaK is well known for its global chaperoning function we hypothesized that its loss might induce strong protein aggregation culminating in a growth arrest. However, *in vivo* aggregation assays revealed that depletion of DnaK at normal growth temperature does not induce a significant increase in the amount of protein aggregates. Instead, our data suggest that induction of the HSR in DnaK-depleted cells is the primary reason for the observed growth inhibition. Reducing the HSR, either by mutating or deleting the heat shock sigma factor RpoH, restores growth in the absence of DnaK in a temperature-dependent manner. Interestingly, the growth defect of DnaK-depleted cells can also be compensated by overexpression of the housekeeping sigma factor RpoD suggesting that high levels of RpoD can outcompete RpoH for binding to the RNA polymerase, hence reducing the HSR. We are currently investigating if *vice versa* high levels of RpoH can outcompete RpoD and in this way directly inhibit growth processes by sigma competition. Alternatively, we are also examining if strong induction of the HSR by RpoH results in increased degradation of growth-promoting proteins through the upregulation of cellular proteases.
In sum, our data provide evidence that in addition to directly protecting the cell against damaged proteins by upregulation of heat shock proteins, induction of the HSR inhibits processes essential for growth. A growth inhibitory role of the HSR might be critical to ensure cellular survival under adverse conditions, when bacteria must rapidly shift gears from a proliferative mode to damage prevention and repair in order to preserve cellular integrity.

STP16

The DxxxQ phosphatase motif of the sensor kinase NreB of *Staphylococcus carnosus*

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Question: The anaerobic nitrate respiration of *Staphylococcus carnosus* is regulated by the two component system NreBC and the nitrate sensor NreA. In the absence of oxygen the sensor kinase NreB is autophosphorylated at a conserved histidine residue (H159). The phosphoryl group is transferred to a conserved aspartate residue of the response regulator NreC which activates the gene expression of the nitrate reductase *narG*. The activity of NreB is modulated by interaction with NreA. Together they form a nitrate/oxygen sensor complex.[1, 2] NreB contains a DxxxQ motif adjacent to the phospho-accepting histidine residue. In the nitrate sensor NarX of *E. coli* this motif is crucial for both the autokinase activity of NarX and the dephosphorylation of the response regulator NarL.[3] It was tested whether the DxxxQ motif regulates NreB activity.
Methods: Variants of NreB with mutated DxxxQ motif were produced by site directed mutagenesis. The variants were tested in an *in vivo narG-lip* reporter gene assay. *In vitro* phosphorylation of the variants as well as the phosphotransfer from sensor to response regulator were tested anaerobically with [γ-^{33}P]-ATP and a phosphoimager.
Results: The DxxxQ variants were tested for their effects on the regulation of the nitrate respiration. While the wild type showed a strong activity under anaerobic growth which was further increased by nitrate, the activity of the variants was strongly influenced. The Q164H variant led to an increased activity under both aerobic and anaerobic conditions independent of nitrate. *In vitro* wild type NreB was phosphorylated and the phosphoryl group was completely transferred to NreC. Although NreC still was phosphorylated the variant Q164H remained partially phosphorylated indicating that the phosphotransfer is affected.
Conclusion: The results indicate that the DxxxQ motif is involved in the activity of NreB. The Q164H variant strongly affected the regulation of the nitrate respiration due to a decreased phosphorylation activity and an incomplete phosphotransfer to NreC.

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STP17

CPH2 – a four colour light sensor produces c-di-gmp as a final output signal

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Questions: The cyanobacterium *Synechocystis* sp. PCC 6803 uses, like many other bacterial species, cyclic di-GMP as a second messenger. Cyanobacteria as well as other phototrophic bacteria encode proteins which contain the c-di-GMP metabolizing domains GGDEF and EAL linked to light sensing domains. One example is the four colour photoreceptor Cph2: it comprises in its whole six domains (GAF-GAF-GGDEF-EAL-CBCR-GGDEF) in which domain 5 (CBCR) is a green/blue light sensing domain, that controls the enzymatic activity of the following GGDEF domain (domain 6). The biological function of this CBCR-GGDEF module has successfully been elucidated in c-di-GMP depending phototaxis.[1] The domains 1 and 2 (GAF-GAF) of Cph2 represent a red/far red light sensing module and have been subject of biochemical research,[2] but the biological function of these two domains is not yet revealed. The red/far-red light sensing by GAF-GAF could on one hand regulate the enzymatic activity of the c-di-GMP metabolizing EAL domain. On the other hand, this red/far-red light could also regulate interactions to other proteins.
Methods: Biochemical enzymatic assays of different Cph2 modules lead to an understanding, how this enzyme integrates different light input signals (red/far red as well as green/blue) to produce a final c-di-GMP output signal. Phototaxis assays as well as *in vivo* and *in vitro* co-purification assays were used to identify possible interaction partners of Cph2.
Results: Upon blue light illumination, domain 6 (GGDEF) enhances the production of c-di-GMP. The EAL domain of Cph2 has been shown to be catalytically active,[1] but a light dependent enzymatic activity still has to be studied. In a yeast two hybrid screen, 11 genes for potential interaction partners were detected.[3] The most significant 6 candidates were genetically inactivated and the constructed mutants were tested for phototaxis under red and far red light. Three candidates showed light-dependent changes in motility responses and were further analysed for possible interactions by *in vivo* and *in vitro* co-purification experiments.
Conclusion: Cph2 is a four-colour light sensor. It integrates different light conditions, to give a final c-di-GMP output signal. Interaction partners further module and possibly fine tune this output.

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STP18

Coordinated control of heme-homeostasis by the ChrSA and HrrSA two-component systems in *Corynebacterium glutamicum*

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Question: Two component systems (TCS) represent a ubiquitous mode bacteria use to sense a variety of different stimuli and respond to environmental conditions. Often bacteria encode up to dozens of different TCS. Therefore maintaining the correct flux of information is crucial for bacterial survival. The Gram-positive soil bacterium *Corynebacterium glutamicum* contains two homologous TCS which are both involved in the regulation of heme homeostasis.
Whereas the HrrSA system is crucial for utilization of heme as an alternative iron source by activating the expression of the heme oxygenase (*hmuO*) under iron limiting conditions, the TCS ChrSA is required to cope with elevated heme levels by activating the expression of a putative heme exporter (*hrtBA*). We are interested in the mechanisms ensuring specificity in signal transduction of these two orthologous systems.
Methods: To study the specificity in signal transduction, growth experiments of the wild type and mutant strains containing specific target gene reporters were conducted. The interaction of the TCS was analyzed using *in vitro* phosphorylation assays, surface plasmon resonance spectroscopy as well as super resolution microscopy.
Results: Activity profiling of the ChrSA and HrrSA target gene reporters and growth experiments with mutant strains revealed that both systems inherit distinct roles in the control of heme homeostasis. Phenotypic analysis of mutant strains as well as *in vitro* phosphorylation assays suggested a high level of cross-talk between the closely related systems. Remarkably, the phosphatase activity appeared to be highly specific for the cognate response regulator [1].
Super-resolution microscopy displayed a homogenous distribution of both sensor kinases in the membrane of *C. glutamicum*. Whereas a constitutive protein level was observed for the sensor kinase HrrS, ChrS expression is strongly induced in response to heme.
Conclusion: Our results emphasize a close interaction of both TCS in the control of heme homeostasis. While both senor kinases (HrrS and ChrS) exhibit a significant level of cross-phosphorylation towards both response regulators (HrrA and ChrA), their highly specific phosphatase activity represents the key mechanism ensuring specific signal transduction. Future studies aim at a comparative analysis of the protein-protein interface of both systems and the identification of specificity residues.

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STP19

PAS4-LuxR solos of *Photorhabdus luminescens*: hormone receptors to sense the host?

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In nature, bacteria live in close association with other organisms, which means that they need to sense each other and to communicate. The best understood chemical language in Gram-negative bacteria is the communication via N-acylhomoserine lactones (AHLs), often produced as an endogenous signal and is called quorum sensing. The prototypical quorum sensing system consists of an AHL synthase belonging to the LuxI-family and a cognate LuxR-family AHL sensor/regulator. Many proteobacteria possess further LuxR-family proteins that lack a cognate LuxI synthase. Initial investigations of those so-called LuxR solos revealed that they have diverse roles in bacteria inter-species and inter-kingdom communication. *Photorhabdus* species harbour a remarkably high number of those LuxR solos (1). Recently, we identified two novel signals of AHL-type LuxR solos in *P. luminescens* and *P. asymbiotica*, respectively. These LuxR solos are part of novel quorum sensing systems that use photopyrones or dialkylresorcinols instead of AHLs as signals (2,3). However, the majority of the LuxR solos in all *Photorhabdus* species have a "PAS4" instead of an "AHL" signal-binding domain. As a first step to investigate the function of PAS-LuxR solos, we generated *P. luminescens* mutants lacking the entire gene clusters *plu0918-0925*, *plu2001-2016*, and *plu2018-2019*, which encode the majority of these receptors. Compared to the wild-type, these mutants exhibited different host specific decrease in pathogenicity, which has given first evidence that PAS4-LuxR receptors might play a central role in sensing the specific insect host. Among others, *plu0258* was identified as a putative target gene of Plu2018 and/or Plu2019. $P_{plu0258}$ activity was inducible with insect homogenate in the wild-type, but not in the mutant lacking PAS4-LuxR receptors Plu2018/Plu2019. This clearly showed that these PAS4-LuxR receptors are involved in inter-kingdom signaling in *P. luminescens*. Stability experiments with insect homogenate revealed that the signaling molecule sensed by Plu2018/Plu2019 could be a hormone-like substance. We currently attempt to isolate this $P_{plu0258}$-inducing signaling molecule from insect homogenate in order identify the first signal of a PAS4-LuxR solo.

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STP20

AntJ is an essential regulator of anthraquinone biosynthesis in *Photorhabdus luminescens*

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Photorhabdus luminescens a Gram-negative, entomopathogenic bacterium is a potent secondary metabolite producer [1,2]. The bacteria produce a set of anthraquinones (AQ). The biosynthesis of the AQs in *P. luminescens* was extensively investigated before[3] and is achieved by a type II PKS and modifying enzymes (AntA-I). However the mechanisms and the signal regulating the production of these pigments remained as unexplored as their biological function.
A DNA protein pull down assay coupled with peptide mass fingerprinting was performed in order to identify transcriptional regulators binding to the promoter region of the AQ biosynthesis gene cluster. The role of candidate regulators in the AQ biosynthesis were examined via deletion and overexpression strains. The absence of one of the identified putative transcriptional regulators, AntJ, resulted in a loss of pigmentation, whereas its overproduction increased AQ production. RNA sequencing analysis showed a downregulation of *antA-I* in the *antJ* deletion strain. We currently perform a YPet reporter assay in order to identify a putative ligand that might bind to the transcriptional regulator to modulate its activity.
Remarkably, *P. luminescens* exists in two different phenotypic forms called primary and secondary that differ in many morphological and phenotypic traits [4]. In primary cells, *antA* promotor activity is heterogeneously distributed at the single cell level [5]. Secondary cells are non-pigmented due to al loss of AQ biosynthesis. Overexpression of *antJ* in secondary cells fully restored pigmentation, which suggests a central role of the novel regulator AntJ in heterogeneous regulation of the AQ production. Identification of the ligand binding to the transcriptional activator AntJ and therefore serving as signal to trigger the AQ production will help to understand the regulation mechanism of AQ biosynthesis and phenotypic heterogeneity in *P. luminescens*.

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STP21

Polyphosphorylation of a *Bacillus subtilis* spore coat protein by a coat-associated Ser/Thr kinase

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The surface of bacterial spores consists of a multi-protein structure, called coat, with functions of protection and interaction with the immediate environment. CotB is an abundant component of the spore coat of *Bacillus subtilis*. During the formation of the coat, CotB protein, of about 46 kDa (CotB-46), undergoes a post-translational modification, which converts it into a species with an electrophoretic mobility of 66 kDa (CotB-66). Two other coat proteins, CotH and CotG, are necessary for the formation of CotB-66. CotH is a morphogenetic protein essential for proper assembly

of the coat, and a structural homologue of Eukaryote-like Ser/Thr kinases. CotB, in turn, shows a C-terminal region with four repeats of a serine/lysine/arginine rich sequence, and two Sm-like domains typically associated with RNA-RNA and RNA-protein interactions, in its N-terminal moiety. While the over-expression of *cotB* in *E. coli* results in the accumulation of a 46kDa protein, the co-expression of *cotB* with *cotG* and *cotH* results in the accumulation of a form of CotB with a mobility of 66 kDa. Furthermore, the absence of CotG or CotH or the production of inactive CotH, bearing a D228Q in its putative active site, prevents formation of CotB-66. We found that CotH undergoes auto-phosphorylation and is insensitive to staurosporine, a broad-spectrum inhibitor. In addition, we show that CotB-46 is phosphorylated by CotH. Therefore, CotH phosphorylates CotB *in vitro*. We discovered that *in vivo* the D228Q substitution results in alterations in the composition, structure and functional properties of spores that approach those seen for a *cotH* deletion mutant. We suggest that during the assembly of the *B. subtilis* coat CotH phosphorylates CotB in its C-terminal, using CotG as a co-factor.

STP22

The translation elongation factor EF-P – regulatory roles during protein translation

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Question: Proline is a poor substrate for the ribosomal peptidyl transferase reaction and distinct XPPX motifs within the nascent peptide chain cause translation arrest. The strength of the stalling effect varies depending on the amino acid up- and downstream of the polyproline cluster. Protein translation is rescued by binding of the bacterial translation elongation factor P (EF-P) to the ribosome, which supports positioning Pro-tRNA at the catalytic center. EF-P is ubiquitous in bacteria and there are orthologs in eukaryots and archaea. Although a remarkably number of proteins contain an EF-P dependent stalling motif, the specific role of those polyproline clusters is still unclear. *E. coli* K-12 contains 95 proteins with at least three consecutive prolines. According to the diversity of these proteins, we suppose different roles of the XPPX motifs. They either can be structural elements or be involved in regulatory mechanisms. Here, we focus on the role of XPPX motifs in three signaling proteins: the histidine kinases EnvZ and EvgS, and the diguanylate cyclase DgcC. Interestingly, the XPPX motif of DgcC is substituted in some *E. coli* strains.
Methods: To assess the role of XPPX motifs in EnvZ, EvgS, and DgcC we constructed *E. coli* K-12 strains, in which the prolines of XPPX were substituted by alanine. Subsequently, we tested the stress response of these mutants and analyzed the copy number of the corresponding proteins. Distribution and conservation of XPPX motifs among approximately 50 *E. coli* strains is analyzed by bioinformatic studies.
Results: Proline to alanine substitutions in XPPX motifs in EvgS and EnvZ lead to mutants that were characterized by altered response to osmotic stress and acid resistance, respectively. Furthermore, the proline to serine substitution in DgcC led to an increased translation level in absence of EF-P and therefore EF-P independency.
Conclusion: It is suggested that in some proteins XPPX motifs influence the protein copy number and hence stress response.

STP23

The role of the cobamide cofactor in the regulation of the organohalide respiration in *Sulfurospirillum halorespirans*.

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Question: *Sulfurospirillum halorespirans* and its close relative *Sulfurospirillum multivorans* are able to conserve energy for growth via organohalide respiration (OHR). The key enzyme in this process is the tetrachloroethene (PCE) reductive dehalogenase (PceA), an iron sulfur protein that harbors a cobamide cofactor (norpseudo-B_{12}) at the active site. Recently, the genome sequences of both organisms became available. The gene encoding PceA is located in close vicinity to a huge gene cluster for cobamide biosynthesis (1). The gene expression of *pceA* is controlled by the enzyme substrate in *S. multivorans* (2). In the absence of PCE the transcription of the *pceA* gene ceased over an unusual high number of more than 100 generations (2). This observation raised the question for the signal, which drives an ongoing expression of the gene and production of functional enzyme while PCE is absent. Since the formation of catalytically active PceA enzyme is strictly dependent on the presence of cobamide, the role of the cofactor in the regulatory network of OHR was investigated in this study. In order to analyze the universality of the long-term phenotype among the reductively dehalogenating epsilonproteobacteria, *S. halorespirans* was included in this survey.
Methods: Both *Sulfurospirillum* isolates were cultivated in a sub-cultivation experiment with nitrate rather than PCE as terminal electron acceptor. The amount of PceA, the enzyme activity, and the transcript of the *pceA* gene was monitored. In addition, the level of cobamide cofactor in the cells was measured as well as the level of transcript of the cobamide biosynthesis genes. The type of cobamide cofactor produced by the cells was manipulated by guided cobamide biosynthesis.
Results: The gene equipment of *S. halorespirans* essential for OHR displays a high sequence identity compared to *S. multivorans*. In the absence of PCE, *S. halorespirans* showed a long-term down-regulation of the OHR similar to its characterized counterpart. The addition of 5,6-dimethylbenzimidazole to cultures of *S. halorespirans* caused the production of nor-B_{12} by the organism, which has been reported previously for *S. multivorans* (3). Under these conditions, the PceA enzyme activity and the amount of PceA was affected and unexpectedly the number of generations needed for the down-regulation of the OHR was reduced significantly in *S. halorespirans*.
Conclusions: From the data obtained, a participation of the cobamide cofactor in the regulatory network of the PCE respiration in *S. halorespirans* was deduced. Currently, investigations are underway to uncover the influence of the type of cobamide cofactor and its intracellular amount on the unusual long-term regulation of the OHR.

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STP24

Sensing and signal transduction by the C_4-dicarboxylate sensor histidine kinase DcuS of *E. coli*

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Question: Two component systems are used in bacteria to respond to the environmental changes and comprise of a sensor kinase and a response regulator. The sensing of C_4-dicarboxylates in *Escherichia coli* is performed by the two component system DcuS-DcuR. The sensor kinase DcuS consists of a periplasmic sensory Per-ARNT-Sim (PAS_P) domain, anchored to the membrane by transmembrane (TM) helices TM1 and TM2, a cytoplasmic PAS (PAS_C) domain and the C-terminal kinase. Effector binding by PAS_P [1] and transmembrane signaling are major issues for understanding DcuS function.
Methods: Changes in TM-topology of TM1 and TM2 were studied by the substituted Cys accessibility method (SCAM).
Results: Transmembrane helix 2 was shifted upon fumarate induction by approximately one helical turn towards the periplasmic side, whereas TM1 remained in a fixed position[2]. The shift corresponds to structural predictions from compaction of the periplasmic binding domain PAS_P.
Conclusion: The transduction of the signal across the membrane is mediated by TM2 of DcuS in a piston-type movement, while TM1 is fixed and serves as an anchor.

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STP25

Computational prediction of ECF σ factor control by a fused C-terminal domain

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The evolutionary conservation of protein-protein interactions is often reflected in statistical co-variations between contacting residues in homologous protein sequences. In recent years this idea has been extensively exploited to predict intra- and inter-domain interactions of proteins, with direct coupling analysis (DCA) being one of the most successful methods. Here used this method to analyze the largest group of extracytoplasmic function (ECF) σ factors, ECF41. In this group the σ factor contains a large C-terminal domain that might function as a fused anti-σ factor [1]. To predict putative residues involved in the interaction between the C-terminal and the σ factor domain, we improved the accuracy of current DCA methods, allowing us to make more reliable predictions even for the limited number (<400) of co-evolved

sequences available. Our analysis predicts that the C-terminal domain, which shares structural similarity with the widespread NTF-2 like protein family, binds both the DNA-binding domain of σ4, as well as the atypically long and conserved linker between the σ2 and σ4 domains. This suggests that the NTF-2 like C-terminal extension constitutes a novel class of anti-σ domains (we termed ASD-III), which inactivates the σ factor by impeding its recognition of the -35 promoter region. Strikingly, the contact predictions in the C-terminal domain can consistently explain previous experiments, in which partial C-terminal truncations lead to the constitutive activation of the σ factor [1]. In the future, experiments will be required to further corroborate our computational predictions.

[1] Wecke T, *et al.* (2012), *MicrobiologyOpen*, 1:194-213.

STP26

Cell signaling PII-like proteins in cyanobacteria

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PII-like proteins are similar and clearly related to classical PII proteins, but nothing known about their functions, targets and regulatory responses. All cyanobacteria analyzed to date contain a GlnB-type PII homologue genes. About 20 % of sequenced cyanobacterial genomes were found to contain a second clear *glnB* paralogue. In addition to *glnB* paralogues, a close examination of available cyanobacteria genomes on CyanoBase revealed further genes with similarity to *glnB* but lacking PII signature sequences, we termed the putative genes products as PII-like proteins. Thus, it is tempting to speculate that PII-like proteins are involved in regulation of different cellular activities, which differ markedly from classical PII proteins in N-metabolism. Even more, the architectural principle of PII superfamily seems to be apparently widely distributed. The available structural information implies that the PII-like proteins have trimeric-protein structures which are highly similar to the classical PII core architecture. Hence, It would be important to find out to which signals PII-like proteins respond, and which targets they regulate for better understanding of cellular function, metabolic responses, and genetic machinery system of different classes of PII/PII-like proteins [1-3]. To figure out *in vivo* cellular function of PII-like proteins, we created different knockout/knockdown mutants to characterize cell physiology of PII-like proteins in three cyanobacterial model organisms (*Anabaena* sp. PCC 7120, *Synechococcus elongatus* PCC 7942, and *Synechocystis* sp. PCC 6803). The PII-like recombinant proteins were used for production of polyclonal antibodies to identify subcellular localization of PII-like proteins. Also, the recombinant proteins were titrated against different effectors molecules to determine the sensory properties of PII-like proteins in comparison to central effector metabolites of classical PII proteins (like, 2-OG). To characterize the interacting partners of PII-like proteins, the tagged PII-like proteins were used, followed by mass spectrometry based pulldown assay to identify potential PII-like targets. Finally, it would be interesting to solve the crystal structures of purified PII-like proteins, to evaluate the building architectural of PII superfamily.

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STP27

The second messenger cyclic di-AMP controls potassium uptake in *Corynebacterium glutamicum* by binding to the RCK domain of the channel protein CglK

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Cyclic di-AMP (c-di-AMP) is a recently discovered nucleotide second messenger found in Gram-positive bacteria, which is involved in the control of many diverse aspects of bacterial physiology such as cell wall metabolism, detection of DNA-damage, potassium uptake, sporulation, and osmotic stress response. In the non-pathogenic *Corynebacterium glutamicum* that, besides its high relevance in biotechnology, serves as a model organism for pathogenic species such as *M. tuberculosis*, c-di-AMP synthesis is catalyzed by the diadenylate cyclase DisA and degradation of c-di-AMP is brought about by the phosphodiesterase PdeA.

Overexpression of *disA* as well as inactivation of *pdeA* severely impaired growth of *C. glutamicum* strains when cultivated in media requiring potassium uptake. Potassium uptake is mediated in *C. glutamicum* exclusively by the channel protein CglK, which consists of the full-length CglK protein and a separate soluble protein harboring only the RCK domain. Binding studies with purified full-length CglK as well as the purified soluble RCK-domain showed that c-di-AMP specifically binds to the RCK domain of CglK. To further analyze effects of c-di-AMP on CglK activity a test system in the potassium uptake deficient strain *E. coli* TK2309 was constructed. *E. coli* TK2309 (pEKEx2-cglK), which carries *cglK* for the *C. glutamicum* potassium channel, is able in difference to the parental strain to grow in potassium deficient, slightly acidic media. *E. coli* does not possess a gene for a di-adenylate cyclase. Upon heterologous expression of *disA* from *C. glutamicum* in *E. coli* TK2309, intracellular accumulation of up to 1.3 mM c-di-AMP was detected in the strain *E. coli* TK2309 (pBad-disA). No growth in potassium deficient medium was observed when expression of *disA* was induced in *E. coli* TK2309 (pBad-disA)(pEKEx2-cglK) by addition of arabinose, indicating that c-di-AMP indeed inhibits CglK activity.
We conclude that activity-control of the *C. glutamicum* potassium channel CglK is mediated by binding of c-di-AMP to the RCK domain, leading to a reduced CglK activity in response to high levels of c-di-AMP.

STP28 – withdrawn

STP29

Identification of the cAMP phosphodiesterase CpdA in *Corynebacterium glutamicum*

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Question: The second messenger cyclic adenosine monophosphate (cAMP) plays a central role in the regulation of important cellular functions in the Gram-positive soil bacterium *Corynebacterium glutamicum*. It serves as effector of the global transcriptional regulator GlxR, a member of the Crp family [1]. When complexed with cAMP, GlxR binds to its DNA target sites and regulates expression of approximately 14 % of all genes in this species [2]. It thus participates in the control of several processes such as respiration, central metabolism or transport. Despite the importance of cAMP, numerous questions regarding the regulation of the intracellular cAMP level and the enzymes that synthesize or degrade cAMP remain to be solved. To date, the adenylate cyclase CyaB is the only cAMP synthesizing enzyme that has been described for *C. glutamicum* [3]. We were especially interested in the degradation of cAMP in this bacterium as this was completely unknown at the beginning of this project.
Methods: Growth experiments were conducted in the Biolector microcultivation system. cAMP phosphodiesterase activity was measured with a coupled enzyme assay monitoring NADH oxidation. Intracellular cAMP concentrations were measured with a cAMP-specific ELISA. Promoter fusions with the venus gene encoding an autofluorescent protein were used for analysis of transcriptional regulation.
Results: We describe the identification of CpdA, a corynebacterial cAMP phosphodiesterase (PDE), with a DELTA-BLAST [4] search using sequences of class II PDEs of other bacteria and lower eukaryotes as query. The cAMP-PDE activity of CdpA was demonstrated both *in vivo* and *in vitro*. Deletion of the *cdpA* gene in *C. glutamicum* resulted in a prolonged lag phase and reduced growth rates on glucose, acetate, citrate, ethanol, or gluconate as carbon sources, while overexpression of *cpdA* had no significant effect on growth. The intracellular cAMP concentration of the Δ*cpdA* strain was increased 8-fold compared to the wild type when cultivated on glucose. Expression of the *cpdA* gene was shown to be positively regulated by GlxR. This effect could be diminished by mutation of a putative GlxR binding site in the *cpdA* promoter.
Conclusion: With CdpA we identified a key enzyme in the control of the intracellular cAMP level in *C. glutamicum* and revealed its important role for growth. On top of that we could demonstrate that the expression of CpdA is transcriptionally activated by the cAMP-GlxR complex, thus providing a feedback loop to counteract elevated intracellular cAMP concentrations.

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STP30

Silencing of cryptic prophage elements by a nucleoid-associated protein in *Corynebacterium glutamicum*

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Question: DNA of viral origin is a major source for strain-specific differences in bacterial species. The acquisition and transfer of phage elements enables organisms to evolve in quantum leaps by taking advantage of phage-encoded beneficial traits, e.g. for the colonisation of new ecological niches. However, the adaption of foreign DNA into the bacterial genome and its integration into host regulatory networks requires strict regulation. Recently, we identified a small nucleoid-associated protein (NAP) CgpS which is associated to prophage DNA in the genome of Corynebacterium glutamicum ATCC 13032. In this study, we set out to analyse the function of CgpS in the control of prophage activity and its impact on the induction of the prophage CGP3, which was previously observed to occur in a small subpopulation (<1 %) even under standard cultivation conditions [1].
Methods: Genome-wide binding profiles of CgpS were analyzed performing affinity purification of CgpS and sequencing of DNA (ChIP-seq) bound by the purified protein. Target regions were validated by electrophoretic mobility shift assays and transcriptome analysis. Induction of the prophage was monitored using real-time PCR or time-lapse microscopy and flow cytometry of reporter strains.
Results: The CgpS-DNA interaction was characterized by ChIP-Seq experiments and showed a preferential binding of the NAP to AT-rich DNA regions. Whereas CgpS accumulated primarily at the cryptic prophage CGP3 element, further potential target genes were identified within the host genome, reflecting the interconnectivity of phage and host regulatory networks. The localization of CgpS to the CGP3 genomic region was further confirmed using fluorescent protein fusions. Remarkably, our studies revealed that cgpS is essential for growth of C. glutamicum ATCC 13032 containing the cryptic prophage CGP3. Inactivation of CgpS was achieved by overproduction of a truncated variant of the NAP led to a strong increase in CGP3 activity which killed the affected cells. Finally, expression of cgpS was shown to complement the phenotype of an Escherichia coli hns mutant, emphasizing an analogous role in the silencing of AT-rich, foreign DNA.
Conclusion: In conclusion, our data reveal the crucial function of the nucleoid-associated protein CgpS for the silencing of phage gene expression and illustrate the interconnectivity of phage and host regulatory circuits.

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STP31

Protein translation machinery is downregulated during heat stress in *Bacillus subtilis*

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Cells of bacterial and eukaryotic organisms survive an otherwise lethal heat shock once they are primed by a mild pre-heat shock, known as thermotolerance. Using *B. subtilis* as a model organism, we investigated the development of thermotolerance by analyzing cell survival, *in vivo* protein aggregate formation and transcriptome changes. Cells of *B. subtilis* respond to the heat shock by increased synthesis of chaperones and proteases which mediate the refolding or removal of unfolded or misfolded proteins and protein aggregates. In these experiments we could identify the thiol stress regulator Spx as a heat shock responsive regulator necessary for thermotolerance development.
In our experiments, we also observed a strong down-regulation of genes e.g. for ribosome biogenesis and translation known to be controlled by stringent response. Furthermore in a *relA* mutant strain, where (p)ppGpp synthesis is elevated and stringent response is turned on, we observed an increased survival to an otherwise lethal heat shock.
According to our current model *B. subtilis* cells respond to heat mediated protein unfolding and aggregation not only by raising the repair capacity (e.g. more (redox) chaperones) but also by decreasing translation involving (p)ppGpp and Spx mediated response to concurrently reduce the load for the cellular protein quality control system.
We will present experiments to further elucidate the molecular and regulatory mechanism orchestrating this stress response.

STP32

The photolyase/cryptochrome member CryB of *Rhodobacter sphaeroides* shows photorepair activity *in vivo*

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The cryptochrome- and photolyase related protein CryB of *R. sphaeroides* was identified as a blue light receptor with signaling and regulating functions, but also shows a contribution to light-dependent photoreactivation after damage by UV-light *in vivo*, attributed to photolyases. These enzymes specifically recognize and repair defined types of DNA damages, usually two different versions of dimerized neighboring thymine bases.
CryB differs from other members of its family in several aspects, most strikingly concerning the composition of the cofactors. Cryptochromes and photolyases bind two light absorbing chromophores. The photo-redox responsive FAD (flavine adenine dinucleotide) is a conserved cofactor and found in all members of this family, and a second variable cofactor serves as an antenna to absorb additional light energy. Additionally, an iron-sulfur cluster was identified as a third, which is unique for this group of proteins, termed CryPro family [1].
We have constructed amino acid substitution variants of CryB by site-directed mutagenesis at relevant cofactor binding residues. These versions have been inserted into a *cryB* knockout strain of *R. sphaeroides* on a plasmid. The strains were analysed for their ability to survive exposure to UV light with subsequent white light illumination to investigate the light-dependent activity of photolyases.
A version which is locked in the oxidized state of FAD is still capable of restoring photoreactivation in Δ*cryB* (survival rate of 70-80 % in wild type and complementation strains compared to non-stressed cells). Lack of the antenna cofactor DLZ has the same effect, while a double mutant shows an impaired photoreactivation comparable to the *cryB* knockout strain (20 % survival).
To investigate the mechanism of photorepair, a photolyase-typical active-site histidine was exchanged to alanine which reduced the survival rate significantly. Currently, we are investigating FAD-free variants to elucidate the involvement of the second light-absorbing cofactor and the iron-sulfur cluster in the repair process.

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STP33

Role of protein phosphorylation in the control of morphological differentiation of *Streptomyces coelicolor* A3(2)

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During morphological differentiation of *Streptomyces coelicolor* A3(2), the sporogenic aerial hyphae are transformed into a chain of more than fifty spores in a highly coordinated manner. Sporulation septation and synthesis of the thickened spore envelope is directed by the *Streptomyces* spore wall synthesizing complex SSSC which resembles the elongasome of rod-shaped bacteria (1). The SSSC also includes the eukaryotic type serine/threonine protein kinase (eSTPK) PkaI, encoded within a cluster of five independently transcribed eSTPK genes (SCO4775-4779).
So far, it is unknown how *Streptomyces* is able to coordinate the simultaneous formation of dozens of sporulation septa during differentiation. Here, we studied whether sporulation septation is controlled by phosphorylation of SSSC proteins.
We inactivated *pkaI* and deleted the complete *SCO4775-4779* cluster. Deletion of *pkaI* alone delayed sporulation and produced some aberrant spores. The five-fold mutant NLΔ4775-4779 had a more severe defect and produced 18 % aberrant spores affected in the integrity of the spore envelope (2). Moreover, overbalancing phosphorylation activity by expressing a second copy of any of these kinases caused a similar defect.

Following co-expression of *pkaI* with either *mreC* or *pbp2* in *E. coli*, phosphorylation of MreC and PBP2 by PkaI was demonstrated and multiple phosphosites were identified by LC-MS/MS (2). By purifying all other SSSC proteins in the presence and absence of each eSTPK, we aim to identify the respective kinase and to determine the specific phosphosites of all SSSC proteins. The effect of protein phosphorylation on the interaction of SSSC proteins is studied by bacterial-two hybrid analyses.
Our data suggest that elaborate protein phosphorylation controls activity of the SSSC to ensure proper sporulation by suppressing premature cross-wall synthesis.

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STP34

Deciphering the function of the redox-sensing system HbpS-SenS-SenR from streptomycetes

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We discovered a novel type of redox-sensing system: the three-component system HbpS-SenS-SenR from streptomycetes. It provides the soil bacteria with an efficient defense mechanism against the toxicity of redox-cycling compounds. Structural and biochemical studies showed that the extracellular HbpS inhibits the autophosphorylation of the membrane-embedded sensor kinase SenS under non-stressed conditions; however, on conditions of oxidative stress HbpS activates SenS activity. SenS in turn phosphorylates the response regulator SenR [1].
We crystallized HbpS and solved its 3D crystal structure that revealed an octomeric assembly which is required for interaction with SenS [2]. Additional studies revealed that HbpS specifically interacts with ferrous ions as well as with the tetrapyrroles heme and cobalamin [3,4]. Recently, we solved the 3D crystal structure of a HbpS-like protein in complex with heme. The crystal structure revealed an unusual *bis*-His heme-coordination by the same histidine residue from adjacent monomers [5].
In order to get insights in to the HbpS-SenS-SenR-mediated transcriptional response, we performed differential RNA sequencing (RNA-Seq) of wild-type *Streptomyces coelicolor* A3(2) and its *hbpS-senS-senR* disruption mutant under non-stressing and oxidative-stressing conditions. RNA-Seq allowed the identification of several differentially expressed genes. Interestingly, some of them are involved in sulfur-related reactions and in the synthesis of cobalamin. We additionally isolated the response regulator SenR. Subsequent gel shift experiments showed that SenR interacts with the regulatory regions of the differentially expressed genes. These findings will be discussed in frame of this presentation.
Since homologs of HbpS-SenS-SenR have been identified in a number of ecologically and medically relevant bacteria, our data serve as a basis to elucidate the role of these homologs.

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STP35

Transcriptomic and proteomic response of *Aspergillus fumigatus* to caspofungin

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Introduction: *Aspergillus fumigatus* is one of the most common human pathogenic fungi and causes a wide range of systemic infections. One therapeutic approach is the use of the lipopeptide antifungal drug caspofungin of the group of echinocandins. It specifically targets the fungal cell wall by inhibiting the synthesis of the polysaccharide β-(1,3)-d-glucan [1]. These changed conditions lead to a fungal response like the adaption of the gene expression and consequently, the protein synthesis.
Objectives: This study aims to detect potential relationships between the transcriptomic and proteomic response of *A. fumigatus* to caspofungin.
Methods: The transcriptomic response of the *A. fumigatus* strain A1163 was measured by RNASeq at 0h, 0.5h, 1h, 4h and 8h after caspofungin treatment. Samples of the proteomic response, taken at 0h, 4h, 8h (synthesised proteins) and at 0h, 24h (secreted proteins) after treatment, were analysed by mass spectrometry. Significantly, differentially regulated mRNA, synthesised and secreted proteins were considered to analyse two potential relationships between the different levels of fungal cell response: The shared response to caspofungin and the shared association with several response pathways.
Results: The comparison of the different cell response levels shows that the overlap of the transcriptomic and proteomic data is relatively low. But different pathway analyses demonstrate the association of the significantly, differentially regulated mRNA, synthesised and secreted proteins with shared and caspofungin-associated pathways. Some of these pathways can only be significantly associated by combining both transcriptomic and proteomic response.
Conclusion: The combination of different OMICs data can help to identify an overall fungal cell response to caspofungin which partially can not be found by analysing either the transcriptome or proteome alone.

[1] Altwasser *et al.* PloS One, 10 (2015):e0136932.

SnMP01

Synthetic Biology approach for the production of D-phenylglycine

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Phenylglycine (Phg) is a rare aproteinogenic amino acid, which only occurs in some natural compounds, such as the streptogramin antibiotics pristinamycin I (PI) and virginiamycin S or the bicyclic peptide antibiotic dityromycin[1]. Sequence analysis of the pristinamycin biosynthetic gene region from *Streptomyces pristinaespiralis* led to the identification of a set of putative Phg biosynthetic genes (*pglA, pglB, pglC, pglD*, and *pglE*)[2]. Successive inactivation of the individual genes resulted in a loss of PI production, whereas production was restored after supplementation with synthetic L-phenylglycine, demonstrating that the *pgl* genes are responsible for Phg biosynthesis[3].
Here we report on the biochemical characterization of the aminotransferase PglE that catalyzes the final reaction step during Phg biosynthesis, which is the transamination from phenylglyoxylate to L-Phg. With regard to the obtained results we postulate a new Phg biosynthesis pathway.
Industrially more important than L-Phg is the enantiomeric isomer D-Phg: D-Phg plays an important role in the fine chemical industry, where it is used as a precursor for the production of semisynthetic β-lactam antibiotics, the antitumor compound taxol or the artificial sweetener aspartame. On the basis of the L-Phg operon from *S. pristinaespiralis* we constructed an artificial D-Phg operon, which we currently express in different host strains to fermentatively produce D-Phg.

1 Al Toma RS, Brieke C, Cryle MJ, Süssmuth RD. (2015) Structural aspects of phenylglycines, their biosynthesis and occurrence in peptide natural products. Nat Prod Rep. 32:1207-1235.
2 Mast Y, Weber T, Gölz M, Ort-Winklbauer R, Gondran A, Wohlleben W, Schinko E. (2011) Characterization of the 'pristinamycin supercluster' of *Streptomyces pristinaespiralis*. Microb Biotechnol. 4:192-206.
3 Mast YJ, Wohlleben W, Schinko E. (2011) Identification and functional characterization of phenylglycine biosynthetic genes involved in pristinamycin biosynthesis in *Streptomyces pristinaespiralis*. J Biotechnol. 155:63-67.

SnMP02

Adapting the SEVA-standard for the needs of *Bacillus subtilis* vectors with exchangeable integration sites

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Bacillus subtilis has the outstanding genetic property of natural competence paired with highly efficient homologous recombination. But so far, most vectors for genome manipulation integrate into a few characterized loci and are combined with a fixed combination of resistance cassettes and additional features such as reporter genes or multiple cloning sites. To fulfill SynBio needs, scientists started working with standardized, reusable parts, such as the Standard European Vector Architecture (SEVA)[1] for *Escherichia coli*.

We aim at adjusting SEVA building blocks to the needs of *B. subtilis* by developing a system to easily create personalized vectors in a standardized manner. Parts from our collection can be assembled with integration loci of choice via the One-Pot "Golden Gate"[2] reaction. Each assembly combines i) two homology regions for integration, ii) one resistance cassette, iii) a multiple cloning site that is flanked by terminators, and iv) an *E. coli* origin of replication.

We designed and created a toolbox for the creation of personalized vectors, featuring 7 *Bacillus* resistance cassettes (bleo, cat, kan, mls, spec, tet, zeo) attached to a pUC18 multiple cloning site with or without a *lacZ*α for blue-white screening. For replication of the final vector in *E. coli*, a low (ROP) or high copy number (ColE1) origin of replication can be chosen. All parts can be assembled via one of five type IIs restriction enzymes (BsaI, BbsI, BtgZI, BsmBI, AarI), depending on the absence of restriction sites in the fragments of choice. Due to its modularity, the *E. coli*-specific vector parts as well as the insert of choice can be exchanged before or after the vector assembly.

We believe that our adaptation of the SEVA-standard provides a powerful and standardized toolkit for the convenient creation of personalized vectors for *B. subtilis*.

1 Silva-Rocha R *et al.*Nucleic Acids Res. 2013 Jan; 41(Database issue):D666-75
2 Engler C *et al.* PLoS ONE 2009 4(5): e5553

SnMP03

Sporobeads – the utilization of *Bacillus subtilis* endospores for protein display

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Bacillus subtilis is a soil bacterium that can form metabolically inactive endospores under nutrient limitations. The process of endospore formation is commenced through an asymmetrical cell division, resulting in the formation of a larger mother cell and the smaller forespore. The forespore provides proteins to protect the DNA against environmental factors like UV-light. Moreover, the spore is encased in three protective layers, the cortex, the coat and the crust, which are produced by the mother cell. The cortex is comprised of peptidoglycan, while the coat and crust are made up of at least 70 different proteins, which are also produced by the mother cell.

Here we provide evidence that the spore surface can be utilized to functionalize the spores, by genetically fusing a gene of interest to a crust protein gene. The resulting spore, a so-called Sporobead, displays a protein with a desired function on the surface, similar to commercially available beads used in filters or in the laboratory. The possible applications of our biological beads are very widespread, from enzymatic functions to filter-function using proteins able to bind to wanted (toxic) compounds.

SnMP04

Design and assembly of large DNA sequences for applications in synthetic biology

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Efficient assembly of large DNA fragments is critical to answer questions in synthetic biology. Often, their sequences are desired to have specific DNA motifs at defined sites but not at others. Other DNA motifs might need to be excluded, as for example restriction sites. Here we present the computer program MARSeG that generates degenerate DNA sequences that do not contain any occurrence of sequences from a user-defined list of DNA motifs. Furthermore, these sequences can also contain defined segments that will not be changed, which allows for more complex designs. For efficient cloning of designed library sequences we modified vectors of the widely used MoClo system (1). Inclusion of the *ccdB* gene results in a highly efficient whole-batch cloning. Furthermore, changing the replication origin to *oriR6K* enabled selective replication of the vectors in *Escherichia coli* strains that contain the Pir protein to facilitate chromosomal integration in *E. coil pir*-strains. Using MARSeG designed sequences and the new vectors we assembled a Fluorescence Repressor Operator System (FROS) array. The periodicity of its DNA sequence allows for the hierarchical assembly from a single primer pair. The FROS system allows visualization of the position and dynamics of genomic loci when integrated into the genome. Indeed, we could use this system to count gene loci based on fluorescence foci in *E. coli*. The design and assembly pipeline presented here can also be used for the efficient, fast and cheap assembly of other large DNA molecules such as synthetic chromosomes (2, 3).

1. Weber E, Engler C, Gruetzner R, Werner S, Marillonnet S (2011) A modular cloning system for standardized assembly of multigene constructs. PLoS ONE 6(2): e16765
2. Messerschmidt S. J., Kemter F. S., Schindler D., Waldminghaus T. (2015). Synthetic secondary chromosomes in *Escherichia coli* based on the replication origin of chromosome II in Vibrio cholerae. Biotechnol. J. 10(2):302-14
3. Schindler D., Waldminghaus T. (2015). Synthetic Chromosomes. FEMS Microbiol Rev. DOI: http://dx.doi.org/10.1093/femsre/fuv030

SnMP05

Characterization of the EF-P arginine rhamnosyltransferase EarP

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During the elongation phase of translation ribosomes stall at polyproline sequence motifs. The eukaryotic and archaeal elongation factor 5A (e/aEF-5A) and its prokaryotic ortholog elongation factor P (EF-P) are capable of alleviating this arrest and enable efficient translation. In order to work properly, both elongation factors depend on post-translational modification. While deoxyhypusination of an invariant lysine residue of EF-5 (IF-5A) is strictly conserved, bacteria use diverse analogous strategies. *Shewanella* and *Pseudomonas* species e.g. employ the recently discovered glycosyltransferase EarP. EarP catalyzes the transfer of rhamnose to an arginine using dTDP-β-L-rhamnose as a nucleotide sugar donor. N-linked protein glycosylation is almost exclusively reported to occur on asparagine residues whereas rhamnose linkage has not been described for arginine before. Thus arginine rhamnosylation constitutes a novel form of sugar modification that is achieved by a yet unknown reaction mechanism.

We used fold recognition via Phyre2 and the I-TASSER server to generate a three-dimensional homology model of EarP and identified a clamp-like structure that is made up by two opposing Rossman-like domains. It is suggested that EarP belongs to the GT-B superfamily of glycosyltransferases. Using the COACH metaserver for molecular modelling and *in vivo* mutational analysis we identified the nucleotide sugar donor binding pocket within the C-domain as well as the functionally important amino acids F191, Y193, F258, R271 E273, D274, S275, Y291 and H298. Using a bacterial two-hybrid approach we were able to detect interaction of EarP with its corresponding ligand acceptor EF-P providing the basis for studies of the protein binding sites.

SnMP06

Post-translational modification strategies to activate translation elongation factor P

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EF-P alleviates ribosome stalling at polyproline stretches by binding to the ribosome between the peptidyl-tRNA binding site (P-site) and tRNA exiting site (E-site) and thereby stimulating peptide bond formation. In bacteria such as *Escherichia coli* a positively charged lysine (K34) at the

tip of the loop region in domain I protrudes towards the peptidyl-transferase-center but has to be post-translationally modified with (*R*)-β-lysine to β-lysinyl-lysine to enhance peptide bond formation. Alternatively, β-proteobacteria have evolved an alternative strategy, namely arginine rhamnosylation by a novel type of glycosyltransferase called EarP, indicating a certain degree of freedom in post-translational activation of EF-P. We now aim to identify novel bacterial modification systems as well as construct synthetic EF-P variants which are constitutively active, independent of species-specific posttranslational modifications.

As a first step in the identification of novel naturally occurring post-translational modifications of EF-P, we selected bacterial strains of *Enterococcus*, *Mycobacterium* and *Helicobacter*, which do not have any of the known EF-P modification enzymes. We analyzed the endogenous EF-P proteins by mass spectrometry and tested the functionality of the unmodified proteins in an *E. coli* reporter strain lacking its own post translational modification system. To generate synthetic EF-P variants we used the "Pyrrolysine Amber-Suppression System" to replace the natural β-lysinyl-lysine moiety by *ε-N*-propyl-, *ε-N*-butyryl-, *ε-N*-valeryl-, *ε-N*-crotonyl- and 2THF-lysine, respectively. Afterwards the resulting synthetically modified EF-Ps were tested for functionality *in vivo* using a β-galactosidase reporter system.

SnMP07

Synthetic microbial pathway for (*R*)-benzylsuccinate production

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Some denitrifying, Fe(III) or sulfate reducing bacteria degrade toluene under anaerobic conditions. The first intermediate of the degradation pathway is (*R*)-benzylsuccinate, an aromatic compound of potential biotechnological interest, e.g. in the production of polymers[1]. We attempt to redesign the metabolism of standard bacteria such as *Escherichia coli* to establish the production of this intermediate in a synthetic process.

We designed a biosynthetic pathway for benzylsuccinate from the fermentation product succinate and exogenous benzoate, using the toluene degradation enzymes in reverse direction, since all of these have been shown to be reversible and active under aerobic or anaerobic conditions.

To enter this synthetic pathway, the precursor benzoate must be transported into the cytosol and activated to benzoyl-CoA. This has been established by cloning the genes for a benzoate transporter and for a benzoate-CoA ligase or alternatively a succinyl-CoA:benzoate CoA-transferase together in an expression vector. This corresponds to a metabolic module for benzoyl-CoA generation, which is useful for many other biosynthetic purposes.

The reverse β-oxidation cycle for benzylsuccinate production from benzoyl-CoA and succinyl-CoA was introduced by cloning the *bbs*-operon (for beta-oxidation of benzylsuccinate)[2] from *Geobacter metallireducens* into a second expression vector. First results on the production of benzylsuccinate and its optimization during different production conditions will be shown. These results establish a general strategy of using enzymes from degradation pathways "in reverse" to compose novel biosynthetic routes for biotechnological purposes.

[1] Guo B, Xu J (2010) Poly(butylene succinate) and its copolymers: research, development and industrialization. Biotechnology Journal 5:1149-1163
[2] Leuthner B, Heider J (2000) Anaerobic Toluene catabolism of *Thauera aromatica*: the *bbs* operon codes for enzymes of beta oxidation of the intermediate benzylsuccinate. Journal of bacteriology 182 (2):272-279*)*.

SnMP08

***Rhodobacter capsulatus* – an alternative microbial platform for terpenoid production**

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Questions: Terpenoids belong to the largest group of natural products, which are characterized by a very high functional and structural variety. Many essential oils containing terpenoids have therapeutical effects, e.g. stimulating the blood flow or exhibiting anti-microbial, anti-fungal, anti-viral, anti-parasitic, anti-inflammatory or anti-cancer activities. Therefore, they are valuable target compounds for the pharmaceutical industry. Since the production of terpenoids in their natural producers, i.e. mostly plants, is not very cost-efficient and convenient, the heterologous production in microorganisms has emerged as a promising alternative. Considering the required precursor supply for the production of terpenoids, it is useful to employ production organisms which are natural terpenoid producers. We aim here to evaluate the suitability of the purple bacterium *Rhodobacter capsulatus*, a facultative phototrophic bacterium, capable of naturally synthesizing the tetraterpene carotenoids spheroidene or spheroidenone in high amounts, therefore it provides the precursors geranyl- (GPP), farnesyl- (FPP) and geranylgeranyl-pyrophosphate (GGPP) through the DXP- (1-deoxy-D-xylulose-5-phosphate) pathway.

Methods: Here, we report on the optimization of the production of sesquiterpenes in *R. capsulatus* exemplarily with the valencene-synthase (oCnVS) from *Callitropsis nootkatensis*. Production of valencene was comparatively evaluated using the expression vectors pRhokHi-2 (weak promoter P_{aphII}, constitutive expression), pRhofHi-2 (weak promoter P_{fru}, inducer: fructose), pRhotHi-2 (strong promoter P_{T7}, inducer: fructose), and pRhon5Hi-2 (P_{nif}, very strong promoter, induction: NH_4^+ limitation). Moreover, co-expression of DXP-pathway-limiting enzyme IspA was tested. Phototrophically grown cultures were overlayed with *n*-dodecane to entrap valencene and enabled straightforward sampling for GC-MS analysis.

Results: We could show that the P_{nif}-expression system is the best choice for effective valencene production and that IspA co-expression significantly improves product formation, in that way the supply of precursors GPP and FPP is increased.

Conclusion: Due to its specific metabolic capacity, *R. capsulatus* appears to be an especially suitable production host for sesquiterpenoid production. The comparative analysis demonstrated that the promoter strength of the expression system as well as the genetic background of isoprenoid precursor biosynthesis play important roles for efficient product formation in *R. capsulatus*.

SnMP09

***P. putida* for plastic monomer utilization**

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All over the world plastic is used and will end up as waste, either on land or in the ocean. These plastics (mainly fossil-based) are mostly not biodegradable on a practical timescale. Poly- (ethylene terephthalate) (PET) is wide spread with an annual production of over 28 million tons per year. Although its recycling is very efficient, currently less than 30 % of all PET is recycled in Europe. Also Polyurethane (PU), in its many forms, is used extensively with an annual production of around 12 million tons per year. Due to the fact that many PU forms are not thermoplastic, this polymer is hardly recycled at all. We propose to use plastic waste as a novel substrate for biotechnology. In order to use PET and PU as substrates these polymers first have to be hydrolyzed, resulting in monomers such as adipic acid (AA), 1, 4-butanediole (BDO), terephthalic acid and diisocyanates. Ethylene glycol (EG) is also a major component of these polymers.

We aim to engineer the metabolism of PET and PU monomers in *Pseudomonas putida*. It´s complex versatile metabolism gives *P. putida* the ability to tolerate environmental stress. The *Pseudomonas* strain used in this work is *P. putida* KT2440, which is one of the first biosafety strains, and has the GRAS status (Generally Regarded As Safe). First tests could show its tolerance towards AA, BDO and EG.

The metabolism of EG in *P. putida* JM37 is reported (Muckschel, *et al.*, 2012) starting with the conversion of EG to glyoxylate which is regulated by the activating regulator AgmR. Further metabolism of glyoxylate proceeds through three parallel pathways: 1] coupling to succinate via the reverse reaction of isocitrate lyase (AceA), 2] forming malate with acetyl CoA via the malate synthase (GlcB), and 3] conversion of two EG molecules to tartronate semialdehyde by tartronate semialdehyde synthase (Gcl). The first two pathways only yield a net conversion of EG to two CO_2 with concomitant generation of energy equivalents. Although *P. putida* KT2440 possesses the genes encoding all of these enzymes, it is unable to grow on EG as sole carbon source. Therefore, the expression of the *gcl*, *glcB* and *aceA* will be tuned in order to balance the distribution of carbon into the three possible pathways. Alternative pathways will also be discussed.

1 Muckschel, B., Simon, O., Klebensberger, J., Graf, N., Rosche, B., Altenbuchner, J., *et al.* (2012) Ethylene Glycol Metabolism by Pseudomonas putida, *Applied and Environmental Microbiology* 78: 8531-8539.

SnMP10
Strain engineering for enhanced rhamnolipid formation in recombinant *P. putida*
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Rhamnolipids are promising biosurfactants for various applications. For instance their tensio-active properties can be exploited when added to cleaning agents or in bioremediation. In nature rhamnolipids are mainly produced by the opportunistic pathogen *Pseudomonas aeruginosa*, which is applied in fermentation processes to gain the product with plant oils as sole energy and carbon source. However, this evokes burdens like difficult downstream processing and safety arrangements.
The recombinant production of rhamnolipids in *Pseudomonas putida* KT2440 in which oils as substrate have been replaced by glucose could be proved to be a safe and competent alternative [Wittgens *et al.*, 2011]. Additionally, this host is a GRAS organism which is fully sequenced and the metabolic network is well understood. We thus introduced the relevant genes for rhamnolipid formation into strain *P. putida* KT2440 and into an engineered derivative for enhanced heterologous gene expression [Martinez-García *et al.*, 2014] encoded on a plasmid and integrated into the genome. We also engineered *P. putida* for enhanced precursor supply and decreased by-product formation.
We obtained mutant strains of *P. putida* KT2440 with constitutive expression of the genes relevant for rhamnolipid formation. It could be shown that product titers can be raised by optimized gene expression in combination with strain engineering. The chromosomal integration of only one copy of the expression cassette yields titers competitive to those with plasmid encoded copies.
The approach to produce rhamnolipids in a system consisting of an expression cassette carrying the relevant genes in a non-pathogenic host turned out to be promising for further modifications. *P. putida* as a bacterial production workhorse can be further engineered towards product formation and thus exploited for other difficult products.

Wittgens A., Tiso T., Arndt T. T., Wenk. P., Hemmerich J., Müller C., Wichmann R., Küpper B., Zwick M., Wilhelm S., Hausmann R., Syldatk C., Rosenau F. and L. M. Blank (2011). Growth independent rhamnolipid production from glucose using the non-pathogenic *Pseudomonas putida* KT2440. *Microb Cell Fact*, 10.
Martinez-García E., Nikel P. I., Aparicio T. and V. de Lorenzo (2014). *Pseudomonas* 2.0: genetic upgrading of *P. putida* KT2440 as an enhanced host for heterologous gene expression. *Microb Cell Fact*, 13.

SnMP11
Development of novel orthogonal genetic circuits based on Extracytoplasmic function (ECF) σ factors
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The rational design of synthetic circuits is often restricted by cross-reactions between circuit components and physiological processes within the heterologous host. In addition, to date most synthetic biology applications rely on a limited set of building blocks consisting of a handful of transcriptional regulators. Our aim is to overcome these restrictions by building synthetic circuits based on Extracytoplasmic function (ECF) σ factors. ECFs are the smallest, simplest and most abundant alternative σ's that specifically recognize orthogonal promoter sequences. For the modular assembly of synthetic ECF circuits in *Escherichia coli*, we generated a toolbox of ECFs, their cognate promoters and anti-σ factors, as well as constitutive and inducible promoters, reporter genes and terminators. After a quantitative evaluation of simple ECF switches under different growth conditions, we used a computational modeling approach to predict the function of more complex ECF circuits. As a first benchmark, we show preliminary results on the rational design and construction of an autonomous timer circuit, which sequentially activates a series of target genes with a defined time delay. Such a circuit could prove useful for biotechnological applications, in which the yield from biosynthetic pathways often heavily relies on a proper timing hierarchy among the expression of individual pathway components.

SnMP12
Implementation of an efficient synthetic carbon fixation cycle *in vitro* and *in vivo*
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Question: Increasing CO_2 emissions requires society to come up with ways of reducing net CO_2 emission via CO_2 fixation. Synthetic biology allows the creation of novel pathways for CO_2 fixation which are advantageous to those present in nature. We propose cyclic synthetic CO_2 fixation pathways which take advantage of the efficient principle of reductive CO_2 fixation, while providing a constant flux of precursors to the central carbon metabolism of selected host organisms. In a first step, the *in vitro* reconstitution of one of these synthetic cycles, termed CETCH pathway, will validate its feasibility. The *in vitro* characterization of this synthetic CO_2 fixation pathway is then followed by its *in vivo* implementation into the alphaproteobacterial model organism *Methylobacterium extorquens*.
Methods: Following purification and characterization of all relevant proteins, the complete CETCH cycle was reconstituted in an *in vitro* platform. Subsequently, cycle intermediates and products were analyzed by liquid chromatography-mass spectrometry. Enzyme activity assays and ^{13}C isotope labeling were used to confirm expression and functionality of this pathway *in vivo*.
Results: Our proposed cycle comprised of twelve purified enzymes was successfully reconstituted *in vitro*. In addition, cofactor regeneration systems were established for *in vitro* characterization of the pathway. Heterologous expression of the majority of proteins in *M. extorquens* was confirmed and their activity was demonstrated by enzyme assays.
Conclusions: Reconstitution of artificial CO_2 fixation pathways *in vitro* is an important breakthrough. This platform allows continuous optimization of the pathway with regard to protein stoichiometry, side-product formation, and cofactor balancing. *In vivo* implementation into *M. extorquens* is ongoing and is expected to yield a synthetic autotrophic organism.

SnMP13
Mechanistic versatility of Corynebacterineae Type III polyketide synthases
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Investigation of the dynamic bacterial metabolome and associated pathways is currently the biggest challenge in understanding microbial physiology. Microbial genome sequencing projects in the past two decades have revealed an unanticipated variety of metabolic, cellular capabilities. In recent years, advances in developments of molecular tools have provided deeper insights into the metabolic pathways for fine-tuned, multifaceted polyketide machinery in bacterial systems. The complex cell wall of members of Corynebacterineae is partly generated by the diversity of lipids that have been attributed to virulence. The first decade after genome sequence revealed biosynthetic pathways and novel proteins like polyketide synthases (PKSs) to be involved in production of virulent lipids many of which are essential for survival. Biosynthesis of small lipidic molecules in Corynebacterineae is still poorly understood. These molecules are crucial for bacterial physiology and pathogenesis. A recently discovered PKS in bacteria that belong to the plant chalcone synthases (CHSs) superfamily of condensing enzymes are type III polyketide synthases (PKSs) that yield complex lipids by repetitive condensation reaction of simple acyl-CoA thioesters. Recent studies have established the role of type III PKS in cell wall modification by producing phenolic lipids in Corynebacterineae which includes *Mycobacterium*, *Nocardia*, *Rhodococcus* and *Streptomyces* genus. The presence of metabolic gene clusters encoding biosynthetic proteins for such phenolic lipids across several bacterial species reveals a new evolutionarily conserved process in microbial physiology. Long chain phenolic lipids (alkylresorcinols and alkylpyrones) replace membrane phospholipids in dormant Azotobacter cells and confer antibiotic resistance in *Streptomyces*. Alkylphloroglucinols are crucial signaling molecules essential for differentiation and development of Dictyostelium molds. Our biochemical, mutational and structural studies provide evidence for an unanticipated potential of these proteins to cyclise a common biosynthetic intermediate to generate chemically and structurally distinct metabolic entities utilizing a single catalytic site and limited pool of precursor molecules. Our study provides novel insights into the functional characteristics of type III PKSs and revealed interesting clues to the mechanistic programming in these proteins and the possible role of small lipid molecules in Corynebacterineae physiology.

SnMP14
Theoretical assessment of context-dependence in synthetic gene regulatory circuits
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Synthetic biology aims at applying rigorous engineering principles to biology. However, even in simple bacterial cells the function of synthetic genetic circuits often depends on their genetic and physiological context, thereby challenging this rational design approach. One major origin of context-dependence derives from sharing the transcription and translation machinery with the host. Here, we address the problem of how to preserve the functionality of a heterologous circuit based on Extracytoplasmic function (ECF) sigma factors. Since these alternative sigma factors compete for a finite pool of RNA polymerases within the cell, we computationally predict an `orthogonality threshold' for transcription parameters, beyond which the heterologous circuits affect endogenous transcription and vice versa. Preliminary experimental data support these predictions and confirm a regime in which different ECF sigma factors can be used simultaneously without affecting the host. In doing so, our study guides the rational design of more complex synthetic circuits consisting of multiple, well-characterized biological parts.

SnMP15
Engineering a glycolytic shunt for carbon degradation of *Escherichia coli* K12 by gene deletion and introduction of Fructose 6-phosphate Aldolase
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Question: Mutations in genes for key enzymes of the Embden-Meyerhof-Parnas route (EMP) have a major impact on growth behavior and survival of cells on distinct C-sources. *Escherichia coli* K12 mutants deficient in phosphofructokinase (PfkA, PfkB) activity are impaired in the use of C-sources entering the cell at or above the level of fructose 6-phosphate (F6P) [1]. Similarly, removal of the phosphoglucose isomerase (PGI) resulted in impaired growth on sugars entering the cell at the level of glucose 6-phosphate (G6P) [2] while a knock out of the G6P-dehydrogenase gene (pentose phosphate pathway) caused only minor effects in growth [3]. A native F6P-Aldolase (FSA) of *E. coli* K12 with unknown physiological function cleaves F6P to dihydroxyacetone (DHA) and glyceraldehyde 3-phosphate and could thus create a glycolytic bypass for carbon degradation [4,5]. We investigate a possible glycolytic shunt using F6P-Aldolases.
Methods: *E. coli* LJ110 triple mutants MT2 (lacking PfkA, PfkB and PGI) and GL3 (deficient in PfkA, PfkB and G6PDH) were created by marker-less gene deletion [6]. The strains were characterized and used for analysis of cells overexpressing FSA wildtype (WT) gene or its variant A129S.
Results: Both strains grew on D-fructose but not on D-glucose. Also no growth was found for MT2 on D-mannitol (C-source entering the EMP at the level of F6P). The growth defects of GL3 in D-glucose and MT2 in D-mannitol could partly be restored by expression of the plasmid FSA variant A129S (AS) but not by the WT gene. FSA A129S overexpression and DHA formation were confirmed by protein and HPLC analysis. By long-term cultivation the generation time of MT2AS could significantly be reduced. 2D-gel analysis indicated a strong FSA A129S overexpression to cause the advance. Whereas MT2 - when grown on rich media with added bile salts - showed drastically reduced cell counts (4 orders of magnitude). The effect of bile salts on MT2 suggests a lack of sedoheptulose 7-phosphate formation, which is needed for the LPS layer.
Conclusion: Restored growth features by FSA A129S expression of MT2 in mannitol and GL3 in glucose strongly indicate that the glycolytic shunt was functional. We propose that F6P was accumulated by MT2 and GL3 when grown on mannitol and glucose respectively. Viability was restored in the A129S strains.

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SnMP16
Towards synthetic RNA devices in cyanobacteria
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Post-transcriptional gene regulation by small RNA molecules (sRNA) is a widespread mechanism for controlling gene expression in eukaryotic and prokaryotic organisms. Due to their favourable kinetic properties and computable structural characteristics, sRNA molecules might serve as useful tools for the rational programming of cellular and inter-cellular molecular networks.
We attempt to develop a newly designed toolbox for cellular computing which will be created following a three-step process: (i) rational design and analysis of RNA-based devices (RNAdev) using computer based approaches, (ii) selecting best performers *in vitro* within highly parallel microfluidic reactors, and (iii) integrating and testing them in living bacterial cells. The synthetic RNAdevs will be regulated by diverse inputs and controlled via fluorescent protein expression as well as via cellular community behavior. Functional RNAdevs will then be integrated into RNA networks *in vivo* to perform complex logical operations. We ultimately aim to exploit these RNA networks for metabolic engineering in *E. coli* and ultimately cyanobacteria.

SsMP01
Measuring the metabolic burden caused by a heterologous load in *Escherichia coli*
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Question: In biotechnological approaches it is often necessary to introduce a multiplicity of genes into a bacterial host system as e.g. *Escherichia coli.* This additional load can lead to metabolic stress, as bacterial cells have a determinate capacity regarding transcriptional, translational and energetic resources. This phenomenon is referred to as Metabolic Burden. Our project addresses the metrological registration, quantification and evaluation of the cellular response of *E. coli* during production of recombinant enzymes. We plan to investigate how cells overcome an additional load, which results in a burden.
A mathematical model which will not only include metabolic fluxes but also transcriptional and translational processes will be designed based on the derived data. This model should be able to calculate and predict energy and resource requirements for a heterologous burden and consequently estimate if and to what extent inserted genes can be expressed.
Methods: First, we needed to establish an indicator system for measuring the metabolic burden. This construct should show proportionality between reporter expression and availability of cellular resources. To this end, we introduced a reporter plasmid expressing the fluorescent protein mCherry under the control of a housekeeping promoter. Therefore, it was necessary to identify suitable housekeeping promoters that are not regulated. Expression from these promoters should only be restricted by the available transcriptional and translational resources. Additionally, we introduced a second plasmid that provides a controllable load. The used pSEVA system supplies resistance cassettes and origins of replication in a highly modular way.
Results: We screened the engineered *laclp* and *gyrBp* as well as *helDp* and *proCp* different native E. coli promoters, in order to find a constitutively active one. In the end we chose two promoters for our reporter system, *laclp* and *gyrBp*, as both promoters are neither dependent on regulation, growth rates nor cultivation parameters such as temperature or substrate. As burden plasmid, which introduces the metabolic load into the cellular system, we used a pSEVA plasmid with tight XylS/Pm promoter that can be induced at different levels and gives variability in load intensity. As burden protein we chose the model protein eGFP. We have performed first experiments to test our system and to monitor and describe the metabolic burden.
Conclusion: We have created a systematic approach for understanding the metabolic burden of *E. coli* regarding cellular resources. This system enables us to systematically analyze genes in respect to their replicative, transcriptional, and translational burden. Finally, with our system it is possible to investigate the impact of additional components, such as antibiotics and inducers.

SsMP02

Transcriptome analysis of *Gluconobacter oxydans* 621H by RNAseq

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Question: The ability to incompletely oxidize a variety of carbohydrates in the periplasm enable the use of the acetic acid bacterium *Gluconobacter oxydans* for several industrial applications. Unfortunately, a broader use is limited by a low biomass yield. Besides the periplasmic oxidation of substrates resulting in low carbon source availability for the cytoplasmic metabolism, a non-functional Embden-Meyerhof pathway and an incomplete citrate cycle also prevent a higher biomass production. Here we applied RNA sequencing to characterize the transcriptome of *G. oxydans* 621H and to identify weakly expressed genes.
Methods: For whole transcriptome analysis from different growth conditions, *G. oxydans* cells were grown in complex medium with mannitol or glucose under non-stress and selected stress conditions. Total RNA was isolated, depleted of rRNA and subjected to a strand-specific library preparation followed by sequencing (MiSeq, Illumina). Data analysis was performed using CLC Genomics Workbench.
Results: Analysis of the RNA sequencing data from *G. oxydans* 621H revealed expression of about 98 % of all annotated genes under the conditions tested. Mapping of the FPKM expression values to the central carbon metabolism of *G. oxydans* 621H revealed moderate to high expression of genes encoding glycolytic enzymes (FPKM 110-733), somewhat higher FPKM values of genes encoding enzymes involved in the pentose phosphate pathway (FPKM 236-1109), and relatively low FPKM values for genes of the Entner-Doudoroff pathway (FPKM 64-81). Lowest expression values were observed for several genes of the citrate cycle (FPKM 34-406). RNA sequencing data also suggest an organization of 967 genes (38 %) in 364 operons. Furthermore, about 325 antisense transcripts (13 %) and about 100 sRNAs were detected.
Conclusions: The application of RNA sequencing revealed global insights into the transcriptome of *G. oxydans* 621H under different conditions. The data support further understanding of regulation and metabolism in *G. oxydans* 621H, indicate potential bottlenecks in the metabolism and support metabolic engineering approaches.

SsMP03

A transcriptome meta-analysis proposes a novel biological role of the antifungal protein AnAFP in *Aspergillus niger*

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Question: Although *Aspergillus (A.) niger* is used since decades in industrial biotechnology for the production of organic acids and proteins, it largely depicts a black box and we are far from understanding how most of the internal cellular processes work on the molecular level. However, the availability of its genome sequence and hundreds of microarray data for this fungus make it now feasible to shed light into this black box. Our interest in AnAFP is due to the fact that the growth-inhibitory effect of the protein and its homologs from other filamentous *Ascomycetes* seems to be restricted to fungi. No detrimental effects have been observed against bacterial, plant and mammalian systems, making this group of proteins interesting for application in red, green and yellow biotechnology.
Methods: We have recently established a database that stores 377 high-throughput microarray data for *A. niger*. The database includes 158 different cultivation conditions related to carbon source and carbon availability, nitrogen metabolism, conditions related to stress, temporal and spatial stages during its asexual life cycle and many more. We have performed a transcriptome-meta analysis of this database, which enabled us to zoom into the gene expression networks and physiological processes under which AnAFP is expressed.
Results: The corresponding transcriptome meta-analysis of *A. niger* suggests a novel prominent biological role of AnAFP. Remarkably**,** *anafp* gene expression is apparently regulated in a non-defense manner. Instead, upon carbon starvation, *anafp* is strongly upregulated and its expression profile resembles that of genes involved in nutrient mobilization and with a predicted role for autophagy. In addition, *anafp* expression strongly increases when the mycelium becomes committed to asexual development. Compared to the wild type, its expression is more than two- to tenfold upregulated in both a *ΔbrlA* or *ΔflbA* background, respectively. As the *flbA* mutant depicts an autolytic phenotype, we propose AnAFP has a function during the asexual life cycle of *A. niger* and is somehow linked to autophagic processes during normal development.
Conclusion: Our in-house transcriptomic database depicts a valuable tool which enabled us to zoom into the gene expression networks and physiological processes of *A. niger*. Further analysis of this database will definitely help to increase our knowledge of the complex regulation of *A. niger's* gene network.

SsMP04

An optimized bioinformatic workflow for metaproteomics of biogas plants

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Question: Metaproteomics provides insights into microbial communities on the functional as well as on the taxonomic level. Identification of proteins with mass spectrometry depends on adequate sequence databases. However, publicly available databases such as UniProtKB/SwissProt or UniProtKB/TrEMBL do not represent the genetic information in microbial communities very well. Therefore, metagenomes are increasingly used as sequence databases for protein identification in metaproteomics. Combining metagenomes of similar biocoenosis for improved identification, however, comes along with an increasing false discovery rate eliminating potential significant protein hits.
Methods: Metaproteomes of industrial-scale biogas plants were searched with the recently published software MetaProteomAnalyzer[1] against UniProtKB/SwissProt as well as against single or combined metagenome databases. The search results were compared regarding the number of identified proteins and the redundancy of sequence data.
Results: The use of metagenome databases generally increased the identification of spectra, peptides and proteins representing the major taxonomies and metabolic pathways in biogas plants[2,3]. Compared to the use of UniProtKB/SwissProt two- to three-fold increase in the identification of the spectra was achieved when the respective metagenome was used. Searches against foreign metagenomes also showed improved identifications. However, searches against combined metagenomes did not improve the number of identifications. The reason was the increased redundancy of the combined databases causing increased numbers of false positive hits. Two strategies seem to be useful: (i) searching against single metagenomes and subsequent combination of results and (ii) removing redundancies from database before searching.
Conclusion: In summary, improvement in mass spectrometry allows the measurement and identification from nearly all environmental samples. Sequenced metagenomes are a valuable alternative to public databases. In order to fully exploit the potential of metaproteomics in analysis of microbial communities, the bioinformatic workflow has to be adapted to the special requirements of metaproteomics.

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SsMP05

Spatial organization of the bacterial transcription and translation machinery in fast growing *E. coli* under drug treatment.

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In fast growing bacterial cells, all processes such as transcription, translation, DNA replication and segregation occur in parallel in one single cellular compartment. The organization of these machineries and their coordination among each other matching all processes remains largely unclear (Xie Annu Rev Biophys 2008, Gahlmann Nat Rev Microbiol 2013, Stracy FEBS Letters 2014, Huang Curr Opin Microbiol 2015).
In recent years, structural as well as dynamical single molecule localization microscopy studies on DNA, RNA polymerase and ribosomes revealed characteristic sub-cellular distributions. In *E. coli*, DNA can form heterogeneous nucleoid structures (Spahn JSB 2014, Spahn MAF 2015), RNA polymerases may cluster, especially on the nucleoid surface (Endesfelder Biophys J 2013, Bakshi Biophys J 2013, Stracy PNAS 2015),

and, in fast growth, transcribe mainly rRNA and further growth-promoting genes (Bremer J Mol Biol 1996). Active polysomes localize at the cellular boundary (Bakshi Mol Microbiol 2012 and 2014).
Those distributions are disturbed in the presence of different drugs or by starvation (Zimmerman J Struct Biol 2002, Cabrera J Bacteriol 2009, English PNAS 2011, Endesfelder Biophys J 2013, Nonejuie PNAS 2013, Bakshi Mol Microbiol 2014, Stracy PNAS 2015). For example, the well-characterized antibiotic rifampicin halts the transcription of DNA in the initiation step by permanently binding to the β-subunit of the RNA polymerase (Campbell Cell 2001) or chloramphenicol stops protein synthesis by binding to 50S ribosomal subunit (Wisseman J Bacteriol 1954). Both treatments cause significant changes in sub-cellular organization of the processes on a short and long period time scale and also change protein expression. In this work we quantitatively describe these changes when reacting to different drugs that influence the processes of transcription and translation.

SsMP06
A systems biology approach to studying sulfur metabolism in the haloalkaliphilic chemolithoautotrophic sulfur-oxidizing bacterium *Thioalkalivibrio thiocyanoxidans* ARh 2^T
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Soda lakes are characterized by their extremely high pH and moderate to high salinity. Carbonates are the dominant anions in solution, leading to a uniquely stable sodium carbonate/bicarbonate system, which has a maximum buffering capacity at pH 9.5-10. Despite these extreme conditions, soda lakes harbor a rich biodiversity that drives active biogeochemical cycles, of which the sulfur cycle is one of the most active. One of the dominant groups of sulfur oxidizers inhabiting soda lakes worldwide is *Thioalkalivibrio*, a genus of high salt-tolerant, alkaliphilic, chemolithoautotrophic *Gammaproteobacteria*. They are capable of oxidizing a variety of inorganic sulfur compounds, such as sulfide, polysulfides, thiosulfate, elemental sulfur and tetrathionate. Some strains also have the ability to use thiocyanate (NCS^-) as electron-donor, sulfur and nitrogen source. Comparative analysis of a large set of *Thioalkalivibrio* genomes sequenced within the Community Science Program of the DOE's Joint Genome Institute has raised additional questions regarding the biochemistry of sulfur oxidation in this group. In the absence of the sulfur dehydrogenase SoxCD, the rDSR pathway is the only known alternative to oxidize the zero-valent sulfur atom to sulfite, but most of the sequenced genomes of *Thioalkalivibrio*, lack the dsr gene cluster as well as soxCD. Here we present the results of transcriptomic analysis of *Thioalkalivibrio thiocyanoxidans* ARh 2^T grown under controlled conditions in thiosulfate- or thiocyanate-limited chemostat cultures. Preliminary analysis indicates a small number of genes that are differentially expressed between thiocyanate and thiosulfate cultures. The strongest change is observed for a small cluster of genes encoding a hypothetical protein, copper resistance proteins and a tat-family transporter. Additionally, on the basis of knowledge from genomes and bacterial physiology, we plan to model the metabolism of and interactions between soda lake organisms. Answering open questions regarding the microbial sulfur cycle brings us closer to a complete understanding of the role of sulfur bacteria in soda lakes and on the application of these organisms in the sustainable treatment of sulfide- and cyanide-containing waste streams.

SsMP07
The regulation of the bacteriochlorophyll biosynthesis in *Dinoroseobacter shibae*
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Question: *Dinoroseobacter shibae* is a member of the Roseobacter clade and belongs to the aerobic anoxygenic phototrophic bacteria (AAnP). AAnPs are using light in the presence of oxygen but without producing oxygen to synthesize organic matter. Therefore, they are using spheroidenone and bacteriochlorophyll *a* (Bchl*a*) as main light harvesting pigments [1]. *D. shibae* serves as our model organism to study the bacteriochlorophyll *a* (Bchl*a*) biosynthesis. Changing light and oxygen conditions result in different expression patterns of photosynthetic genes [2,3]. Interestingly, bacteriochlorophyll *a* is synthesized in the dark and gets rapidly degraded under high light conditions. Thus, we raised the question, how the bacteriochlorophyll biosynthesis in *D. shibae* is regulated by light and which genes are involved.
Methods: We used our *D. shibae* transposon library and established a high-throughput screening [4]. Transposon mutants were grown on 96 micro well plates and we used *in vivo* UV/ Vis spectroscopy to identify mutants with an altered absorption spectra compared to the wildtype strain. In addition, we screened for the accumulation of the Bchl*a* biosynthesis intermediate magnesium-protoporphyrin IX monomethyester (MPE) which is fluorescing under blue light.
Results: We identified gene mutants encoding enzymes of almost every step of the Bchl*a* biosynthesis. Moreover, we could identify homologs of the photosynthetic gene regulators PpsR and PpaA. In *Rhodobacter sphaeroides* both regulators are known as repressors of photosynthetic genes in the presence of oxygen [5]. Since in *D. shibae* Bchl*a* is synthesized in the presence of oxygen, the function of these regulators in *D. shibae* is unknown yet. Moreover, mutants in *clpX* and *clpP* genes, encoding proteases, showed altered absorption spectra, indicating a role in bacteriochlorophyll biosynthesis.
Conclusion: These mutants will be investigated by transcriptome and proteome analyses to define the bacteriochlorophyll biosynthesis regulation by the photosynthetic gene regulators and the connections of yet unknown protagonists.

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SsMP08
Broadening the application range of compartmented eukaryotic metabolic models for cases of unknown cellular localization of amino acid synthesis pathways
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The rapidly growing field of fluxomics has proven to be invaluable for the physiological characterization of microbial systems as well as the design of rational metabolic engineering approaches. While recent advances in availability and applicability of genetic tools for non-*Saccharomyces* yeasts raise high hopes regarding industrial potentials of such yeasts, respective fluxomics data is scarce and has so far rarely aided in this development. The compartmentation of eukaryotic systems is the main issue adding model complexity and models usually require precise definition of pathway localizations, which may be unknown and are tedious to investigate. A small scale model for ^{13}C-based metabolic flux analysis of central yeast carbon metabolism was developed, which is universally valid and does not depend on localization information regarding amino acid anabolism. The feature of variability in compartmental origin of traced metabolites allows for the application of the model to yeasts with uncertain genomic and transcriptional background. The model was shown to find the same solution for resolvable fluxes in an undefined localization setting and in a setting with constraints based on curated or computationally predicted localization information for a *S. cerevisiae* and a *Hansenula polymorpha* dataset respectively, while finding false solutions with false settings. This indicates the potentially adverse effect of assuming *Saccharomyces*-like constraints, as well as the validity of discarding those constraints for a small scale metabolic model. The model was specifically designed to investigate the intracellular metabolism of various wild-type yeasts under various stress conditions but is expected to be a useful basis for modelling other eukaryotic systems as well.

SsMP09
Impact of intermediate toxicity on the regulation of fungal metabolic pathways
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The assumption that biological processes follow optimality principles due to evolutionary adaption has provided valuable insights in complex systems like the metabolism of fungal species. The understanding of metabolic networks and their regulation is crucial for the the industrial use of fungi like *Saccharomyces cerevisiae* and to combat pathogenic fungi such as *Aspergillus fumigatus* and *Candida albicans*. With the rather novel

approach of dynamic optimization we were able to uncover time-resolved optimality principles behind the regulatory strategies controlling metabolic pathways [1, 2]. In the present study, we focus on the impact of toxic intermediates on the regulatory strategies controlling metabolic pathways. We found that toxic intermediates are controlled by a tight regulation of upstream enzymes preventing their accumulation. Consequently, this changes the position of the mainly regulated enzyme, also called key enzyme. These ndings can explain a sparse regulation by key enzymes at various positions of a pathway and not mainly at the rst and last position, as it was observed previously. These results were validated in a large scale data set from prokaryotes with known genome and metabolic structure and latest results for *Saccharomyces cerevisiae* show that these principles are transferable to fungi. Further, our results provide new opportunities for antifungal drugs by targeting highly regulated enzymes and introducing a self-produced and accumulated toxic intermediate.

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Personalia aus der Mikrobiologie 2015

Habilitationen

Christian Riedel habilitierte sich am 27. Mai 2015 an der Universität Ulm (Genomic and Molecular Analysis of Bifidobacteria-Host Interactions).

Anne Samland habilitierte sich am 20. Juli 2015 an der Universität Stuttgart (Untersuchungen zu Struktur-Funktionsbeziehungen in der Enzymfamilie der Transaldolasen).

Fabian M. Commichau habilitierte sich am 28. Juli 2015 an der Universität Göttingen (Glutamate homeostasis in the Gram-positive model organism *Bacillus subtilis*).

Christoph Albermann habilitierte sich am 16. Dezember 2015 an der Universität Stuttgart (Rekombinante Darstellung und Modifikation von Naturstoffen durch Biologische Synthese und Synthese Biologie).

Ruf angenommen

Hannes Link von der ETH Zürich übernahm am 1. Januar 2015 die Position als Gruppenleiter der Emmy-Noether-Gruppe „Dynamic control of metabolic networks" am Max-Planck-Institut für terrestrische Mikrobiologie, Marburg.

Tanja Schneider von der Universität Bonn übernahm am 4. Februar 2015 die W2-Professur für den Lehrstuhl für Pharmazeutische Mikrobiologie an der Universität Bonn.

Thorsten Mascher von der Universität München übernahm am 1. April 2015 die W3-Professur für Allgemeine Mikrobiologie an der Technischen Universität Dresden.

Dina Grohmann von der Universität Braunschweig übernahm am 1. April 2015 die W2-Professur für den Lehrstuhl für Mikrobiologie an der Universität Regensburg.

Matthias Brock von der Universität Jena übernahm am 1. April 2015 die Assistenzprofessur für Fungal Biology and Genetics an der University of Nottingham.

Guntram Graßl von der Universität Kiel, Institut für Experimentelle Medizin sowie dem Forschungszentrum Borstel übernahm am 1. April 2015 die W2-Professur für Medizinische Mikrobiomforschung am Institut für Medizinische Mikrobiologie und Krankenhaushygiene der Medizinischen Hochschule Hannover.

Takashi Fujishiro vom Max-Planck-Institut für terrestrische Mikrobiologie in Marburg übernahm am 1. April 2015 die Assistant Professur für die "Graduate School of Science and Engineering" an der Saitama University (Japan).

Alexander Elsholz von der Harvard University übernahm am 1. Juni 2015 die Position als Gruppenleiter der Forschungsgruppe „Post-translational control of bacterial cell differentiation" am Max-Planck-Institut für terrestrische Mikrobiologie, Marburg.

Tim Urich von der Universität Wien übernahm am 1. Juli 2015 die W2-Professur für Bakterienphysiologie an der Universität Greifswald.

Kai Papenfort von der Princeton University, USA, übernahm am 1. September 2015 die W2-Professur für Mikrobiologie an der Ludwig-Maximilians-Universität München.

Andriy Luzhetskyy vom Helmholtz-Institut für Pharmazeutische Forschung Saarland (HIPS) übernahm am 1. Oktober 2015 die W3-Professur für den Lehrstuhl Pharmazeutische Biotechnologie an der Universität des Saarlandes.

Haike Antelmann von der Universität Greifswald übernahm am 1. Oktober 2015 die W3-Professur für Mikrobiologie an der Freien Universität Berlin.

Bruno Bühler von der TU Dortmund übernahm am 1. Oktober 2015 die W2-Universitätsprofessur für den Lehrstuhl Angewandte Biokatalyse an der Universität Halle-Wittenberg in gemeinsamer Berufung mit dem Helmholtz-Zentrums für Umweltforschung GmbH – UFZ, Department Solare Materialien.

Marc Dumont vom Max-Planck-Institut für terrestrische Mikrobiologie in Marburg übernahm am 1. Oktober 2015 eine Dozentenstelle an der Universität in Southampton (UK).

Martin Thanbichler von der Universität Marburg übernahm am 1. Oktober 2015 den Ruf als Max Planck Fellow der Gruppe „Bacterial cell biology" am Max-Planck-Institut für terrestrische Mikrobiologie, Marburg.

Knut Drescher vom Max-Planck-Institut, Marburg übernahm am 14. Oktober 2015 die W2-Professur für Biophysik an der Universität Marburg.

Katja Bühler von der TU Dortmund übernahm am 1. Dezember 2015 die W2-Universitätsprofessur für den Lehrstuhl Technologie Produktiver Biofilme an der Technischen Universität Dresden in gemeinsamer Berufung mit dem Helmholtz-Zentrums für Umweltforschung GmbH – UFZ, Department Solare Materialien.

Pensionierungen

Gerold Barth vom Institut für Mikrobiologie an der Technischen Universität Dresden wurde am 31. März 2015 emeritiert.

Helmut König vom Institut für Mikrobiologie und Weinforschung der Universität, Mainz wurde am 30. September 2015 pensioniert.

Friedrich Götz vom Interfakultären Institut für Mikrobiologie und Infektionsmedizin (IMIT), Mikrobielle Genetik an der Universität Tübingen wurde am 1. Oktober 2015 als Seniorprofessor emeritiert.

Wissenschaftliche Preise

Antje Boetius vom Max-Planck-Institut für Marine Mikrobiologie, Bremen, MARUM, Universität Bremen und vom Alfred-Wegener-Institut Helmholtz Zentrum für Polar- und Meeresforschung, Bremerhaven erhielt im Jahr 2015 Fellowship der American Academy of Microbiology für ihre Expertise auf dem Gebiet der Biogeochemie und besonders in der Tiefsee- und Polarforschung und wurde in 2015 als gewähltes Mitglied in die European Academy of Microbiology aufgenommen.

Ulrike Endesfelder vom Max-Planck-Institut für terrestrische Mikrobiologie Marburg erhielt in 2015 die Auszeichnung "Elected Member of the Young Academy der Deutschen Akademie der Wissenschaften Leopoldina

Alexander Grünberger (FZ Jülich), **Isabel Kolinko** (LMU München), **Daniela Münch** (Universität Bonn) und **Alexander Probst** (Universität Regensburg) erhielten am 2. März 2015 in Marburg die Promotionspreise der VAAM.

Christian Hertweck vom Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie – Hans-Knöll-Institut erhielt am 3. März 2015 Gottfried Wilhelm Leibniz-Preis der DFG für seine Forschungen zu bioaktiven Naturstoffen.

Hannes Beims erhielt von der Arbeitsgemeinschaft der Institute für Bienenforschung e. V. am 30. März 2015 den Evenius-Preis 2015 für seinen Vortrag „Einsatz von Bakteriophagen im Kampf gegen Amerikanische Faulbrut".

Qian Chen vom Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie – Hans-Knöll-Institut in Jena erhielt am 10. April 2015 den Wissenschaftspreis des Beutenberg Campus e. V., am 26. Juni 2015 den Promotionspreis der Biologisch-Pharmazeutischen Fakultät der Universität Jena und am 15. September 2015 den Rainer-Greger-Promotionspreis der Deutschen Gesellschaft für Nephrologie für ihre immunologischen Arbeiten über die Nierenerkrankung MPGN.

Benjamin Schurr von der TU München erhielt am 14. April 2015 den Preis der Dr. Nienaber-Stiftung für seine Arbeit über „Dissection of the molecular mechanism of hop inhibition in *Lactobacillus brevis*".

Cynthia Sharma von der Universität Würzburg erhielt am 5. Mai 2015 den Heinz Maier-Leibnitz-Preis der DFG.

Uwe Bornscheuer von der Universität Greifswald erhielt am 5. Mai 2015 den „Stephen S. Chang Award" der American Oil Chemists' Society für seine Arbeiten über die enzymatische Modifikation von Lipiden.

Rudolf K. Thauer vom Max-Planck-Institut für terrestrische Mikrobiologie Marburg erhielt am 11. Juni 2015 den André-Lwoff-Award of the Federation of European Microbiology Societies für seine herausragenden Verdienste um die Mikrobiologie in Europa.

Florian Kloss vom Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie – Hans-Knöll-Institut erhielt am 26. Juni 2015 den Promotionspreis der Chemisch-Geowissenschaftlichen Fakultät der Universität Jena für seine Arbeiten zum mikrobiellen Wirkstoff Closthioamid.

Stephan Binder und Georg Schaumann vom Institut für Bio- und Geowissenschaften, IBG-1: Biotechnologie am Forschungszentrum Jülich erhielten am 3. Juli 2015 den Preis „Innovatoren unter 35" von Technology Review für ihr Ausgründungsprojekt „SenseUP Biotechnology".

Sonja Mayer, Marcel Prax und **Christopher Schuster** von der Universität Tübingen erhielten am 22. Juli 2015 die Promotionspreise der Reinhold-und-Maria-Teufel-Stiftung 2015 für ihre Dissertationen.

Falk Harnisch von der Universität Leipzig erhielt am 18. August 2015 den Science for Solving Society's Problem Award of ECS and Bill and Melinda Gates Foundation für seine Arbeiten über A fully cardboard-based microbial fuel cell energy generation sowie am 25. November 2015 den UFZ-Forschungspreis für seine Arbeiten über Mikrobielle Bioelektrokatalyse und Bioelektrotechnologie.

Annina Schulz von der Philipps-Universität Marburg erhielt im September 2015 den „For Women in Science-Preis" der UNESCO-Kommission und der L'Oréal-Stiftung.

Christian Siegel von der Universität Würzburg erhielt im September 2015 den DGHM-Promotionspreis für seine Dissertation mit dem Titel „Regulation des Tumorsuppressors p53 während *Chlamydia tarachomatis* Infektion.

Axel Brakhage vom Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie – Hans-Knöll-Institut – und der Universität Jena wurde am 16. September 2015 zum Ehrenmitglied der Deutschsprachigen Mykologischen Gesellschaft für seine Arbeiten über die Infektionsbiologie und Sekundärstoffbildung bei humanpathogenen Pilzen ernannt.

Ilse Jacobsen vom Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie – Hans-Knöll-Institut – und der Universität Jena erhielt am 16. September 2015 den Forschungsförderpreis der Deutschsprachigen Mykologischen Gesellschaft für ihre Arbeiten über *in vivo*-Infektionsmodelle.

Sascha Brunke vom Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie – Hans-Knöll-Institut erhielt am 16. September 2015 den Wissenschaftspreis der Stiftung der Deutschsprachigen Mykologischen Gesellschaft für seine Arbeiten über die Infektionsbiologie von *Candida albicans.*

Katja Graf vom Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie – Hans-Knöll-Institut erhielt am 16. September 2015 den Wissenschaftspreis der Stiftung der Deutschsprachigen Mykologischen Gesellschaft für ihre Arbeiten über die Infektionsbiologie von *Candida albicans.*

Bettina Böttcher vom Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie – Hans-Knöll-Institut erhielt am 16. September 2015 den Hans-Rieth-Preis der Stiftung der Deutschsprachigen Mykologischen Gesellschaft für ihre Arbeiten zu evolutionären Prozessen und Mechanismen von *Candida albicans* und *C. glabrata* während der Infektion.

Martin Klapper vom Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie – Hans-Knöll-Institut erhielt am 16. September 2015 das Hoechst Doktorandenstipendium der Stiftung Stipendien-Fonds des Verbandes der Chemischen Industrie (VCI) in Kooperation mit der Aventis Foundation für seinen herausragenden Studienabschluss im Fach Chemische Biologie.

Anna Müller von der Universität Bonn erhielt im Rahmen der DGHM-Tagung am 27. September 2015 den DZIF-Promotionspreis für ihre Dissertation mit dem Titel „Mechanistic studies of new inhibitors of Gram-positive cell envelope biosynthetic pathways".

Beate Henrichfreise von der Universität Bonn erhielt am 29. Oktober 2015 den Phoenix Pharmazie Wissenschaftspreis für ihre Arbeiten über „Identifizierung eines neuen Penicillin-Targets in Chlamydien".

Nick Wierckx von der RWTH Aachen, Institut für Angewandte Mikrobiologie – iAMB – erhielt am 2. November 2015 den Preis „BioSC Supervision Award" für seine herausragende Betreuung von Promovierenden im Themenbereich Angewandte Mikrobiologie.

Thiemo Zambanini, von der RWTH Aachen, Institut für Angewandte Mikrobiologie – IAMB – erhielt am 2. November 2015 den Preis „BioSC PhD-Competence Award" für seine Arbeiten über die biotechnologische Umwandlung von industriellen Abfallströmen zu wertvollen Plattform-Chemikalien

Gulimila Shabuer, Keishi Ishida, Sacha Pidot, Martin Roth und **Hans-Martin Dahse** vom Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie – Hans-Knöll-Institut erhielten am 2. November 2015 den medac-Forschungspreis der medac GmbH, Wedel, für ihre Arbeiten auf dem Gebiet der Wirkstoffe aus anaeroben Bakterien.

Markus Greßler, Florian Meyer, Daniel Heine und **Peter Hortschansky** vom Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie –Hans-Knöll-Institut erhielten am 2. November 2015 den medac-Forschungspreis der medac GmbH, Wedel, für ihre Arbeiten auf dem Gebiet der Wirkstoffbildung durch *Aspergillus terreus.*

Anja Wartenberg, Jörg Linde, Ronny Martin, Maria Schreiner, Fabian Horn, Thomas Wolf und **Sascha Brunke** vom Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie – Hans-Knöll-Institut erhielten am 2. November 2015 den medac-Forschungspreis der medac GmbH, Wedel, für ihre Arbeiten auf dem Gebiet der Interaktion von *Candida albicans* mit Immunzellen.

Alexander Steinbüchel und seine Arbeitsgruppe von der Westfälischen Wilhelms-Universität Münster erhielten am 3. November 2015 zusammen mit der ausgegründeten Cysal GmbH den Sybille-Hahne-Gründerpreis für die Entwicklung einer biotechnologischen Methode zur Produktion bestimmter Protein-Bausteine in industriellem Maßstab.

Sarah Willkomm von der Universität Regensburg erhielt am 4. November 2015 den Heinrich-Dräger-Wissenschaftspreis für ihre Arbeiten über „Minimal mechanistic model of siRNA-dependent target RNA slicing by recombinant human Argonaute 2 protein".

Michael Jahn von der Universität Leipzig erhielt am 25. November 2015 den UFZ-Promotionspreis für seine Arbeiten über Characterization of population heterogeneity in a model biotechnological process using *Pseudomonas putida.*

Promotionen 2015

RWTH Aachen

Yulei Zhao: The molecular basis of symptom formation in *Sporisorium reilianum*
Betreuer: Jan Schirawski

Alana Poloni: Investigation of host specificity mechanisms of *Sporisorium reilianum* in maize and sorghum
Betreuer: Jan Schirawski

Elena Geiser: Itaconic Acid Production by *Ustilago maydis*
Betreuer: Lars M. Blank

Bernd Leuchtle: Mikrobiologische Kontamination von Heizöl – Ursachen und Auswirkungen auf Brennstoff und Tank
Betreuer: Lars M. Blank

Humboldt-Universität Berlin

Franziska Kirsch: Analyse der Substratbindestelle, der Stöchiometrie und der Transportfunktion von S-Einheiten bakterieller ECF-Transporter
Betreuer: Thomas Eitinger

Yvonne Kohlmann: Charakterisierung des Proteoms von *Ralstonia eutropha* H16 unter lithoautotrophen und anaeroben Bedingungen
Betreuerin: Bärbel Friedrich

Freie Universität Berlin

Carmen Bednorz: Influence of Feed Supplements on the Porcine Intestinal *Escherichia coli* Microbiota
Betreuer: Lothar Wieler, Rupert Mutzel

Mara Wittig: hBD-2 and hBD-3 are induced in *S. pneumoniae* infected human macrophages via distinct signaling pathways but exhibit similar immune modulatory functions
Betreuer: Norbert Suttorp, Rupert Mutzel

Universität Bielefeld

Hanna Berger: The regulatory network adjusting light-harvesting in the model green alga *Chlamydomonas reinhardtii*
Betreuer: Olaf Kruse

Marius Herbst: Genome reduction of *Corynebacterium glutamicum* and its impact on L-lysine production
Betreuer: Volker Wendisch

Jaide Vold Korgaard Jensen: Metabolic engineering of *Corynebacterium glutamicum* for production of glutamate derivatives
Betreuer: Volker Wendisch

Robert Kulis-Horn: Untersuchung der L-Histidinbiosynthese in *Corynebacterium glutamicum* ATCC 13032 zur Erzeugung eines L-Histidinproduzenten
Betreuer: Jörn Kalinowski

Lennart Leßmeier: Metabolic engineering of *Corynebacterium glutamicum* toward the utilization of methanol
Betreuer: Volker Wendisch

Christian Matano: Alternative carbon sources for *Corynebacterium glutamicum*: chitin and its derivates
Betreuer: Volker Wendisch

Quynh Anh Nguyen: Metabolic eningeering for improving putrescine production in *C. glutamicum*
Betreuer: Volker Wendisch

Armin Neshat: Transcriptome Analysis of Industrially Relevant Bacteria by Next-Generation Sequencing
Betreuer: Jörn Kalinowski

Vimac Nolla Ardevol: Anaerobic digestion of the microalga *Spirulina* at alkaline conditions (pH~10; 2.0 M Na+) – Biogas production and metagenome analysis
Betreuer: Olaf Kruse

Johannes Pfeifenschneider: Metabolic engineering of *Bacillus methanolicus* and *Corynebacterium glutamicum* for the production of cadaverine from methanol
Betreuer: Volker Wendisch

Katharina Pfeifer: Entwicklung der Transkriptomsequenzierung und Anwendung zur Analyse des Transkriptoms von *Corynebacterium glutamicum*
Betreuer: Jörn Kalinowski

Daniel Siebert: Genome reduction of *Corynebacterium glutamicum*: biotechnological applications in context of peptidase deficiency and production of 1,2-propanediol and 1-propanol
Betreuer: Volker Wendisch

Frederik Walter: Etablierung einer LC-ESI-MS-Plattform und Analyse der Biosynthese von ι-Arginin und γ-Glutamyl-Dipeptiden in *Corynebacterium glutamicum*
Betreuer: Karsten Niehaus

Sergej Wendler: Comprehensive proteome analysis of *Actinoplanes* sp. SE50/110, the producer of the medically important secondary metabolite acarbose
Betreuer: Alfred Pühler

Universität Bochum

Kordula Becker: Funktionelle Genomanalysen zur Regulation von Morphogenese und Sekundärmetabolismus in dem industriellen Penicillin-Produzenten *Penicillium chrysogenum*
Betreuer: Ulrich Kück

Anna Beier: Die STRIPAK-assoziierte katalytische Untereinheit 1 der Proteinphosphatase 2A reguliert die sexuelle Entwicklung des Hyphenpilzes *Sordaria macrospora*
Betreuer: Ulrich Kück

Tim Alexander Dahlmann: Vergleichende Genom- und Transkriptomanalysen zum Nachweis chromosomaler Rekombinationen und kleiner RNAs im Penicillin-Produzenten *Penicillium chrysogenum*
Betreuer: Ulrich Kück

Olga Reifschneider: Spleißosomähnliche Komplexe in Chloroplasten der Grünalge *Chlamydomonas reinhardtii*
Betreuer: Ulrich Kück

Eva Katharina Steffens: Entwicklungsbiologie bei dem Hyphenpilz *Sordaria macrospora*: Zielgene des Transkriptionsfaktors PRO1 kodieren für Komponenten des Zellwandintegritäts-Signalweges
Betreuer: Ulrich Kück

Linna Danne: Membranbindemechanismen bakterieller Phospholipid *N*-Methyltransferasen
Betreuer: Franz Narberhaus

Julia Schwach: Funktion und Struktur der Phycobiliproteinlyasen aus *Prochlorococcus marinus* SS120und dem Cyanophagen P-HM1
Betreuerin: Nicole Frankenberg-Dinkel

Universität Bonn

Kevin Denkmann: Biochemische, strukturelle und kinetische Charakterisierung des ungewöhnlichen *c*-Typ Cytochroms Thiosulfatdehydrogenase
Betreuerin: Christiane Dahl

Anna Müller: Mechanistic studies of new inhibitors of Gram-positive cell envelope biosynthetic pathways
Betreuer: Hans-Georg Sahl

Stefanie Claudia Berger: Energy conservation in aceticlastic methanogenic archaea and human gut archaeon *Methanomassiliicoccus luminyensis*
Betreuer: Uwe Deppenmeier

Jessica Zeiser: Charakterisierung von PQQ-abhängigen Dehydrogenasen aus *Spingomonas wittichii* RW1 und Entwicklung eines enzymatischen Verfahrens zur Quantifizierung von PQQ
Betreuer: Uwe Deppenmeier

Technische Universität Braunschweig

José Manuel Borrero de Acuña: Membrane-associated higher-ordered protein mega-complexes for denitrification and motility in *Pseudomonas aeruginosa*
Betreuer: Dieter Jahn, Kenneth Timmis

Tobias Knuuti: Natürliche Kompetenz und Proteinexport in *Bacillus megaterium* – Grundlagen und biotechnologische Anwendungen
Betreuer: Dieter Jahn, Michael Steinert

Melanie Kühner: Kristallisation des anaeroben Radical SAM Proteins SkfB aus *Bacillus subtilis*
Betreuer: Gunhild Layer, Dieter Jahn

Constanze Finger: Rekombinante Proteinproduktion, Codonusage und tRNA Coproduktion in *Bacillus megaterium* sowie Studien zu Protein-Protein-Interaktionen
Betreuer: Dieter Jahn, Gunhild Layer

Franziska Schuster: Underlying principles of bistability in the expression of the pivotal virulence regulator RovA of *Yersinia pseudotuberculosis* and its role for virulence
Betreuerin: Petra Dersch

DSMZ/TU Braunschweig

Anne Fiebig: Genomic and phenotypic characterization of members of the *Roseobacter* clade (*Rhodobacteraceae*)
Betreuer: Hans-Peter Klenk, Markus Göker

Palani Kannan Kandavel: Inferring organismal and character evolution from functional genome features
Betreuer: Hans-Peter Klenk, Markus Göker

DSMZ/Jomo Kenyatta University, Nairobi, Kenia

Juliah K. Akhwale: Isolation and characterization of novel bacteria and bacteriophages from the haloalkaline lake elmenteita
Betreuer: Hans-Peter Klenk, Hamadi Boga

Universität Bremen/MPI für Marine Mikrobiologie Bremen

Gerdhard Jessen: Ecosystems response to hypoxia: what can marine benthos tell us?
Betreuerin: Antje Boetius

Judith Zimmermann: Diversity, specificity and evolutionary history of marine invertebrate symbioses and functions of the sulphur-oxidizing symbionts
Betreuerin: Nicole Dubilier

Marina Zure: Biogeography of *Rhodopirellula* in European coastal sediments
Betreuer: Jens Harder

Judith Lucas: Spatiotemporal dynamic of the bacterial community in the German Bight
Betreuer: Rudolf Amann

Marion Helen Stagars: Ecophysiology of key sulfate-reducing bacteria involved in anaerobic hydrocarbon degradation at marine gas and oil seeps
Betreuer: Rudolf Amann

Jimena Barrero Canosa: Systematic optimization of the detection of single genes in microorganism by fluorescence *in situ* hybridization (Gene FISH)
Betreuer: Rudolf Amann

Robert Marmulla: The linalool metabolism in *Castellaniella defragrans* 65Phen and *Thauera linaloolentis* 47Lol
Betreuer: Jens Harder

Judith M. Klatt: Cyanobacteria and hydrogen sulfide: Insights into an (ancient) love-hate relationship
Betreuer: Friedrich Widdel

Viola Krukenberg: Novel insights into the physiology and genomics of thermophilic anaerobic methane-oxidizing consortia
Betreuerin: Antje Boetius

Jacobs Universität Bremen/MPI für Marine Mikrobiologie Bremen

Julia Schnetzer: On the feasibility to engage heterogeneous communities in data gathering, charing and enrichment
Betreuer: Frank Oliver Glöckner

Joost Waldmann: Reliable taxonomic classification of metagenome fragments from varying marine bacterial communities
Betreuer: Frank Oliver Glöckner

Technische Universität Darmstadt

Michael Martin Rudolph: Entwicklung und Anwendung synthetischer RNA-Schalter zur Regulation der Genexpression
Betreuerin: Beatrix Süß

Florian Groher: Kontrolle des prä-mRNA-Spleißens durch synthetische Riboswitche
Betreuerin: Beatrix Süß

Nona Heueis: Charakterisierung kleiner, nicht-kodierender RNAs in *Streptomyces coelicolor*
Betreuerin: Beatrix Süß

Christine Spohr: Etablierung einer 3R-Alternativmethode für die Chargenprüfung von bovinem Tuberkulin unter Berücksichtigung mykobakterieller Lipidantigene im Meerschweinchenmodell.
Betreuer: Ger van Zandbergen, Jörg Simon

Lucia Carrillo: Application of high-resolution membrane capacitance measurements in the study of exocytosis and endocytosis in *Saccharomyces cerevisiae*
Betreuer: Adam Bertl

Technische Universität Dortmund

Christian Dusny: Microfluidics enable quantitative physiology of individual microorganisms in controlled extracellular environments
Betreuer: Andreas Schmid

Technische Universität Dresden

Nicole Matschiavelli: Analyse der Formiat-Bildung in *Methanosarcina acetivorans*
Betreuer: Michael Rother

Christoph Loderer: Strukturelle Determinanten der Stereoselektivität und Substratspezifität Zink-abhängiger Alkoholdehydrogenasen
Betreuerin: Marion Ansorge-Schumacher

Liane Flor: Untersuchungen zur Biosynthese von chlorierten Sekundärmetaboliten aus *Aster tataricus*, *Talaromyces islandicus* und *Streptomyces albogriseolus*
Betreuer: Karl-Heinz van Pée

Universität Düsseldorf

Marc Swidergall: Mechanismen der Msb2-vermittelten Virulenz des humanpathogenen Pilzes *Candida albicans*
Betreuer: Joachim Ernst

Thorsten Langner: Charakterisierung der chitinolytischen Maschinerie aus *Ustilago maydis*
Betreuer: Michael Feldbrügge

Universität Düsseldorf/ Forschungszentrum Jülich

Philana van Summeren-Wesenhagen: Metabolic engineering of *Escherichia coli* for the production of plant phenylpropanoid derived compounds
Betreuer: Michael Bott

Eva Hentschel: Interaction of the two-component systems HrrSA and ChrSa in *Corynebacterium glutamicum*
Betreuer: Julia Frunzke

Xenia Schuplezow: Strukturelle und funktionelle Untersuchungen zur Kupfer-Homöostase in *Corynebacterium glutamicum*
Betreuer: Michael Bott

Universität Duisburg-Essen

Julia Christin Verheyen: *Sulfolobus acidocaldarius* & *Sulfolobus solfataricus*: Exploitation of thermoacidophilic Archaea for biotechnological applications
Betreuerin: Bettina Siebers

Universität Erlangen-Nürnberg

Camila Azevedo Antunes de Oliveira: Characterization of the multi-functional virulence factor DIP0733 among other pathogenicity properties of *Corynebacterium diphtheria*
Betreuer: Andreas Burkovski

Judith Lind: Role of different secreted or translocated effector molecules of the gastric pathogen *Helicobacter pylori* – Studies on different pathogenicity factors leading to immune system activation
Betreuer: Steffen Backert

Universität Frankfurt am Main

Alexander Farwick: Engineering of D-xylose transport in *Saccharomyces cerevisiae*
Betreuer: Eckhard Boles

Sophie Korn: Functional analysis of the lantibiotic immunity proteins SpaI from *Bacillus subtilis* and *NisI* from *Lactococcus lactis*
Betreuer: Karl-Dieter Entian

Kai Schuchmann: A hydrogen-dependent CO_2 reductase: enzyme properties, applications and implications for the energy metabolism of the acetogenic bacterium *Acetobacterium woodii*
Betreuer: Volker Müller

Friederike Nollmann: Characterization and Synthesis of selected secondary metabolites produced by *Xenorhabdus* and *Photorhabdus*
Betreuer: Helge B. Bode

Anna Proschak: Identification and characterization of secondary metabolites and biosynthesis pathways from *Xenorhabdus* and *Acinetobacter*
Betreuer: Helge B. Bode

Tilman Ahrendt: Structure and function of selected metabolites from *Myxococcus* and *Legionella*
Betreuer: Helge B. Bode

Universität Freiburg

Sebastian Estelmann: Ungewöhnliche Elektronentransferprozesse im anaeroben Aromatenabbau
Betreuer: Matthias Boll

Universität Gießen

Fazal Adnan: Protein- and RNA-based regulation of the photooxidative stress response in *Rhodobacter sphaeroides*
Betreuerin: Gabriele Klug

Bernhard Remes: Regulatory links between iron metabolism and oxidative stress response in *Rhodobacter sphaeroides*
Betreuerin: Gabriele Klug

Bernd Christoph Lochbühler: The potential of yeast proteins to substitute for traditional fining agents – technological and sensory aspects
Betreuerin: Sylvia Schnell

Universität Göttingen

Stefan Frey: The STRIPAK complex and its role in fruiting-body development of the filamentous fungus *Sordaria macrospora*
Betreuerin: Stefanie Pöggeler

Stephanie Großhennig: Novel virulence determinants in *Mycoplasma pneumoniae*: contribution of transport systems and H_2S production to viability and hemolysis
Betreuer: Jörg Stülke

Jan Gerwig: Control of biofilm formation in *Bacillus subtilis*
Betreuer: Jörg Stülke

Lorena Stannek: Control of glutamate homeostasis in the Gram-positive model organism *Bacillus subtilis*
Betreuer: Fabian M. Commichau

Universität Greifswald

Andreas Bäumgen: Untersuchungen zur enzymatischen Fixierung von CO_2
Betreuer: Uwe Bornscheuer

Mechthild Gall: Biotransformation von Flavonoiden
Betreuer: Uwe Bornscheuer

Fabian Steffen-Munsberg: Structure- and sequence-function relationships in (*S*)-amine transaminases and related enzymes
Betreuer: Uwe Bornscheuer

Christin Peters: Kopplung von Oxidoreduktasen in synthetischen Enzymkaskaden
Betreuer: Uwe Bornscheuer

Sandy Schmidt: An artificial enzyme cascade for the biocatalytic synthesis of polymer building blocks
Betreuer: Uwe Bornscheuer

Janett Müller: Untersuchungen zur Acyltransferaseaktivität der Lipase A aus *Candida antarctica*
Betreuer: Uwe Bornscheuer

Hannes Kohls: Biocatalytic synthesis of amino alcohols
Betreuer: Uwe Bornscheuer, Matthias Höhne

Müller, Marret: Charakterisierung des alkalischen Schock Proteins Asp23 in *Staphylococcus aureus*
Betreuer: Michael Hecker

Bonn, Florian: Analyse der Antibiotikastress-Antwort von *Staphylococcus aureus*
Betreuerin: Dörte Becher

Gerlach, Torsten: Entwicklung neuer Testsysteme zur Erfassung östrogener und androgener Wirkungen im Rahmen der Umweltanalytik
Betreuer: Rüdiger Bode

Chamas, Alexandre: Development of novel Surface Plasmon Resonance-based biosensors with purified recombinant human HER-2 and progesterone receptor produced in two different yeast species
Betreuer: Rüdiger Bode

Rauter, Marion: Herstellung rekombinanter Alkoholdehydrogenasen in der Hefe *Arxula adeninivorans* und ihre Verwendung für die Synthese enantiomerenreiner Alkohole
Betreuer: Frieder Schauer

Mohammed Redha Abdullah: Functional and structural characterization of DacB and PccL, lipoproteins contributing to pneumococcal pathogenesis
Betreuer: Sven Hammerschmidt

Christian Schulz: The influence of regulatory proteins on the physiology and virulence of *Streptococcus pneumoniae*
Betreuer: Sven Hammerschmidt

Universität Halle-Wittenberg

Nicole Wiesemann: Mechanismus der Goldresistenz in *Cupriavidus metallidurans*
Betreuer: Dietrich H. Nies

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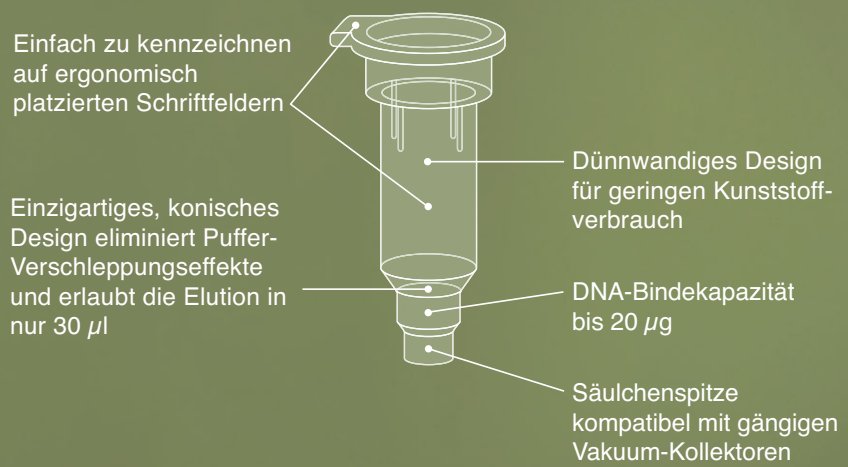

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