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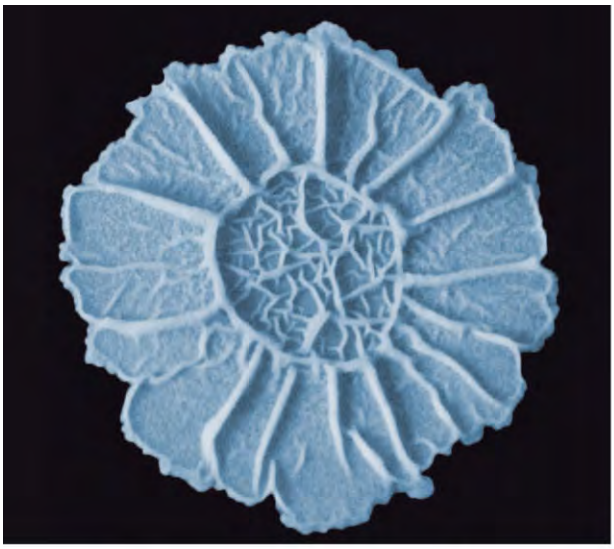

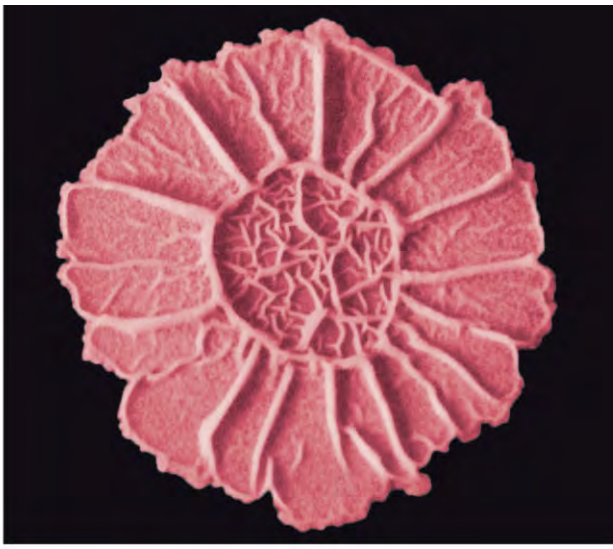

2015
Sonderausgabe

Tagungsband zur

VAAM-Jahrestagung 2015

1.–4. März in Marburg/Lahn

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Jahrestagung der Vereinigung für Allgemeine und Angewandte Mikrobiologie (VAAM)

Programme
Annual Conference 2015 of the Association for General and Applied Microbiology (VAAM)

1 – 4 March 2015 in Marburg, Germany

Conference Chair
Prof. Dr. Erhard Bremer
Philipps-University of Marburg
Department of Biology
Laboratory for Microbiology
Karl-von-Frisch-Str. 8
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Organizing Society of the Conference
VAAM
Vereinigung für Allgemeine und Angewandte Mikrobiologie
Präsident: Prof. Dr. Dieter Jahn
Geschäftsstelle:
Mörfelder Landstraße 125
60598 Frankfurt am Main
Leiterin: Dr. Katrin Muth

About the cover:
Upper row: Color-enhanced photographs of *Bacillus subtilis* NCIB3610 grown under biofilm-inducing conditions on an agar plate. The "wild" *B. subtilis* NCIB3610 isolate is a close relative of the most commonly used *B. subtilis* laboratory strain 168. *B. subtilis* 168 has been *domesticated* by bacterial geneticists and has thereby accidentally lost the ability to form robust biofilms, a property that has been retained by the *B. subtilis* NCIB3610 strain. The ancestor of the *B. subtilis* 168 strains was isolated around 1899 at the University of Marburg by Meyer and Gottheil and was defined in 1930 by Conn as the *B. subtilis* type strain. (© Tamara Hoffmann and Patricia Wagner)
Lower row: Impressions about life and buildings in the University city of Marburg. (© Sabine Feuersänger, Markus Farnung, Oliver Geyer)

Dear members of the VAAM, Dear members of the *Microbial Community*,

■ It's a pleasure once again to welcome the members of the VAAM and other guests to Marburg for the Annual Microbiology Meeting. The last VAAM meeting held here was in 1989. I know that some of you were attendees and have special memories about this meeting. To more youthful visitors who find 1989 to be on the borderline to ancient history, I extend a particularly warm welcome.

We invited a panel of internationally highly recognized speakers both from Germany and abroad who will present and discuss with us the latest developments in the field of Microbial Evolution, Synthetic Microbiology, CRISPR-Systems and Viruses, Microbial Cell Biology, and Evolution. In addition to the plenary sessions, a broad range of topics in the area of General Microbiology will be covered in numerous symposia and in poster sessions that will, in particular, give students and young scientists the opportunity to present the central findings of their ongoing studies. It is these presentations that make the annual VAAM meetings so vibrant.

I would like to take the opportunity to thank our invited speakers for coming to Marburg and we are exceptionally grateful to the numerous authors of oral and poster presentations for their contributions to this conference. We are very thankful to our main sponsors and industrial partners, as well as the German Research Foundation (DFG), the Federation of European Microbiological Societies (FEMS) and the local DFG-funded Collaborative Research Center SFB-987 for the generous financial support of this meeting. I thank the president of the Philipps-University, Prof. Dr. Katharina Krause, for generously allowing us to use the facilities of the central lecture building to hold this conference.

2015 is a special year for the VAAM since our society will be 30 years old. This is a time for reflection, but also for looking in a different way at our beloved bacteria, fungi and phages. The comedian Vince Ebert will present a refreshing outside view of the fascinating world of microorganisms.

I thank all my colleagues from the Philipps University, the Max-Planck Institute for Terrestrial Microbiology, the LOEWE Center for Synthetic Microbiology, and the microbiology-oriented SFB-987 for their kind help in putting the program for this conference together. I am very grateful for their advice and support!

Marburg is one of the oldest university towns in Germany. History, culture, and science are interwoven in this city and all contribute to its attractiveness. Allow yourself to be enchanted in its charm and spirit! The members of the organizing committee welcome you all wholeheartedly for four days of exciting science, meeting colleagues and friends, making new acquaintances, and – of course – also for some fun!

I hope that you all will say when you go back home again after the end of this conference on March 4th: science was great, we enjoyed ourselves and – we all will definitely come back to Marburg for a new VAAM Meeting! Fortunately, I certainly will be retired by then and unavailable to serve as an organizer for this future conference. ■

For the *Microbial Community* in Marburg,

Erhard Bremer, Conference Chair

Welcome of the President of the VAAM to the Annual Conference 2015

■ Dear Colleagues and Friends, the highlight of each year for our society is its annual meeting. Exactly 25 years after the conference took place in Marburg for the first time, we meet for the 30th anniversary of the VAAM again in this beautiful city. I have a special relationship to the city and university of Marburg. The Philipps-Universität Marburg is the old *alma mater* of my wife Martina and me. Both our sons were born here.

It is also very special that we will celebrate the 30th anniversary of the VAAM in Marburg. We look back on 30 exciting years of scientific and educational activities covering the full scope of microbiology, with a continuously increasing number of members and a special commitment to the support of young scientists. Clearly, the strength and success of our organization is largely built on a long series of very successful conferences, where students and professionals from academia and industry come together for an intense scientific and social exchange. Additionally, the attractiveness of these conferences is ensured by the high quality of the speakers and presentations. This year, the organizers have selected again a variety of very interesting, state-of-the-art topics including Synthetic Microbiology, Bacterial Cell Biology, Microbial Evolution, CRISPR-Systems and Viruses, and Symbiosis.

Finally, I want to thank especially Erhard Bremer from the Microbial Underground in Marburg, all members of the Organization Committee, Conventus, and Katrin Muth for their great efforts and commitment in organizing the Annual Conference in Marburg. I would also like to thank all scientists for their contributions. I am convinced that we will have an exciting conference with stimulating discussions and I would like to encourage you to join us in Marburg for this outstanding scientific event. ■

Enjoy the Conference and Happy Birthday VAAM !

Dieter Jahn

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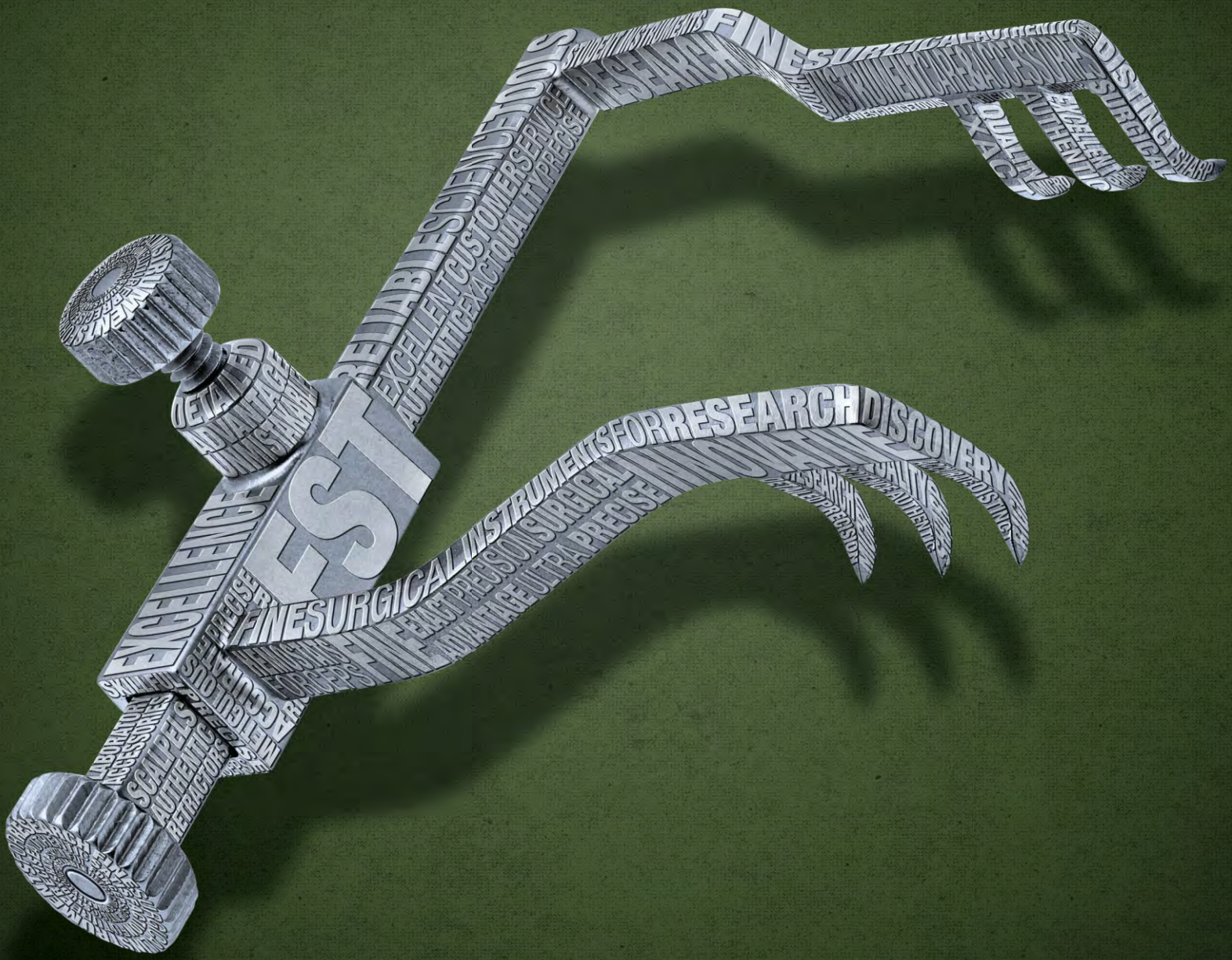

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Microbes and Microbiologists in Marburg

■ If a microbiologist thinks about Marburg, a sleepy little University town in Hesse, two names come immediately to mind: Emil von Behring and the Marburg virus. Both names are connected with the *Hygiene-Institute* of the Philipps-University that was founded in 1885 and whose first director was Max Rubner. R. Siegert, a long-time director (1957–1982) of this institute, succinctly summarized the history and impressive accomplishments of the members of the *Hygiene-Institute* in the fields of bacteriology, public health, immunology, and virology. His overview article [1] appeared in the abstract booklet of the VAAM Annual Meeting held in Marburg in 1989, the first-ever abstract booklet published by the VAAM in connection with its yearly scientific meeting. This meeting (Fig. 1) was organized together with Section I of the *Deutsche Gesellschaft für Hygiene und Mikrobiologie* (DGHM) and the *Nederlandse Vereniging voor Microbiologie* (NVvM); about 1 200 microbiologists came to Marburg to participate in this memorable conference from the 19th to the 22nd of March 1989.

Fig. 1. The keynote speaker of the 1989 VAAM Meeting in Marburg, Julius Adler (Madison, WI, USA), in conversation with Hans Gerhard Schwick (CEO of the Behringwerke AG) and Wolfgang Buckel (co-organizer of the 1989 VAAM Meeting).

Emil von Behring

Without doubt, Emil von Behring is the most prominent member of the *Hygiene-Institute* (director from 1895–1916). His ground-breaking work in the field of microbiology and the discovery of serum therapy saved countless lives and earned him in 1901 the Nobel Prize, the first ever awarded in the fields of medicine and physiology. In his work, Emil von Behring connected excellence in science with entrepreneurship through the foundation of the *Behring Werke* that produced and marketed sera against various kinds of deadly infectious diseases such as tetanus and diphtheria. In remembrance of Emil von Behring, the Philips-University Marburg awards on a regular basis a prestigious prize that honors outstanding contributions in the fields of microbial infection biology, immunology and virology (http://de.wikipedia.org/wiki/Emil-von-Behring-Preis). The **Emil von Behring-Prize** is currently proudly sponsored and funded by one of the successor companies of the former *Behring Werke*, Novartis Vaccines.

Marburg virus

The arrival of the Marburg virus in Germany was connected with research conducted at the *Behring Werke* in 1967. The likely origin of the virus were green macaques, apes that were imported by this pharmaceutical company from Africa in connection with experiments to establish kidney cell cultures that were needed to develop a vaccine against poliomyelitis. In a tour de force, and without precise knowledge of the danger that lurked in handling infected patients, tissue, and body fluids (seven patients died during outbreaks in Marburg and Frankfurt), the infectious agent was identified as a virus in a fast-paced collaboration between members of the *Hygiene-Institute* in Marburg (Slenczka) and the Bernhard-Nocht-Institute in Hamburg (Peters and Müller). This type of virus had a morphology not seen before; it became the founding member of the *Filoviridae* family. The christening of the virus put Marburg on the "world map" of the most deadly infectious agents.

The Marburg virus has a close relative; the notorious Ebola virus. Its recent uncontrolled outbreak in Africa reminds us how vulnerable mankind is against infectious diseases that jump from animal reservoirs to humans. The Institute for Virology of the Philipps-University under the successive leadership of Hans-Dieter Klenk and Stephan Becker has been at the forefront of research on the biology of the Marburg and Ebola viruses and actively participates in efforts to develop remedies against infections by these deadly viruses. A state of the art *biosafety level 4* (BSL4) high security facility, one of the only two laboratories of this type that currently operate in Germany, allows advanced work with these highly contagious viruses.

Microbiology in the Medical School

From 1974 on, the Marburg Hygiene Institute developed into a center comprising the Institute of Medical Microbiology and Hospital Hygiene, the Institute of Immunology and the Institute of Virology, whose present members and their scientific interests are listed in Fig. 2. The three Institutes moved in 2007 into the newly built Biomedical Research Center located on the Campus Lahnberge of the Philipps-University. In Fig. 2 also the Institute of Cytobiology and Cytopathology is listed. These are the four institutes, with which the microbiologists outside the medical school in Marburg (Fig. 3) collaborate in Graduate Schools and Collaborative Research Centers (see below).

Microbiology in the Department of Biology

Until the beginning of the 1960s, bacteria were considered by botanists to belong to the lower plants and cyanobacteria to the algae. In the Botanical Institute at the Department

[1] **Siegert, R.** (1989) 100 Jahre Hygiene-Institute der Philipps-University Marburg; 1885–1985. Forum Mikrobiologie 1–2/89: 3–6.

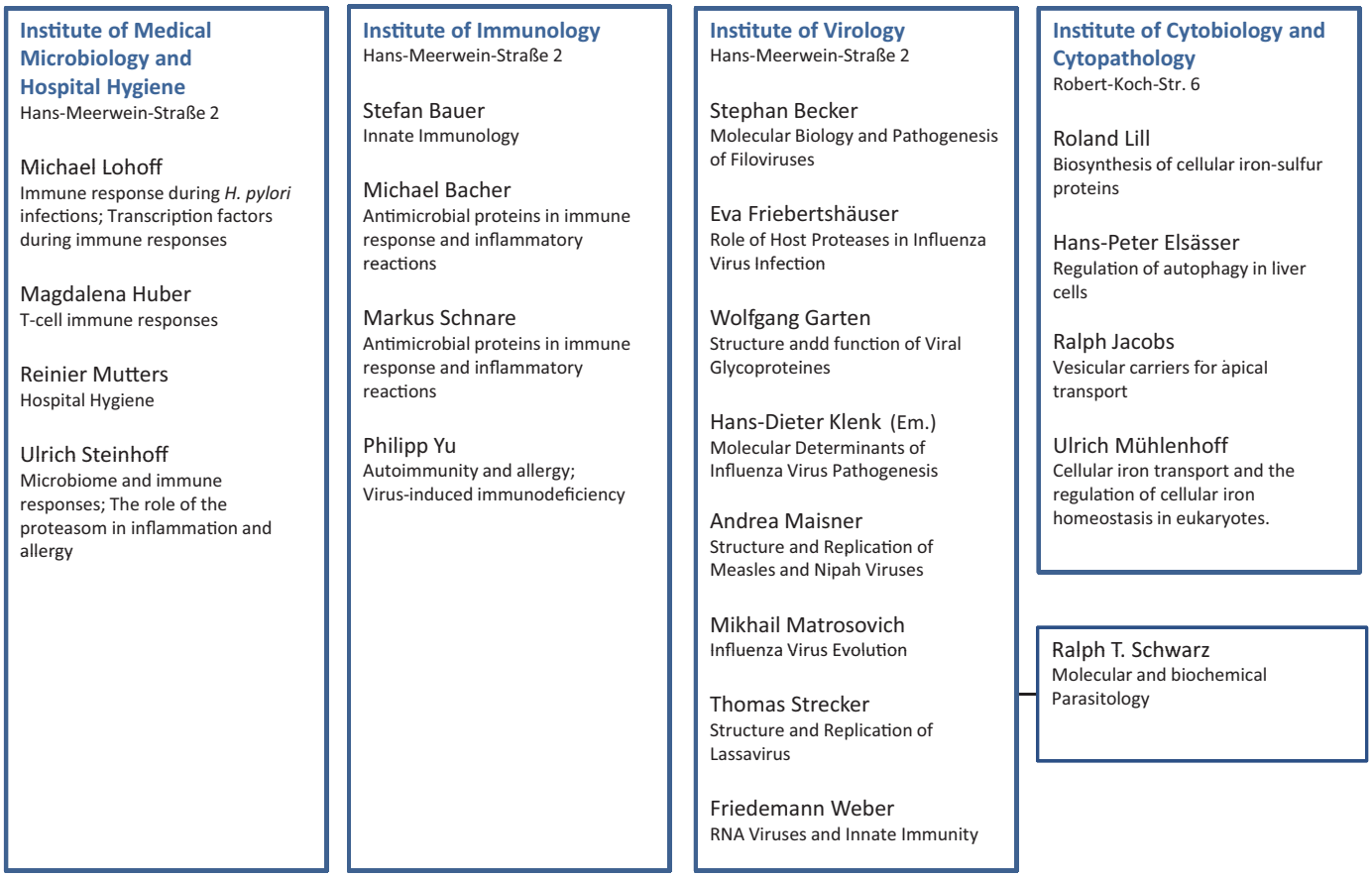


Fig. 2. Scientists working in the area of infection biology and cytobiology at the Philipps-University Marburg. www.uni-marburg.de/fb20/medmikrobio; www.uni-marburg.de/fb20/immunologie; www.uni-marburg.de/fb20/virologie; www.uni-marburg.de/fb20/cyto.

of Biology of the Philipps University, A. Henssen explored *Actinomycetes*, D. Werner studied *Rhizobia* and W. Wehrmeyer worked with *Cyanobacteria*, to name only a few botanists from the Dept. of Biology interested in microbes. In 1976, a dedicated professorship for microbiology was founded at the Dept. of Biology of the Philipps-University.

The establishment of the *Fachgebiet Mikrobiologie* at the Dept. of Biology marked an important event in the development of this field at the Philipps-University; Rolf Thauer was appointed as the first professor of General Microbiology. In 1980, a second professorship for Microbiology was established and Achim Kröger was its first incumbent. When Kröger left Marburg to take up a full professorship at the University of Frankfurt, Berhard Schink came to Marburg in 1985 only to be called away already in the year 1987 by an offer for full professorship at the University of Tübingen. Wolfgang Buckel then filled this vacant professorship in the same year and he held this position until his formal retirement in 2008. Subsequently, he became a Max Planck Fellow and continues to this day his work on the mechanisms of enzymes in anaerobic bacteria in the Laboratory for Microbiology at the Philipps-University. In connection with the foundation of the Max Planck Institute for Terrestrial Microbiology in 1991, a third professorship was established to strengthen the field of Molecular Microbiology at the Philipps-University. This position was filled in 1995 with Erhard Bremer who originally came to Marburg in 1992 as a tenured group leader to work in the Dept. of Biochemistry at the MPI for Terrestrial Microbiology. The retirement of R. Thauer from his duties at the Philipps-University in 2005 enabled the Dept. of Biology to hire Martin Thanbichler, first as a tenure track Junior Professor (2008–2014) and, subsequent to an offer from the LMU München, as professor for Cellular Microbiology (since 2014). The professorship formerly held by W. Buckel was awarded in 2008 to Johann Heider, who is a microbial biochemist.

Microbial Genetics

One of the drivers of modern Microbiology is the field of microbial genetics. In recognizing this, the Philipps-University established a professorship for this discipline at the Dept. of Biology that was filled in 1983 with Albrecht Klein. A second professorship in genetics was founded in 1989 and Bernhard Erni took on these duties. After Erni left Marburg for the University of Bern (Switzerland), Michael Bölker became professor for Genetics in the year 1997 and Hans-Ulrich Mösch was hired in 2004 by the Dept. of Biology to replace Klein upon his retirement. Klein, Erni, Bölker, and Mösch are all microbial geneticists that work/ed either with *Archaea* (Klein) *Bacteria* (Erni) or *Fungi* (Bölker and Mösch). Right from the beginning, the laboratories for Microbiology and Genetics at the Dept. of Biology co-operated closely, both in research and in teaching.

MPI for Terrestrial Microbiology

A game-changing event for Microbiology in Marburg was the foundation of the Max Planck Institute for Terrestrial Microbiology in the year 1991. Rolf Thauer served as the founding director and held joint appointments both at the Philipps-University and the MPI in the years between 1991 and 2005. He then served exclusively as head of the Dept. of Biochemistry at the MPI and since 2007 as the leader of an Emeritus research group; he

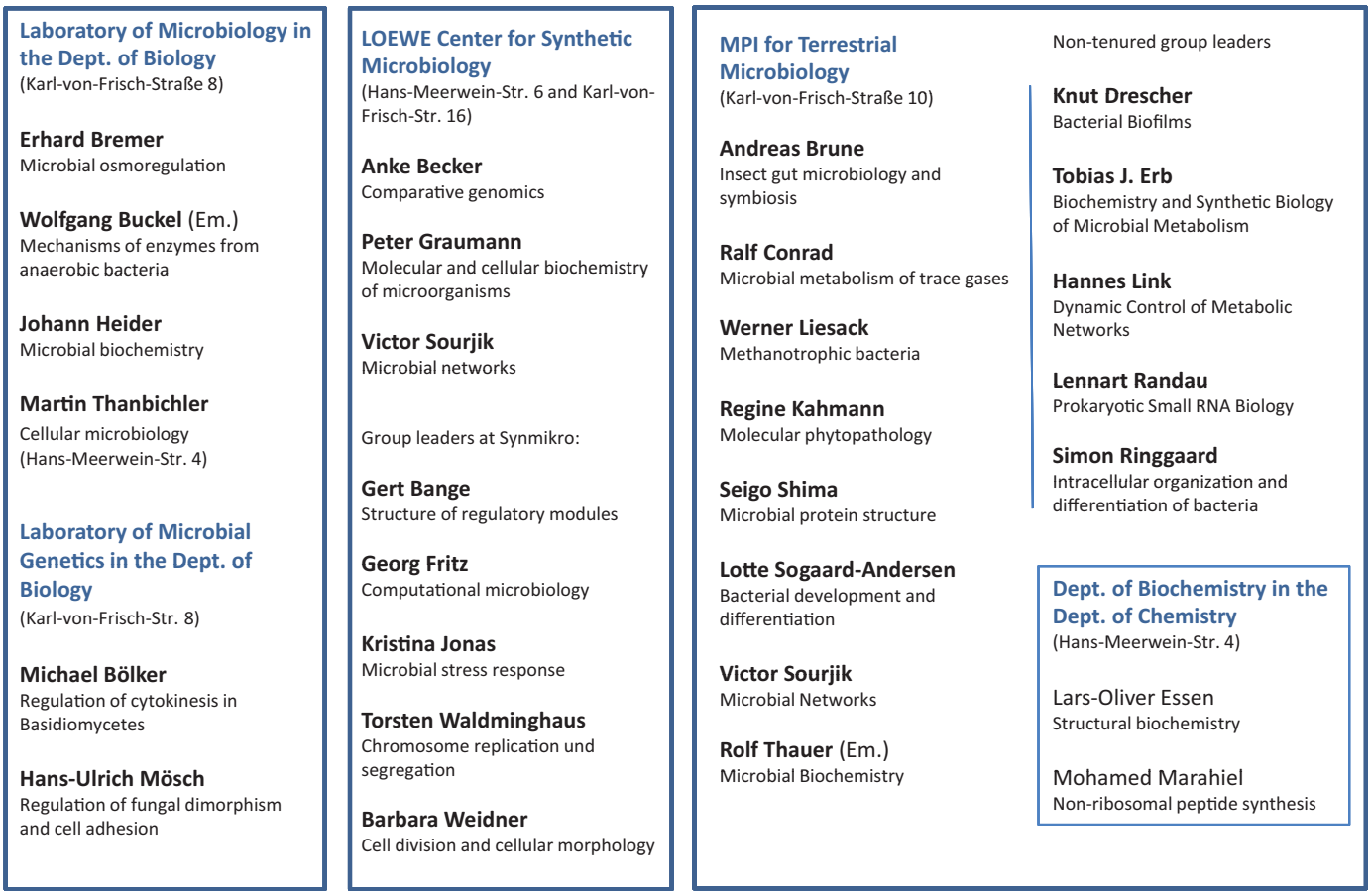


Fig. 3. Scientists working in the area of general and applied microbiology at the Philipps-University Marburg and at the Max Planck Institute for Microbiology. www.uni-marburg.de/fb17/fachgebiete/mikrobio/; www.uni-marburg.de/fb17/fachgebiete/genetik/; www.synmikro.com/en/research/; www.mpi-marburg.mpg.de/research/; https://www.uni-marburg.de/fb15/fachgebiete/bio/.

retired at the end of 2014. The other directors of the MPI are Ralf Conrad (head of the Dept. of Biogeochemistry; since 1991), Regine Kahmann (head of the Dept. of Organismic Interactions; since 2000), Lotte Sogaard-Andersen (head of the Dept. of Ecophysiology; since 2004) and Victor Sourjik (head of the Dept. of Systems and Synthetic Microbiology; since 2014). In addition, Andreas Brune holds a senior research position at the Dept. of Biogeochemistry (since 2003). A considerable number of permanent and temporary research groups are housed by the Max Planck Institute (Fig. 3) and have significantly strengthened the research activities in various fields of microbiology in Marburg.

Free floaters

A highly successful tool of the Max Planck Society to nurture the careers of young scientists is the so-called *Free-Floater* program. The leaders of these independent research groups are chosen through a highly selective evaluation procedure and then can choose (hence the name!) at which institute of the Max Planck Society they want to work. It tells its own tale that, since start of the program in 2006, six of the selected group leaders chose Marburg to establish their laboratories: (i) Sonja-Verena Albers (now professor at the University in Freiburg), (ii) Martin Thanbichler (now professor at the University in Marburg), (iii) Eva Stukenbrock (now professor at the University in Kiel), (iv) Lennart Randau (since 2010), (v) Tobias Erb (since 2014), and (vi) Knut Drescher (since 2014).

Joint appointments and activities

The building of the MPI for Terrestrial Microbiology is located right next to the building of the Dept. of Biology of the Philipps-University (Fig. 4). This close spatial neighborhood signalized the establishment of fruitful ties between these two institutions in research and teaching. A reflection of these connections is the co-appointment of R. Kahmann and L. Sogaard-Andersen as full professors at the Dept. of Biology; R. Conrad, V. Sourjik and A. Brune were awarded honorary professorships by the Philipps-University. These ties between the Philipps-University and the MPI for Terrestrial Microbiology considerably enriched the research activities in various fields of microbiology in Marburg. It allowed the sharing of facilities such as the mechanics workshop and lecture halls, and of equipment, and mobilized additional financial resources and expertise for teaching.

International Max Planck Research School

Based on these joint activities, the International *Max Planck Research School for Environmental, Cellular and Molecular Microbiology* (IMPRS-Mic; http://www.imprs-microbiology.mpg.de/) was established in 2003 as a joint effort between the Philipps-University and the Max Planck Society. It went through two rounds of competitive peer review and, as a reflection of its success and international visibility, is now in its third funding period (until 2020). Striving for excellence, the IMPRS-Mic recruits outstanding PhD students from abroad and from within Germany. In addition to their 3-year PhD research projects, they are trained through lectures, seminars and practical courses in advanced techniques in a broad range of topics and learn the

skills required for a successful career in science or industry. As a hallmark of the IMPRS-Mic, each student chooses his own thesis advisory committee. Typically members from both the MPI and the Philipps-University serve on the committees and participate in supervising and directing the research efforts of the PhD students. Currently, approximately 30 experienced group leaders and professors are involved in the IMPRS-Mic. Lotte Sogaard-Andersen spearheaded the intense training efforts of the IMPRS-Mic for many years.

Fig. 4. Building of the MPI for Terrestrial Microbiology and its close spatial location to the building of the Dept. of Biology of the Philipps-University Marburg.

DFG-funded collaborations

The close collaborations of groups from various departments from the Philipps-University and subsequently with the MPI for Terrestrial Microbiology enabled the microbiologically oriented research community in Marburg to successfully compete for DFG-funded collaborative research projects. This started in 1990 with the *Graduiertenkolleg* "Enzyme Chemistry" (1990–2000), followed by the *Graduiertenkolleg* "Protein Function at the Atomic Level" (1999–2006). Groups from the Laboratories of Microbiology and Genetics and the MPI for Terrestrial Microbiology also participate in the *Graduiertenkolleg* "Intra- and Intercellular Transport and Communication" (http://www.iitc-marburg.de/objective.html). In 1996, the Collaborative Research Center SFB-395 was established that thematically focused on "Interaction, Adaptation and Catalytic Capability of Soil Microorganisms". In 2012 a new SFB (987) started its work with a scientific focus on "Microbial Diversity in Environmental Signal Response" (http://www.sfb987.de/). Groups at the MPI also participate in the work of the SFB-593, which focuses on the "Mechanisms of Cellular Compartmentalization and the Relevance for Disease" (2003–2014) (http://www.uni-marburg.de/sfb593).

Microbial Diversity

The title of the ongoing SFB-987 heralds one of the real strengths of microbial research in Marburg. Instead of focusing just on a few model microorganisms, the research groups work with a large variety of *Bacteria*, *Archaea*, and *Fungi* and study their properties both under controlled laboratory conditions and in their natural habitats. A breadth of approaches and techniques are applied with the aim to understand specific activities and signaling processes of defined species, the behavior of individual cells and microbial communities, the interactions of microorganisms between each other and with their eukaryotic hosts, and the countless contributions that microorganisms make to global cycles operating on our planet. Microorganisms are diverse and thus the studied objects and processes must be diverse as well; it is that simple! Microbial diversity lies at the heart of evolution and prepares microbial cells, masters of change, for whatever challenges might lie ahead.

Undergraduate and graduate teaching

The varied research activities and the large number of microorganisms studied in Marburg transcends into the focused teaching activities to train the next generation of microbiologists in the best possible ways. Teaching is high on the agenda of the microbiologists working in Marburg, be it through lectures, seminars, practical courses, lab rotations, Bachelor, Master and PhD thesis projects and through concerted efforts of graduate schools. Students enrolled in studying microbiology in Marburg are offered a broad choice both with respect to the organisms and processes they might want to study. After their studies, many of them have found satisfying and influential positions in industry or academia. This shows that Marburg is a good place to study Microbiology!

Training young researchers

There is one other aspect of *Microbial Diversity* that we want to point out here since it is often overlooked. If our work in Marburg was focused exclusively on just a few model microorganisms (let's say *Bacillus* and *Ustilago*), it would over time become increasingly hard for aspiring young group leaders to find appropriate positions in academia, regardless how brilliant they might be in their respective research fields. Given that the research activities of the many young group leaders in the fields of microbiology and microbial genetics are diverse in Marburg, there is plenty of room for each of them to flourish and successfully compete for academic positions. This concept is a success story and in Table 1 we list all those 54 individuals who have been trained in Marburg and went on to become respected colleagues in our profession; we are proud of each of them!

Center for Synthetic Microbiology

A second game-changing event for Microbiology in Marburg was the foundation of the LOEWE Center Synthetic Microbiology (*Synmikro*) in the year 2009. LOEWE is the acronym for „Landes-Offensive zur Entwicklung Wissenschaftlich-ökonomischer Exzellenz (https://wissenschaft.hessen.de/loewe). For the center three new colleagues joined Marburg: Anke Becker (Comparative Genomics), Peter Graumann (Molecular and Cellular Biochemistry of Microorganisms), and Victor Sourjik (Microbial Networks), who is also Director at the Max Planck Institute. Additionally, the center allowed establishing

1976-1990
Michael Bott (KFA Jülich; University Düsseldorf)
Gabriele Diekert (University Jena)
Bernd Eikmanns (University Ulm)
Georg Fuchs (University Freiburg)
Ken Hammel (University of Wisconsin, USA)
Jacob Kristjansson (University of Iceland at Reykjavik)
Kesen Ma (University of Waterloo, Canada)
Sabine Rospert (University Freiburg)
Paul Scherer (University Hamburg)
Peter Schönheit (University Kiel)
Alfred Spormann (Stanford University)
Fritz Widdel (MPI Bremen)
Ajit Varma (Neruh University, New Delhi, India)

Since 1991
Sonja-Verena Albers (University Freiburg)
Susumu Asakawa (Kumamoto University, Japan)
Erhard Bremer (University Marburg)
Matthias Brock (University Nottingham, UK)
Petra Dersch (Technical University Braunschweig)
Gunther Döhlemann (University Köln)
Evert Duin (Auburn University, Alabama, USA)
Peter Dunfield (University Calgary, Canada)
Michael Feldbrügge (University Düsseldorf)
Reinhard Fischer (University Karlsruhe)
Philipp Franken (Humboldt-University Berlin)
Michael Friedrich (University Bremen)

Since 1991 (continued)
Penelope Higgs (Wayne State University, Detroit, USA)
Peter Janssen (AgResearch, Palmerston North, NZ)
Dieter Jahn (Technical University Braunschweig)
Mohamed Jebbar (University of Brest; France)
Jörg Kämper (University Karlsruhe)
Jihoe Kim (Yeungnam University, Korea)
Fuli Li (CAS Institute for Biotechnology, Qingdao, China)
Yahai Lu (Agricultural University, Beijing, China)
Erica Lyon (Roger Williams University, RI, USA)
Matthias Mack (Fachhochschule Mannheim)
Georgi Muskhelishvili (Jacobs-University Bremen)
Antonio Pierik (University Kaiserslautern)
Barbara Reinhold (University Bremen)
Karin Sauer (State University at Binghampton, NY, USA)
Gary Sawers (University Halle)
Jan Schirawski (RWTH Aachen)
Ruth Schmitz (University Kiel)
Sylvia Schnell (University Giessen)
Thorsten Selmer (Fachhochschule Aachen)
Gero Steinberg (University of Exeter, GB)
Eva Stukenbrock (University Kiel)
Martin Thanbichler (University Marburg)
Kai Thormann (University Giessen)
Matthias Ulrich (Jacobs-University Bremen)
Helle Ulrich (Institute for Molecular Biology, Mainz)
Uwe Völker (University Greifswald)
Julia Vorholt (ETH Zürich)
David Weiss (University of Iowa, USA)
Alga Zuccaro (University Köln)

Table 1. 54 microbiologists who were trained either at the Laboratory of Microbiology of the Dept. of Biology of the Philipps-University, or at the Max Planck Institute for Terrestrial Microbiology, or were research group leaders there and are now professors.

four non-tenured junior groups that tackle scientific problems in microbial cell biology (Kristina Jonas), structural biology of microbial proteins and protein complexes (Gert Bange), chromosome biology of microorganisms (Torsten Waldminghaus) and computational microbiology (Georg Fritz). We remember with great respect our colleague Alexander Böhm who came to Marburg at the beginning of 2012 as a junior group leader for the *Synmikro* Center and unexpectedly died at the end of 2012. Whereas the groups of Anke Becker, Peter Graumann and the four junior groups are currently housed in renovated labs of the Philipps-University in Hans-Meerwein Str. 6, the group of Victor Sourjik is housed in a newly erected building that is located between the Max Planck Institute and Hans-Meerwein-Str. 6.

The director of the *Synmikro Center* is Bruno Eckhardt, a colleague from the Dept. of Physics; Anke Becker serves as the vice-director. The choice of a physicist as the director of an endeavor that focuses on microorganisms seems unusual at first sight, but *Synmikro* is an initiative that embraces groups from the fields of biology, chemistry, pharmacy, medicine, mathematics and physics to look at microorganisms in a new way; this requires the deviation from well-traveled roads. The main goal of the *Synmikro* Center in Marburg is to further the understanding of the inner workings of microbial cells and their communication with the outside world. Using synthetic approaches, these processes are not only described from a biological, genetic and biochemical point of view but also in a quantitative manner, and this requires also the modeling of cellular processes. The considerable expertise in various fields of microbiology that we have here in Marburg also helps to exploit the boundless biosynthetic potential of microorganisms for practical purposes.

Cell biology

The field of microbiology is highly dynamic. Technical revolutions such as whole genome sequencing, meta-genomics, proteomics, and meta-transcriptomics provide incentives and opportunities to tackle scientific questions from viewpoints that were not experimentally approachable before. Due to their small size, bacterial cells were long seen as unsuitable objects to study them from the perspective of a cell biologist. Striking improvements in microscopy and the advent of fluorescent labels and proteins changed all that and have provided already fascinating new insights into the makeup of cellular structures, developmental processes and the dynamics of individual proteins and protein complexes within single cells. Microbial cell biology is certainly a blooming field. Recognizing this early on, concerted efforts were made in recent years in Marburg to strengthen and expand our expertise in this area; the hiring of Martin Thanbichler and Peter Graumann testifies to this. The increased local activities in cellular microbiology were only possible through the generous funding within the framework of the LOEWE initiative by the government of Hesse.

A new building for *Synmikro*

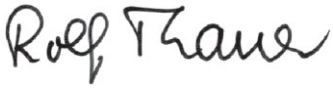
In a highly welcomed decision, the Central Science Advisory Committee to the German government (*Wissenschaftsrat*) recommended in April 2014 the funding of a large new building to house the microbiologically oriented groups and activities of *Synmikro* in Marburg under one roof. This new building will have space of about 6 000 qm^2 and about 61 million EUR have been allocated to its construction and equipping. It will be located on the expanding campus on the Lahnberge right next to the MPI for Terrestrial Microbiology, the already existing facilities of *Synmikro* and the buildings of the Dept. of Biology and Chemistry (just newly built) of the Philipps-University. It is expected to be operational in 2019/2020. We anticipate that this new building will foster even closer ties between groups, the cost-effective sharing of equipment and joint efforts in teaching. Nothing is better for the advancement of science than chatting with a colleague or a student over a cup of coffee!

Swan Song

We took the Annual Meeting of the VAAM in March 2015 as an opportunity to reflect on the past development and future of microbiological-oriented research and teaching in Marburg. With gratitude to our colleagues, co-workers and countless students, we conclude that microbiology in Marburg is alive and well. The members of the local *Microbial Community* head enthusiastically into the future! ■

Marburg, Dec. 2014

Erhard Bremer



Rolf Thauer

Mikrobiologie – das bewährte Laborhandbuch

Eckhard Bast
Mikrobiologische Methoden
3., überarb. u. erg. Aufl. 2014, XVIII, 472 S. 31 Abb. Brosch.
ISBN 978-3-8274-1813-5
€ (D) 39,95 | € (A) 41,11 | *sFr 50,00

Toptitel

Dieses bewährte Laborhandbuch richtet sich an Studierende und Dozenten der Biologie, Biotechnologie und Medizin, an Biologielehrer, an technische Assistenten und an Wissenschaftler in Forschung, Industrie und Untersuchungslabors. Es bietet auch in der Neuauflage präzise und reproduzierbare „Man-nehme"-Vorschriften der wichtigsten mikrobiologischen Methoden sowie theoretische Grundlagen und Hinweise zur Auswertung, zur Leistungsfähigkeit und zu den Grenzen der behandelten Arbeitstechniken. Das Buch hat sich als unentbehrlicher Begleiter für alle erwiesen, die erste Erfahrungen im Umgang mit Mikroorganismen, insbesondere mit Bakterien, sammeln und sich über die gängigen Standardmethoden informieren wollen.

Für die **3. Auflage** wurde der Text überarbeitet und an zahlreichen Stellen ergänzt. Unter anderem wurden die Regeln der Biostoffverordnung, Schnelltests zur Gramfärbung und die Epifluoreszenzmikroskopie mit einer Reihe von Färbeverfahren neu aufgenommen.

Venue

Philipps-University of Marburg
Hörsaalgebäude
Biegenstraße 14
35032 Marburg (DE)

Address for correspondence

Conventus Congressmanagement & Marketing GmbH
Alexandra Vogel
Carl-Pulfrich-Strasse 1
07745 Jena (DE)
Phone: +49 (0)3641 31 16-361
Fax: +49 (0)3641 31 16-243
www.vaam-kongress.de

Registration

Please register online at www.vaam-kongress2015.de.

Conference Tickets

	Regular Registration (from 21/12/2014)
Member VAAM/GBM/DECHEMA[1]	230 EUR
Student (Member VAAM/GBM/DECHEMA)[1]	85 EUR
Member Retiree	100 EUR
Non-Member	295 EUR
Student (Non-Member)[1]	110 EUR
Non Member Retiree	140 EUR

Day Tickets

Member VAAM/GBM/DECHEMA[1]	95 EUR
Student (Member VAAM/GBM/DECHEMA)[1]	40 EUR
Member Retiree	40 EUR
Non-Member	120 EUR
Student (Non-Member)[1]	110 EUR

[1] Proof of status required. Please send via e-mail to registrierung@conventus.de, via Fax +49 3641 31 16-244 or postal at Conventus GmbH · Keyword: VAAM 2015 · Carl-Pulfrich-Straße 1 · 07745 Jena (DE)

Social Programme[2]

Welcome Reception, 1 March 2015 – for participants	included
Welcome Reception, 1 March 2015 – accompanying person	15 EUR
Mixer, 3 March 2015 – for participants	included
Mixer, 3 March 2015 – accompanying person	25 EUR

[2] Registration required.

KombiTicket (public transport)

You may purchase Conference Ticket from Conventus GmbH to use public transport in Marburg during the Annual Conference of the Association for General and Applied Microbiology (VAAM).

KombiTicket	5 EUR

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
- Welcome Reception
- 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.

General Terms and Conditions

You can find the general terms and conditions on our conference website www.vaam-kongress2015.de.

Check-In

The Check-In will be at the entrance of the Philipps-University of Marburg.

No 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	13^{00}–21^{00} h	09^{30}–18^{00} h	10^{00}–18^{00} h	10^{30}–11^{30} 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

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 Philipps-University Marburg.

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 Philipps-University Marburg, we suggest to first successfully connect at least one time with the eduroam network at your home university.

Voucher

Every participant has the opportunity to buy a voucher at the Check-In. The voucher is valid for the conference time and costs 2.50 EUR per device.

Conference Language

The conference language is English.

Poster Session

The poster presentations are divided in two sessions. The posters should be switched by the authors in the time between Monday evening (19:30 h) and Tuesday morning (10:00 h).

Poster Session I (from Sunday to Monday, 19:30 hrs):
Cell Biology and Cell Cycle
Environmental Microbiology
Free Topics
Microbial Ecology
Microbial Evolution
Microbial Interactions
Protein Folding and Degradation
Single Cell Analysis
Symbiosis
Toxin-/Anti-Toxins
Translocation of Large Molecules across Membranes and Protein Targeting
Translocation of Small Molecules across Membranes

Poster Session II (from Tuesday to Wednesday)
Biotechnology
CRISPR-Cas, Viruses and Regulatory RNAs
Gene Regulation
Metabolism, Enzymes and Cofactors
Microbial Stress Responses
Microbiology of Anaerobes
Secondary Messengers
Secondary Metabolites
Sensing and Adaptation
Synthetic Microbiology

Travel and City Map

Travel by Car
Address: Biegenstraße 14, 35037 Marburg
Please be reminded that car parking in Marburg is severely limited! If you are driving to Marburg from Frankfurt, you take the highway A5, following the signs directing you towards the city Kassel. At the "Gambacher Kreuz" take highway A45. This will take you to Marburg via Giessen. At the "Südkreuz Gießen" take highway A485 and follow the signs directing you towards Marburg. This highway leads into the through-road B3 (keep left) which then goes directly to Marburg.
Once you are on the B3 and have reached Marburg, take the exit "Marburg-Mitte" and then turn right. At the next traffic lights turn right into the "Erlenring" and follow the signs to the "Stadtmitte". Please follow now the instructions listed in section 3.

If you are coming from Kassel, you will also need to get on the through-road B3 (depending on your departure location, first the A49 then the B3), and follow the signs directing you to Marburg. Once you are on the B3 and have reached Marburg, take the exit "Marburg-Mitte" and turn right at the next traffic lights into the "Erlenring". Follow the signs to the "Stadtmitte".

The "Erlenring" leads you over a bridge across the river Lahn. Keep in the right lane when crossing the bridge. After crossing the bridge, take the first right turn into the "Rudolphsplatz". This will take you to downtown Marburg and the university. After 300 meters you can see the "Hörsaalgebäude" on the left side.

At the "Hörsaalgebäude" there will be no parking spaces available. We recommend to use the underground car park Lahncenter/Welcome Hotel or the car park Pilgrimstein. These car parks are only 5 walking minutes away from the conference venue.

Underground Car Park Lahncenter/Welcome Hotel
Address: Biegenstraße 12, 35037 Marburg
Maximum daily fee: 14 EUR

Car Park Pilgrimstein
Address: Am Pilgrimstein 17, 35037 Marburg
Maximum daily fee: 14 EUR

After you passed the "Hörsaalgebäude" follow the main street and turn left on the next crossing. After about 500 meters turn left into "Pilgrimstein". Almost at the end of the street you can see the car park.

More information about the Car Parks in Marburg can be found on the congress homepage: www.vaam-kongress2015.de.

Travel by Public Transport
Once you have arrived in Marburg main station (this is the station "Marburg/Lahn"; please do NOT leave the train at "Marburg/Südbahnhof"), you must cross the main street (cross the lanes travelling in both directions) and go to the bus stop on the right side of the road. You can reach the center of town and the university easily from the train station: The buses with the numbers 1, 2, 4, 5, 6 und 7 will take you into the city. To get to the university, you would exit the bus at the station "Stadthalle".

Travel by Train

Acting today for tomorrow: Travel by train from 99 Euro with 100% green power to the Annual Conference 2015 of the VAAM.

In cooperation with Conventus Congressmanagement & Marketing GmbH and Deutsche Bahn you travel safely and conveniently to the Annual Conference 2015 of the VAAM in Marburg! Your way to save the environment: Travel with 100% green power to your event with Deutsche Bahn long-distance services. We guarantee to get the energy you needed for your journey in Germany from 100% renewable sources.

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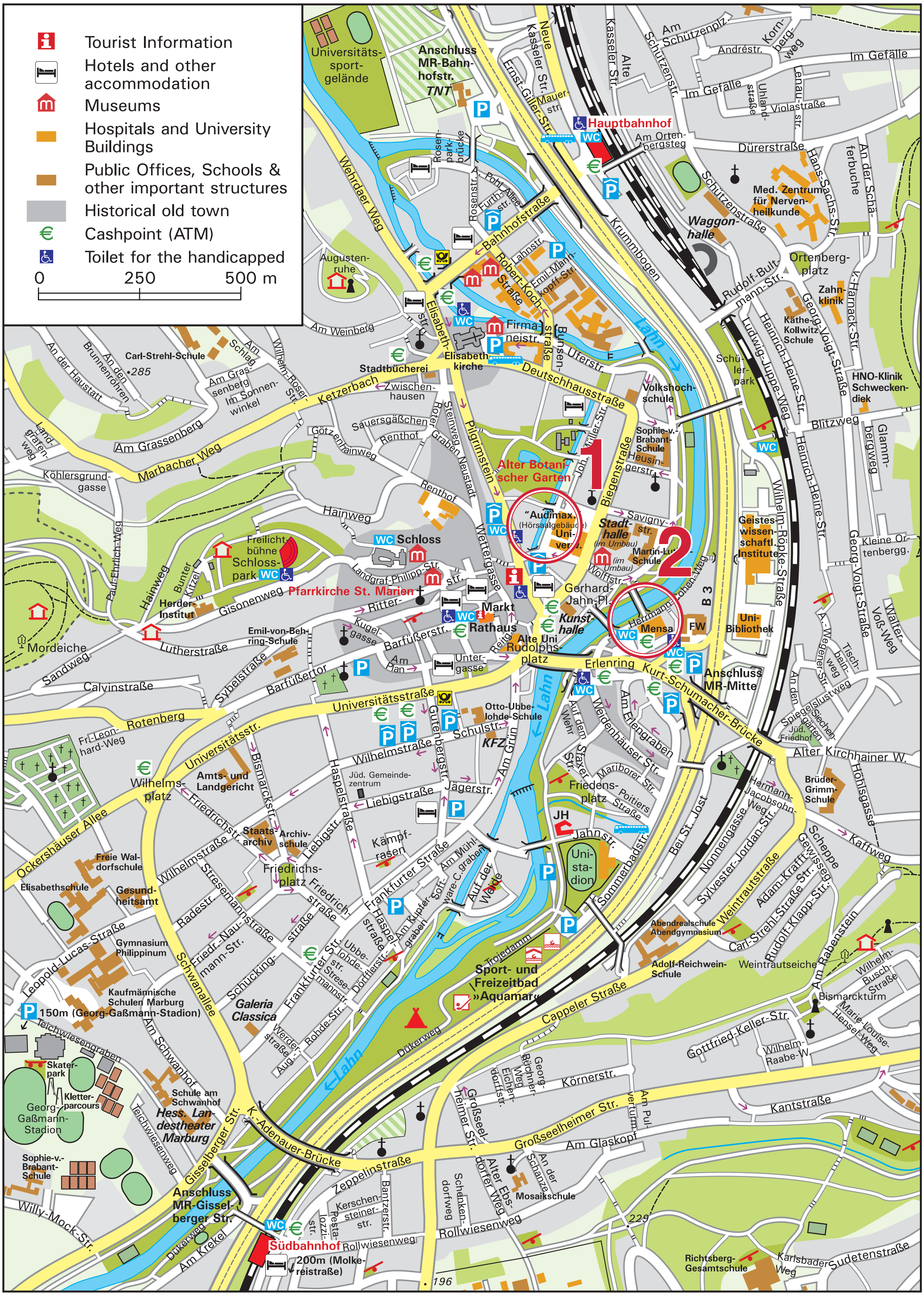
2nd class	99 EUR	139 EUR
1st class	159 EUR	199 EUR

Our call center is glad to inform you about the ticket price for international journeys. This special offer is valid for all congresses of Conventus Congressmanagement & Marketing GmbH in 2014.
To book call +49 (0)180 6 31 11 53** and quote "CONVENTUS" as reference. Have your credit card ready please.

* An advance booking of at least three days is required. Changes and reimbursement before the first day of validity are EUR 15 excluded from the first day of validity onwards. Passengers restrict themselves to a particular train and travel times. For a supplement of EUR 40 full flexible tickets are also available for domestic travels within Germany.

** The booking line is available from Monday to Saturday 0700–2200 h. Calls will be charged at 0.40 EUR per call, from mobiles 0.60 EUR per call maximum.

City Map



© Marburg Tourismus und Marketing GmbH 1 Venue 2 Mixer

Karrieresymposium

Vorstellung verschiedener Berufsbilder in den Biowissenschaften – Anregungen und Tipps

Dienstag, 3. März 2015

15.00 – 16.30 Uhr, Hörsaal 00/0070

Christian Kandt, Bonn

„Promotion, Postdoc, Gruppenleiter und dann?“

Praktische Erfahrungen aus dem Wissenschaftsbetrieb

* * *

Anke Werse, Darmstadt
Thermo Fisher Scientific

„Kinder, Küche und Karriere“

Ein Erfahrungsbericht als Managerin in einem großen Konzern

* * *

Julia Morzfeld, Wiesbaden
Bundeskriminalamt, Abteilung Humanspuren

„100% Bio“

Das Berufsbild von Biologen in der Kriminaltechnik

Hessisches Ministerium für Wirtschaft,
Energie, Verkehr und Landesentwicklung

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- organisiert Workshops, Fachtagungen und Messebeteiligungen
- vernetzt Akteure aus Pharma, Biotechnologie und Medizintechnik
- vermarktet Biotechnologie in Hessen
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VAAM Jahrestagung 2015

Hessen-Biotech HAUPTSPONSOR
1.–4. März 2015, Marburg

Synmikro Symposium 2015

Hessen-Biotech KOOPERATIONSPARTNER
Microbial Biosensors & Regulatory Circuits
Mittwoch, 22. April 2015

synmikro
LOEWE-Zentrum für Synthetische Mikrobiologie

Philipps Universität Marburg

An Hessen führt kein Weg vorbei.

Hessen Biotech

HESSEN TRADE & INVEST

EUROPÄISCHE UNION:
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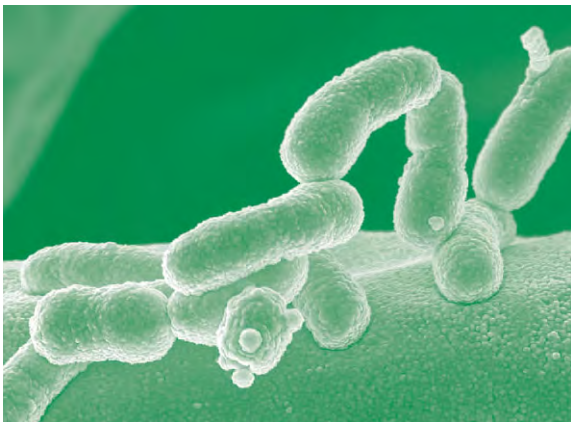
VAAM JAHRESTAGUNG

ANNUAL CONFERENCE

of the Association for General and Applied Microbiology 2016



13–16 MARCH 2016

JENA GERMANY

CONFERENCE CHAIR

Prof. Dr. Axel Brakhage
Friedrich Schiller University Jena
Institute of Microbiology and
Leibniz Institute for Natural Product
Research and Infection Biology
Hans Knoell Institute Jena

VENUE

Friedrich Schiller University Jena
Campus Ernst-Abbe-Platz
Carl-Zeiss-Straße 3
07743 Jena, Germany

TOPICS

- Microbial communication
- Natural products
- Infection biology
- Biotechnology/Synthetic microbiology
- Biodiversity and ecosystem functions
- Bio-Geo-interactions
- Fungal biology
- Systems microbiology
- Biodegradation
- Open topics

WWW.VAAM-KONGRESS.DE

Einladung zur Mitgliederversammlung der VAAM

■ Hiermit lade ich alle Mitglieder der VAAM zur Mitgliederversammlung ein. Sie wird am Dienstag, den 3. März 2015, um 18.00 Uhr in Hörsaal 00/0030 in Marburg stattfinden.

Vorläufige Tagesordnung:

1. Festlegung der Tagesordnung und Genehmigung der Niederschrift der Mitgliederversammlung vom 07.10.2014 in Dresden (siehe BIOspektrum 7/14, Seiten 785 und 786)
2. Bericht aus dem Vorstand, u.a. Haushalt 2014 und Haushaltsplan 2015, Ort und Zeit der nächsten Jahrestagung, Aktivitäten der Fachgruppen, DGHM
3. VBIO
4. Bericht der Kassenprüfer
5. Entlastung des Vorstandes
6. Wahl des Präsidiums (Präsident, 1. Vizepräsident, Schatzmeister, Schriftführer) und drei der sechs Mitglieder des Beirates (geheime Wahl während der Mitgliederversammlung)
7. Mikrobe des Jahres
8. Verschiedenes

Hiermit bitte ich alle Mitglieder, Vorschläge zur Wahl des Präsidiums und des Beirates beim Präsidenten einzureichen (bis 14 Tage vor der Mitgliederversammlung), wobei Vorschläge für das Präsidium von zehn VAAM-Mitgliedern und für den Beirat von drei Mitgliedern unterschrieben sein müssen. Ich möchte auch darauf hinweisen, dass der Vorstand der VAAM den jetzigen 1. Vizepräsidenten entsprechend der Geschäftsordnung (siehe Homepage der VAAM) zur Wahl zum Präsidenten vorschlagen wird. Ordentliche und studentische Mitglieder haben auf der Mitgliederversammlung gleiches Stimmrecht.

Reisekostenzuschüsse für studentische Mitglieder können bei fristgerecht eingegangenen Anträgen und bei Vorliegen der sonstigen Voraussetzungen **nur persönlich ab Dienstag, den 3. März 2015, 14.00 Uhr bis Mittwoch, den 4. März 2015, 13.00 Uhr** im Tagungsbüro abgeholt werden. ■

Hubert Bahl
Schriftführer

Social Programme

Sunday, 1 March 2015 · 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, 3 March 2015 · 30 YEARS VAAM
An exciting outside view on microorganisms and microbiologists presented by the comedian Vince Ebert on the occasion of the 30th birthday of the VAAM.
Time 17:00 h to 17:45 h
Place Audimax in the main lecture hall

MIXER
We like to invite you to the MIXER for speakers, participants and exhibitors. The wonderful music group “Lounge-Band” will play for your entertainment. Food and drinks will be provided.
Time 19:30 h
Place Mensa Erlenring
Erlenring 5, 35037 Marburg

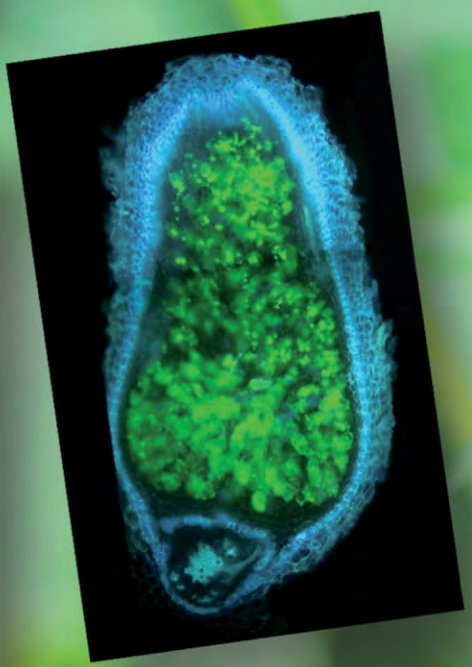
Bring your copy of the - BIOspektrum Sonderausgabe 2015; at the meeting it will cost 20 Euro

Mikrobe des Jahres 2015

Rhizobium

Knöllchenbakterien

– leben in Symbiose mit Leguminosen
– fixieren Luftstickstoff in Wurzelknöllchen
– machen Pflanzen unabhängig von Stickstoffsalzen
– düngen den Boden auf natürliche Weise
– steigern den Ertrag von Agrarflächen
– sichern so einen Teil unserer Ernährung
und
– sind eine evolutionäre Erfolgsgeschichte



Fotos: H. Engelhardt, Martinsried

Foto (oben): U. Mathesius, Canberra

Weitere Informationen unter: www.mikrobe-des-jahres.de

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Sponsors Poster Awards

BIOspektrum (Heidelberg)

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Georg Thieme Verlag KG
SYNFACTS, SYNLETT, SYNTHESIS

Springer Spektrum I Springer-Verlag GmbH
BIOspektrum

S. Karger AG
Journal of Molecular Microbiology and Biotechnology

Wiley-VCH Verlag GmbH & Co. KGaA
GIT Laborfachzeitschrift

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Analytik Jena AG	28
AppliChem PanReac	12
BaseClear	2
BioFroxx GmbH	24
Bio-ITech BV	13
Bruker Daltonik GmbH	20
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Forschungszentrum Jülich GmbH	25
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LGC Standards GmbH	18
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m2p-labs GmbH	17
MACHEREY-NAGEL GmbH & Co. KG	21
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Springer Fachmedien Wiesbaden GmbH	
Takara Bio Europe	8
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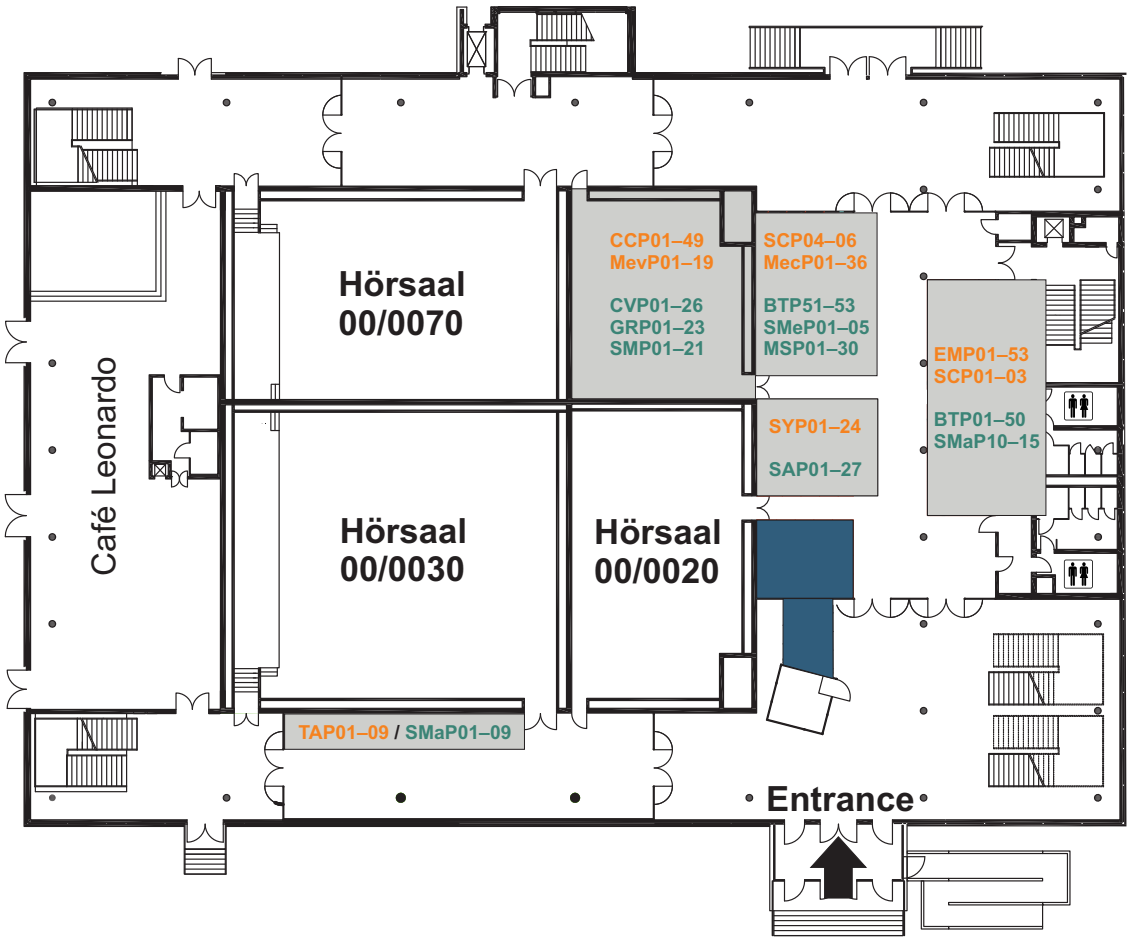
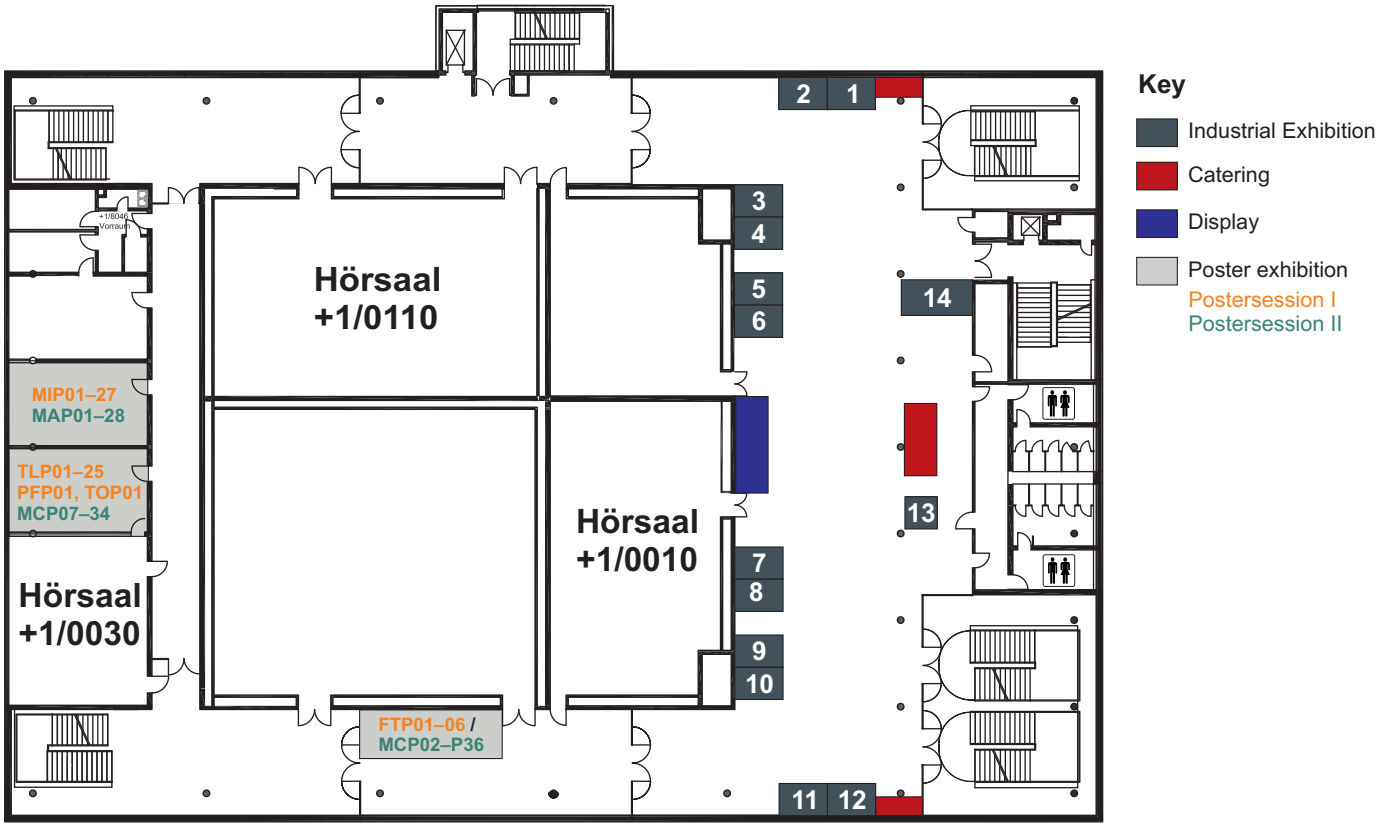
Zymo Research Europe GmbH	22

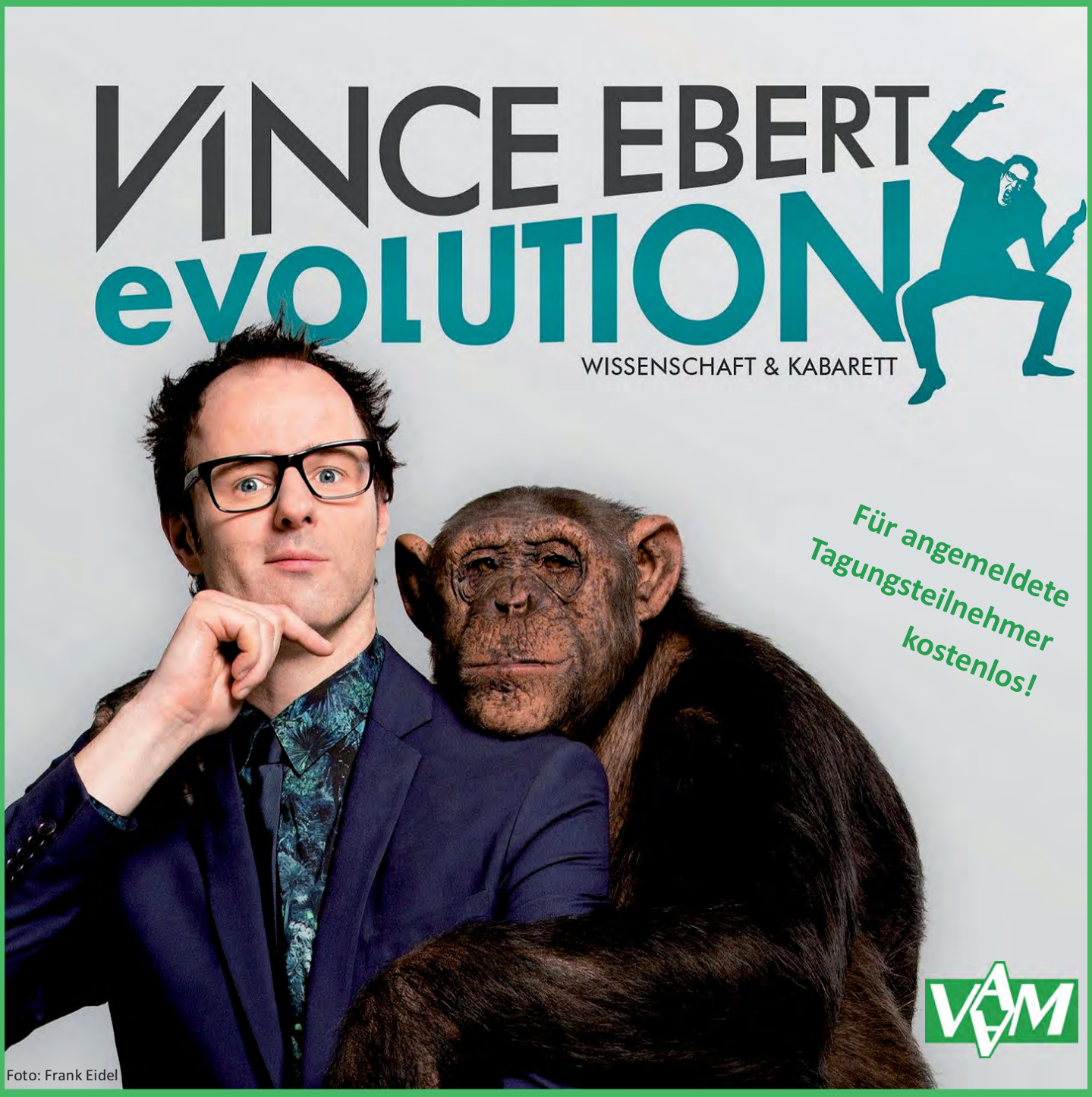
Ground floor



1st floor



Zum 30. Geburtstag der VAAM:

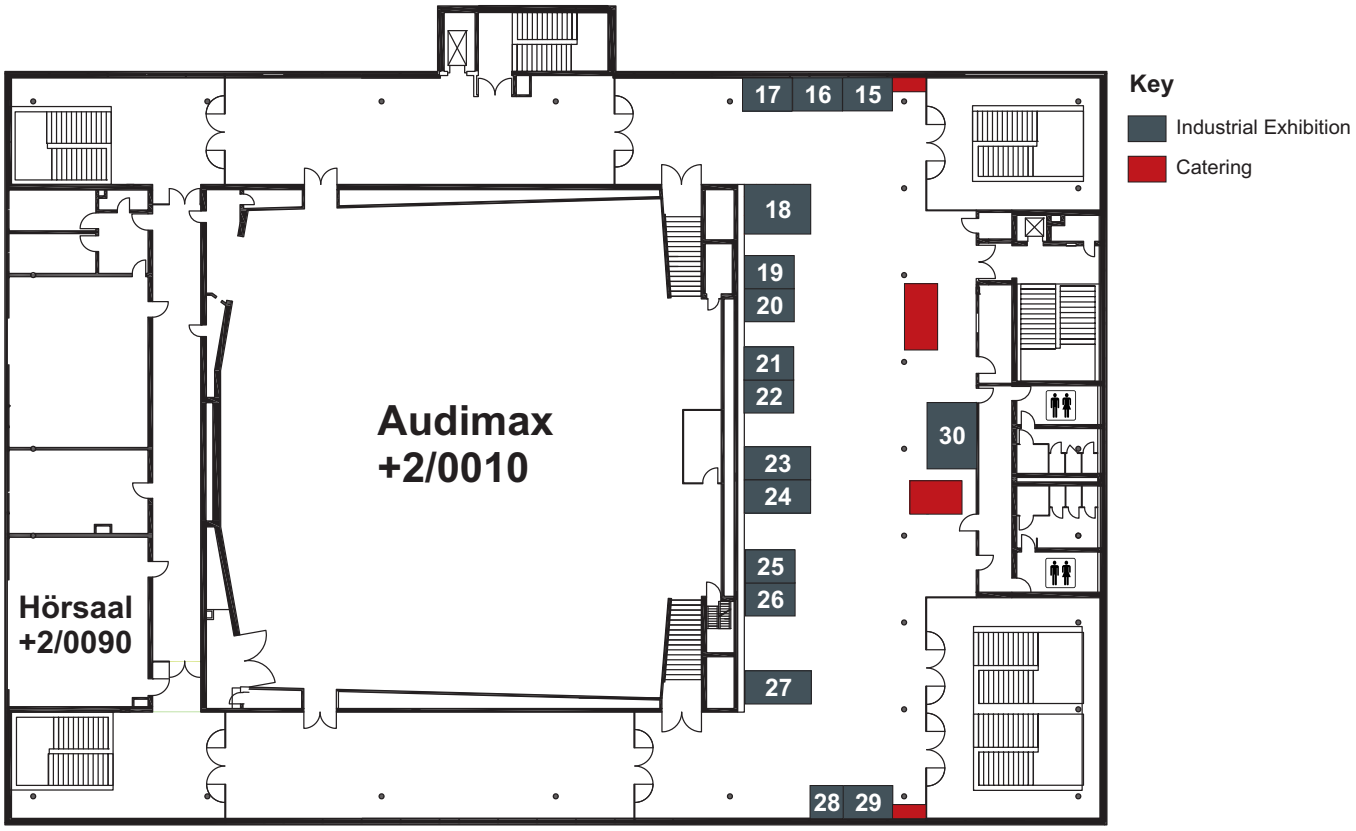


Der Wissenschaftskabarettist Vince Ebert beschäftigt sich in einem Auszug seines aktuellen Programms mit dem größten Thema überhaupt: dem Geheimnis des Lebens. Vor rund 3,5 Milliarden Jahren entstand die erste Lebensform - und natürlich standen von Anfang an Mikroorganismen im Mittelpunkt. Vince Ebert erklärt naturwissenschaftlich fundiert, welche Bedeutung die kleinen Lebewesen für den Menschen haben - Seitenhiebe auf den *Homo sapiens* dürfen natürlich nicht fehlen. Lassen Sie sich überraschen!

www.vince-ebert.de

Di., 3. März um 17.00 Uhr im Rahmen der VAAM-Jahrestagung in Marburg

2nd floor



Bring your copy of the BIOspektrum Sonderausgabe 2015; at the meeting it will cost 20 Euro

Fachgruppe Archaea

Die Fachgruppe Archaea richtete im September 2014 ihre jährliche Tagung in Schmitten im Taunus aus, die vor allem jüngeren Wissenschaftlern eine Plattform bietet, ihre Forschungsergebnisse zu präsentieren und mit anderen kritisch zu diskutieren. Es wurden Forschungsbeiträge über Enzyme, Archaellen, die Wirkung kleiner RNAs, Daten zur Transkriptions- und Translationsregulation, zur Ribosomengenese und zur Biofilmbildung einem fachkundigen und sehr interessierten Publikum vorgestellt. Vier Übersichtsvorträge über *High-throughput sequencing,* Genomannotationen, CRISPR und Gasvesikel regten zudem zu weiterführenden Diskussionen an.

Der Erfolg dieser seit 15 Jahren von Jörg Soppa (Frankfurt) organisierten Archaea-Tagung beruht vor allem auf den sehr fachnahen Diskussion und der Möglichkeit, sich über neuere Entwicklungen bei Methoden und Materialien auszutauschen. Methanogene, hyperthermophile und halophile Archaea sind physiologisch sehr vielfältig, und ihre extreme Lebensweise schafft bei der Arbeit im Labor besondere Probleme, die im Gespräch mit anderen „Extremophilen" oft schneller gelöst werden können.

Im Herbst 2014 wurde der Fortbestand der Fachgruppe Archaea über eine Mitgliederbefragung bestätigt und eine neue Sprecherin bzw. Stellvertreterin gewählt. Sonja Verena Albers, Professorin an der Universität Freiburg, wird die Fachgruppe für die nächsten zwei Jahre führen und dabei von ihrer Stellvertreterin Ruth Schmitz-Streit von der Universität Kiel tatkräftig unterstützt. Beiden wünschen wir viel Erfolg bei dieser Aufgabe.

Felicitas Pfeifer, Universität Darmstadt (Fachgruppen-Sprecherin bis Oktober 2014)

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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 genauso 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 der Fachgruppe. 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 bakterieller 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 Kolleg/inn/en der Mikrobiologie auch solche 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 2015 in Frankfurt stattfinden.

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Fachgruppe Fungal Biology and Biotechnology/Experimentelle Mykologie

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Im Rahmen der VAAM-Jahrestagung 2015 in Marburg wird am 2.3.2015 ein Minisymposium der Fachgruppe stattfinden. Gestaltet wird es von Nachwuchswissenschaftler/inne/n der fünf „Marie Skodowska Curie Innovative Training Networks“, die aktuell im Bereich der Pilzforschung aktiv sind: QuantFung, Ariadne, FungiBrain, ImResFun und YEASTCELL. Koordiniert wird dieses Minisymposium von der QuantFung-Teilnehmerin Danielle Troppens (Göttingen).

Vom 7. bis 9. Oktober 2015 wird die Tagung „Molecular Biology of Fungi“ der Fachgruppe in Berlin stattfinden. Sie wird von Vera Meyer (TU Berlin) organisiert und soll vor allem Nachwuchswissenschaftler/inne/n die Gelegenheit geben, ihre Ergebnisse (inter)nationalen Forschergruppen vorzustellen. Adressiert wird Grundlagenforschung im Bereich der Stoffwechselphysiologie, Entwicklungsbiologie und Pathogenität von Pilzen sowie anwendungsorientierte Forschung im Bereich der roten und weißen Biotechnologie. Mitglieder der Fachgruppe und Gäste sind herzlich nach Berlin eingeladen.

Fachgruppe Wasser/Abwasser

Hans-Curt Flemming war 19 Jahre lang Sprecher der Fachgruppe Wasser/Abwasser und hielt sie mit viel Engagement und Kreativität lebendig. Die Fachgruppe bedankt sich bei ihm für die äußerst erfolgreiche Arbeit und wünscht ihm nach dem Ausscheiden aus dem aktiven Berufsleben viel Zeit und Muße für all die schönen Dinge des Lebens, für die ihm bislang zu wenig Zeit zur Verfügung stand.

Als großer Erfolg erwies sich die von Flemming ins Leben gerufene Konferenz mit dem provokativen Titel „How dead is dead?“ Seit 2009 findet diese Tagung alle zwei Jahre statt. Die nächste HDID IV findet am 21. und 22. Mai 2015 bei der Eawag, Dübendorf, Schweiz unter der Leitung von Frederik Hammes statt und wird von der Fachgruppe Wasser/Abwasser mit organisiert und finanziell mit einem Posterpreis unterstützt. Zu den spannenden Fragestellungen rund um die VBNC (*viable but non culturable*)-Stadien von medizinisch relevanten Bakterien und Umweltbakterien werden wieder international renommierte Wissenschaftler wie Slava Epstein (Northeastern University, USA), Jim Oliver (University of North Carolina, USA) und Bill Keevil (University of Southampton, UK) Plenarvorträge halten. Das Programm kann auf der Konferenz-Website www.hdid-conference.de eingesehen werden.

Auf der Fachgruppensitzung am 6. Oktober 2014 während der VAAM/DGHM-Tagung in Dresden wurde über die zukünftige inhaltliche Ausrichtung der Fachgruppe diskutiert. Neben der Weiterführung der HDID-Tagung, die über den Horizont der Wasser- und Abwasserthemen hinausblickt, wird eine intensivere Zusammenarbeit mit der Fachgruppe Umweltmikrobiologie, in der ebenfalls mikrobiologische Wasser-Aspekte abgedeckt werden, angestrebt. Hier ließe sich eine gute Synergie etablieren, wobei eine Fachgruppe mehr über Abbauprozesse im Wasser, die andere mehr über hygienische Relevanz und Ökologie von Mikroorganismen beiträgt. Weiterhin soll in Fragen zur Mikrobiologie in Oberflächengewässern eine engere Anbindung an die Bundesanstalt für Gewässerkunde, Koblenz bzw. die Universität Koblenz/Landau (Werner Manz) erreicht werden. Außerdem wurde die Wahl der neuen Sprecher im Online-Verfahren vorbereitet.

Im November 2014 wurde Bernd Bendinger (Hamburg) als neuer Sprecher der Fachgruppe gewählt und Ulrich Szewzyk (Berlin) als lang bewährter stellvertretender Sprecher in seinem Amt bestätigt.

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Fachgruppe Identifizierung und Systematik

In den letzten Jahrzehnten beruhten Arbeiten zur Bakterientaxonomie und Beschreibung neuer Arten hauptsächlich auf der Analyse phänotypischer Merkmale (z. B. Morphologie, Physiologie, Biochemie, Fettsäuren, Chinone, Peptidoglykanstruktur, polare Lipide und Polyamine) sowie der 16S rRNA-basierten Phylogenie. Dabei ist zu beobachten, dass besonders die eigentlich schon alte Methode der Analyse von Lipidprofilen zur Klassifizierung von Bakterien (wichtige Arbeiten dazu gehen auf die 1960er und 1970er Jahre zurück) wieder verstärkt eingesetzt wird. Auch wenn diese klassischen Techniken in der nächsten Zeit sicherlich noch eine bedeutende Rolle spielen werden, so befinden wir uns an einem Punkt, an dem die Rolle von Genomen zur Klassifizierung von Bakterien dringend berücksichtigt werden sollte, nicht zuletzt auch deshalb, weil die Kosten für eine Genomsequenzierung inzwischen in einer Größenordnung liegen, die bezahlbar erscheint. Aus diesem Grund wurde bei der VAAM-Jahrestagung in Dresden (2014) dazu ein Minisymposium abgehalten, und dasselbe Thema wird auch in einem Minisymposium der Fachgruppe im Rahmen der VAAM-Jahrestagung in Marburg (2015) weitergeführt und vertieft.

Im Rahmen der VAAM/DGHM-Jahrestagung in Dresden richtete die Fachgruppe Identifizierung und Systematik gemeinsam mit der DGHM-Fachgruppe Mikrobielle Systematik, Populationsgenetik und Infektionsepidemiologie ein Minisymposium mit dem Titel „Evolutionary Genomics“ aus. Dazu gab es zwei Vorträge von eingeladenen Sprechern, Ulrich Vogel (Würzburg) und Helena Seth-Smith (Zürich) sowie zwei Kurzvorträge. Diese Veranstaltung war gut besucht und genug Motivation, auch weiterhin im Rahmen der VAAM-Jahrestagungen Minisymposien zu organisieren.

Weiterhin wurde in Dresden eine Mitgliedergliederversammlung abgehalten. Einziger Punkt auf der Agenda war die Wahl des Fachgruppensprechers und des Stellvertreters. Eine Stimmenabgabe für diese Wahl war sowohl vorab per Email möglich gewesen als auch bei der Mitgliederversammlung. Als Ersatz für Brian Tindall als Stellvertreter hatte sich André Lipski zur Wahl gestellt. Er wurde ebenso wie Hans-Jürgen Busse einstimmig gewählt. Bei der Mitgliederversammlung wurde diskutiert, unter welchem Thema das Minisymposium im Rahmen der VAAM-Jahrestagung in Marburg organisiert werden sollte. Die Mehrheit sprach sich dabei dafür aus, noch einmal Genome in den Vordergrund zu stellen. Es gab aber auch den Wunsch nach einem Symposium über Klassifizierung von Bakterien (eher allgemein). Daher ist angedacht, 2016 tatsächlich ein Minisymposium zu diesem Thema zu veranstalten.

Für das Minisymposium in Marburg unter dem Titel „The Place of Genomics in Taxonomy – or vice versa?“ konnten als Vortragende gewonnen werden: Hans-Peter Klenk (Newcastle, UK), „The impact of the genomic Enzyclopaedia of Bacteria and Archaea on microbial taxonomy“; Kostas Konstantinidis (Atlanta, USA), „Bacterial species in the light of genomics and metagenomics” und Jörn Kalinowski (Bielefeld), „Genome-Based Taxonomy of the Genus *Corynebacterium*“. Wir erhoffen uns spannende Erkenntnisse für die Zukunft der Bakterientaxonomie und eine Einschätzung über die Bedeutung der Genomanalytik in dieser Disziplin.

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Fachgruppe Lebensmittelmikrobiologie

Auf der 4. gemeinsamen Jahrestagung der VAAM und der DGHM in Dresden im Oktober 2014 veranstaltete die FG Lebensmittelmikrobiologie gemeinsam mit der Fachgruppe Lebensmittelmikrobiologie und –hygiene der DGHM an zwei Tagen das Minisymposium „Food Microbiology and Food Hygiene 1 & 2“ mit zwölf interessanten Vorträgen und weiteren Posterpräsentationen.

Die erfolgreiche Zusammenarbeit der beiden Fachgruppen soll zukünftig weiter intensiviert werden. Vorgesehen ist die Gründung einer gemeinsamen Fachgruppe mit einem gemeinsamen Vorstand. Über die geplante Fusion soll auf der nächsten Mitgliederversammlung der Fachgruppe im Rahmen des 15. Fachsymposiums Lebensmittelmikrobiologie (15.–17. April 2015) mit den Mitgliedern diskutiert und abgestimmt werden. Alle Mitglieder sind daher herzlich eingeladen, diesen Vorgang aktiv mitzugestalten und sich einzubringen. Kommentare und Ideen können gerne auch im Vorfeld an die Sprecher der Fachgruppe gerichtet werden.

Nachdem 2014 kein gemeinsames Fachsymposium außerhalb der Jahrestagung stattgefunden hat, nehmen wir diese Tradition 2015 wieder auf und laden Mitglieder und Nichtmitglieder herzlich zur Teilnahme am Fachsymposium vom 15. bis 17. April 2015 im Bildungszentrum Kardinal-Döpfner-Haus in Freising ein. Weitere Informationen zur Veranstaltung finden Sie unter www.akademie.ziel.tum.de/index.php?id=351.

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Fachgruppe (VAAM-DGHM) Mikrobielle Pathogenität

■ Auf der 4. gemeinsamen Jahrestagung der DGHM und VAAM in Dresden stellten Themen zur mikrobiellen Infektion und Erreger-Wirt Interaktion einen der Tagungsschwerpunkte dar. Dieser Forschungsschwerpunkt wird gleichermaßen durch über 500 Naturwissenschaftler und Mediziner der gemeinsamen Fachgruppe Mikrobielle Pathogenität der DGHM und VAAM vertreten. Nach einem gemeinsamen Übergangsvorstand wurde jetzt der erste gemeinsame Vorstand berufen, der aus einem Naturwissenschaftler (Sprecher) und Medizinern besteht. Als Sprecher fungiert Sven Hammerschmidt (Greifswald), stellvertretender Sprecher ist Holger Rohde (Hamburg) und Schriftführerin ist Alexandra Schubert-Unkmeir (Würzburg).

Die Fachgruppe hat sich an der diesjährigen gemeinsamen DGHM- und VAAM-Jahrestagung „Microbiology and Infection“ in Dresden an sechs verschiedenen Workshops mit insgesamt 44 Vorträgen und zahlreichen Posterpräsentationen beteiligt. Zudem richtete sie einen weiteren gemeinsamen Workshop mit der Fachgruppe Regulation und Signaltransduktion sowie mit der Fachgruppe Eukaryotische Krankheitserreger aus. Von besonderem Interesse waren dabei unter anderem Mechanismen der Wirtszellmodulation durch Adhäsine und Effektoren, die Anheftung der Staphylokokken mittels der WTA, die Rolle eines trimeren Autotransporter von *Acinetobacter baumannii*, wie das c-di-AMP-Signalling in Staphylokokken funktioniert, oder auch wie der unterschiedliche Metabolismus von *Campylobacter* species die gewebespezifische Kolonisierung beeinflusst.

Für das Jahr 2015 sind neben der Ausrichtung eines Symposiums auf der VAAM- sowie einiger Workshops auf der DGHM-Jahrestagung (27.–30.09.2015 in Münster), der 13. Deutsche Chlamydien Workshop in Wien (Februar 2015), das Internationale Symposium über Bartonellen (Frankfurt, 2015), ein zweites Treffen der Pneumokokken/Streptokokken-Forscher (2. German Pneumococcal and Streptococcal Symposium) und ein Meeting am HZI in Braunschweig zum Thema „Sensory and regulatory RNAs“ mit dem Schwerpunkt „Rolle von regulatorischen RNAs bei der Infektion“ geplant. ■

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Fachgruppe Mikrobielle Zellbiologie

■ Die Fachgruppe veranstaltete im März 2014 zum ersten Mal eine Fachtagung außerhalb der regelmäßig durchgeführten Minisymposien auf den Jahrestagungen der VAAM. Die Diskussionstagung war mit rund 40 Teilnehmer/inne/n bewusst im Kreis der FG-Mitglieder gehalten. Das Ambiente des Tagungsschlosses Rauischholzhausen bei Marburg, die abendlichen Runden im Schlosskeller, aber vor allem die wissenschaftlichen Vorträge und intensiven Gespräche zu verschiedenen Aspekten der mikrobiellen Zellbiologie haben uns bewogen, diese Treffen alle zwei Jahre im Wechsel mit den Minisymposien zu organisieren. Der nächste Termin wird also 2016 sein. Eine kurze Zusammenfassung der Tagung und der Eindrücke können Sie im BIOspektrum 4/2014 auf Seite 438 lesen.

Unser diesjähriges Symposium auf der Jahrestagung in Marburg widmet sich einem speziellen Aspekt der mikrobiellen Zellbiologie: der Bildung von Membrandomänen (Membrane Domains in Microbes). Lipid rafts sind eine bekannte Struktur der funktionellen Kompartimentierung eukaryotischer Membranen, die wesentlich von der Verteilung des eingelagerten Cholesterins und spezieller Lipide gebildet wird und zur Segregation von Membranproteinen führt. In Bakterien beobachtet man einen Einfluss durch Hopanoide, dem bakteriellen Äquivalent zu Cholesterin, und durch Proteine des Cytoskeletts und der Flotilline, die Membrandomänen schaffen. Wir werden zu den verschiedenen Mechanismen der Mikrostrukturbildung jeweils Vorträge von eingeladenen Rednern hören. Die Frage nach divergenten und konvergenten Lösungen der funktionellen Domänenbildung in Lipidmembranen unterschiedlicher Organismen wird sicherlich die Diskussionen beschäftigen.

Unsere Fachgruppe hat in den letzten beiden Jahren einen erfreulichen Zuspruch erfahren und ist weiter gewachsen. Wir wollen die zukünftige Entwicklung unserer Aktivitäten auf einer FG-Versammlung in Marburg diskutieren und auch die nächste Fachtagung Microbial Cell Biology 2016 vorbereiten. Dazu laden wir alle Fachgruppenmitglieder herzlich ein! Das Mitgliedertreffen findet unmittelbar im Anschluss an unser Symposium am 2. März 2015 im gleichen Raum statt.
Wir freuen uns auf Ihr Kommen! ■

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Fachgruppe Qualitätssicherung und Diagnostik

In Marburg wird die Fachgruppe in einem Symposium die Themen „Control, detection and prevention of microorganisms during use in clinical and work environment“ mit fünf Referenten präsentieren. Das Spektrum der Themen reicht von der Erfassung von Bioaerosolen in belasteten Arbeitsumgebungen über Detektion in Biofilmen mittels eines neuen Biosensors bis hin zur Desinfektion und der „schnellen Mikrobiologie“ im Bereich neuartiger, zuzulassender Biopharmazeutika. Daher erwarten wir hier viel Anlass für Diskussionen und Austausch untereinander.

Der Hauptfokus der Fachgruppe liegt auf Themen der mikrobiologischen Qualitätskontrolle und dazugehörigen Themen der Diagnostik. Die Mitglieder und Interessenten der anwendungsorientierten VAAM-Fachgruppe beschäftigen sich mit aktuellen Fragen und neuen Entwicklungen aus diesem Themenbereich. Zur mikrobiologischen Qualitätssicherung gehören beispielsweise Prüfungen von Rohstoffen und Medien für die Herstellung von pharmazeutischen Wirkstoffen, die Überwachung von Reinräumen oder Fertigarzneimittel. Die Analyseergebnisse der mikrobiologischen Qualitätskontrollen sowie die Identifizierung der detektierten Keime sind Grundvoraussetzung für adäquate korrigierende und vorbeugende Maßnahmen in regulierten Betrieben. Sie bieten manchmal sogar die einzige Möglichkeit zum Nachweis von mikrobiell verursachten Problemen am Arbeitsplatz oder in Versorgungsbetrieben. Erfahrungen dazu werden innerhalb der Fachgruppe diskutiert. Durch den Austausch untereinander können individuelle Lösungen erarbeitet oder neue Methoden etabliert werden. Zudem werden neue behördliche Anforderungen sowie neuartige Methoden zur mikrobiologischen Qualitätssicherung diskutiert. Hierzu arbeiten wir auch mittels gemeinsamer Symposien mit der Ständigen AG Diagnostische Verfahren (StAG DV) der DGHM zusammen. So konnten wir auf der gemeinsamen Tagung in Dresden zwei von beiden Gruppen organisierte und sehr gut besuchte Symposien abhalten.

Die Mitglieder der Fachgruppe kommen aus vielen unterschiedlichen Bereichen der Qualitätssicherung & Diagnostik wie Pharmabetrieben und Behörden, aber auch aus den Hochschulen. Zudem sprechen wir anwendungsorientiert arbeitende Mikrobiologen aller Hersteller von Diagnostik-Methoden und -geräten (Systemanbieter) an und stärken die Interaktion zwischen Entwicklung und Anwendung.

Wir weisen schon jetzt auf unser Fachgruppen-Treffen am 11. September 2015 in Düsseldorf hin, wo wir uns mit Unterstützung der Firma LGC im „Mercure-Hotel Düsseldorf City Center“ treffen werden. Am Vorabend besteht Gelegenheit, sich in lockerem Kreis zusammenzufinden. Gerne nehmen wir Ihre Vorschläge für Vorträge bereits jetzt entgegen und freuen uns über viele interessierte Teilnehmer.

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Fachgruppe (VAAM-Dechema) Biotransformation

Die Fachgruppe Biotransformation der VAAM wurde 1996 gegründet und 2009 erweitert durch Zusammengehen mit dem DECHEMA-Arbeitsausschuss „Biologische Grundlagen der Stoffproduktion“. Da sich die Arbeit beider Gremien in vielen Bereichen stark überschnitt, war ihre Zusammenlegung im Sinne einer deutlichen Erhöhung der Effektivität und gleichzeitig auch einer deutlich gesteigerten Mitgliederzahl bei gemeinsamen Veranstaltungen und Aktionen sehr sinnvoll. Im Moment hat die Fachgruppe dadurch etwa 350 registrierte Mitglieder.

Nach Änderung der DECHEMA-Satzung 2013 erfolgte in Absprache mit der VAAM auf elektronischem Wege mit guter Beteiligung in diesem Jahr die Wahl eines Lenkungskreises von 25 Personen, für den sich alle Mitglieder bewerben konnten. Dieser Lenkungskreis ist nahezu anteilig zusammengesetzt aus Vertretern aus Hochschulen und Industrie. Als neue Sprecher wurden im Oktober für die VAAM und als Vertreter der Industrie Jürgen Eck (BRAIN AG, Zwingenberg) und für die DECHEMA und als Vertreter der Hochschulen Andreas Liese (TU HH-Harburg) gewählt.

Neben regelmäßigen eigenständigen meist ein- bis zweitägigen Veranstaltungen zu ausgewählten Themen, die auch mit ausländischen Fachgruppen und/oder anderen VAAM- oder DECHEMA-Fachgruppen durchgeführt werden, 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 (siehe Bericht zur Sommerschule 2014 im BIOspektrum 7/2014).

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Fachgruppe Regulation und Signaltransduktion in Prokaryoten

Das Überleben in der mikrobiellen Welt verlangt von jeder Zelle ein kontinuierliches Überwachen zahlreicher extra- wie auch intrazellulärer Parameter, um rechtzeitig und adäquat auf Veränderungen reagieren und die zelluläre Physiologie entsprechend anpassen zu können. Zur Umsetzung solcher Prozesse benötigen Bakterien sensitive Sensoren und spezifische Regulatoren, die einen äußeren Reiz in eine zelluläre Antwort übersetzen. Zur Reizerkennung und -weiterleitung bedienen sich Prokaryoten hierbei Ein- und Zweikomponentensysteme sowie alternativer Sigmafaktoren oder kleiner regulatorischer RNA-Moleküle. Daneben werden Signalstoffe aber auch aktiv von Bakterien produziert, um zum Beispiel multizelluläre Differenzierungsprogramme sowohl extrazellulär über zelldichteabhängige Prozesse (*Quorum sensing*) als auch intrazellulär mittels sekundärer Botenstoffe wie dem zyklischen di-GMP zu koordinieren.

Mit all diesen Facetten beschäftigt sich die Fachgruppe Regulation und Signaltransduktion in Prokaryoten. Sie bietet allen an solchen Prozessen interessierten Wissenschaftler/inne/n innerhalb der VAAM ein entsprechendes Forum zum Erfahrungsaustausch und zum Vorantreiben von Kooperationsprojekten. Zu diesem Zweck organisiert die Fachgruppe jährliche Minisymposien während der VAAM-Tagung, die sich in den letzten Jahren mit verschiedenen Aspekten der Regulation und Signaltransduktion auseinandersetzten. Das Thema des letzten Minisymposiums, das unsere Fachgruppe gemeinsam mit der FG Mikrobielle Pathogenität während der VAAM/DGHM-Jahrestagung in Dresden organisiert hatte, widmete sich neuartigen sekundären Botenstoffen und deren Rolle für die bakterielle Signaltransduktion und Pathogenität. Zu diesem Thema konnte als Plenarsprecherin Angelika Gründling (London) gewonnen werden, die einen sehr schönen Überblick über die Funktion von c-di-AMP bei der Signaltransduktion von *Staphylococcus aureus* lieferte. Weitere Sprecher adressierten die strukturellen Aspekte der c-di-AMP-abhängigen Signaltransduktion (Gregor Witte, München), die ppGpp-abhängige stringente Antwort in *S. aureus* (Benjamin Kästle, Tübingen) sowie die c-di-GMP-abhängige Regulation der Alginatproduktion in *Pseudomonas aeruginosa* (Annika Schmidt, Tübingen).

In diesem Jahr widmet sich das Mini-Symposium der Rolle von alternativen (*ExtraCytoplasmic Function*, ECF-) Sigmafaktoren, die nach den Ein- und Zweikomponentensystemen den drittwichtigsten Mechanismus bakterieller Signaltransduktion repräsentieren. Hierfür konnten mit John D. Helmann (Ithaca, USA), Anke Becker (Marburg) und Jacques Cove (Grenoble, Frankreich) drei hochkarätige Sprecher aus dem In- und Ausland gewonnen werden.

Eine weitere wichtige Aktivität der Fachgruppe ist die alle zwei Jahre stattfindende VAAM-Sommerschule, die sich aus dem traditionsreichen Plasmid-Meeting heraus entwickelt hat. Dieses Symposium hat sich in den letzten Jahren immer stärker zu einem Forum vor allem für Nachwuchswissenschaftler/innen entwickelt, die in einer anregenden und informellen Atmosphäre über ihre Forschung berichten und sich mit anderen vernetzen können. Im Oktober vergangenen Jahres fand das Symposium Mechanisms of Gene Regulation zum 30. Mal statt und wurde von Julia Frunzke und Kolleg/inne/n in Düsseldorf ausgerichtet (siehe hierzu separaten Bericht im BIOspektrum 1/2015).

Auch in diesem Jahr findet während der VAAM-Tagung in Marburg im direkten Anschluss an das Mini-Symposium eine Mitgliederversammlung der Fachgruppe statt, zu der wir Sie auf diesem Wege recht herzlich einladen möchten. Neben aktuellen Berichten, z. B. über neue thematisch relevante Förderinitiativen zur bakteriellen Regulation und Signaltransduktion, steht in diesem Jahr auch wieder die Wahl der Sprecher/in und Stellvertreter/in auf dem Programm.

Sprecher: Thorsten Mascher, Ludwig-Maximilians-Universität München
Email: mascher@lmu.de

Stellvertretende Sprecherin: Julia Frunzke, Forschungszentrum Jülich
Email: j.frunzke@fz-juelich.de

Fachgruppe Hefe

Während der letzten VAAM-Jahrestagung in Dresden fand am 6. Oktober 2014 ein Symposium der Fachgruppe Hefe statt. Acht Vortragsthemen umfassten die Bereiche Transport niedermolekularer Substanzen, Killerfaktoren, Ribosomen-Biogenese, Hefe-Biotechnologie und Säuretoleranz. Die Veranstaltung fand regen Zuspruch und die Vorträge wurden intensiv diskutiert. Auf dem anschließenden Treffen der Fachgruppe Hefe wurde Karl-Dieter Entian für weitere zwei Jahre als Sprecher der Fachgruppe Hefe gewählt.

Die Fachgruppe freut sich über weitere aktive Mitglieder, inhaltliche und organisatorische Unterstützung und sucht derzeit eine/n stellvertretende/n Fachgruppen-Sprecher!

Sprecher: Karl-Dieter Entian, Universität Frankfurt
Email: entian@bio.uni-frankfurt.de

Fachgruppe Symbiotische Interaktionen

Die Fachgruppe Symbiotische Interaktionen hat sich zum Ziel gesetzt, den regelmäßigen Kontakt und Austausch zwischen den Arbeitsgruppen 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.

Die Fachgruppe richtete auf der vergangenen Jahrestagung 2014 in Dresden ein Minisymposium mit dem Titel „Plant-Microbe Interactions" aus, das Sylvia Schnell und Massimiliano Cardinale (Universität Gießen) organisierten. Den Einführungsvortrag hielt die Gastsprecherin Gabriele Berg (Universität Graz, Österreich) zum Thema „Deciphering the plant microbial network". Weitere Vorträge beschäftigten sich mit der Rolle von Wurzelendophyten in *Medicago*, mit den Auswirkungen bakterieller Inokulation auf pflanzliche Fitness unter Salzstress, der Charakterisierung von Enzymen des pflanzenpathogenen Bakteriums *Pseudomonas syringae* sowie der Transkriptionsantwort der Modellpflanzen *Arabidopsis* und *Solanum* auf Pflanzenwachstum-stimulierende Endophyten, die „plant growth promoting bacteria" (PGPB). Die Zuhörerschaft in diesem Symposium war erfreulich groß, was das Interesse an der Thematik von Pflanzen-Bakterien-Interaktionen widerspiegelte.

Auf der kommenden Jahrestagung 2015 in Marburg wird unsere Fachgruppe erstmalig ein Minisymposium zum Thema „Microbe–Insect Symbioses" unter der Leitung von Andreas Brune (MPI Marburg) veranstalten. Erfreulicherweise konnten wir mit den internationalen Gastrednern Angela E. Douglas (Cornell University, USA) und Yuichi Hongoh (Tokyo Institute of Technology, Japan) zwei ausgewiesene Spezialisten auf dem Gebiet der Insekten-Mikrobiologie gewinnen, die über Funktion und metabolische Koevolution der bakteriellen Symbionten im Intestinaltrakt und in speziellen Organen der Wirtsorganismen sowie in den Zellulose-abbauenden Flagellaten des Termitendarms referieren werden. Zusammen mit dem im Rahmen einer Plenarsitzung vorgesehenen Vortrag der bekannten Evolutionsbiologin Nancy A. Moran (University of Texas) über die bakteriellen Gemeinschaften im Darm von Honigbienen sowie mehreren Kurzvorträgen deutscher und niederländischer Arbeitsgruppen verspricht die Veranstaltung einen Überblick über die vielfältigen Interaktionen von Mikroorganismen und Insekten. Die Beiträge reichen von der Rolle der Darmmikrobiota bei der Überwindung der chemischen Abwehr der Wirtspflanzen und der Ergänzung einer vitaminarmen Kost bis zum bakteriellen Schutz von Insekteneiern vor Pilzbefall und einer überraschenden Selektion bestimmter Bakterienlinien bei der experimentellen Besiedlung von keimfreien Schaben mit artfremden mikrobiellen Gemeinschaften.

Internet: www.helmholtz-muenchen.de/en/symbiotic-interactions/homeaims/index.html

Sprecherin: Ute Hentschel Humeida,
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Fachgruppe Umweltmikrobiologie

Die Fachgruppe Umweltmikrobiologie führte auf der VAAM-Tagung in Dresden wieder ein interessantes und gut besuchtes Minisymposium durch. Das Thema – die Umsetzung von Spurengasen in Böden – hatten Markus Horn und Steffen Kolb aus Bayreuth organisiert. Auf der anschließenden Fachgruppensitzung wurden die bisherigen Sprecher Rainer Meckenstock (bisher Helmholtz-Zentrum München und seit Oktober 2014 Universität Duisburg-Essen) und Karl-Heinz Engesser als Stellvertreter (Uni Stuttgart) bestätigt. Das Minisymposium war ein gutes Beispiel, wie die Fachgruppe Nachwuchswissenschaftler/inne/n ein Forum bieten kann, um ihre Themen im größeren Rahmen zu diskutieren. Dieser Rahmen steht allen offen und besonders junge Wissenschaftler/innen sind eingeladen, sich mit dem Sprecher in Verbindung zu setzen, um ein Mini-Symposium zu organisieren.

Dieses Jahr wird auf der VAAM-Tagung in Marburg ein Minisymposium zum Thema „Single cell technologies in environmental microbiology" durchgeführt. Wir haben eine Reihe hochkarätiger Redner gewinnen können, die Single-Cell-Anwendungen aus verschiedenen Blickwinkeln diskutieren werden. Nach den exzellenten Entwicklungen der molekularen Methoden in der Umweltmikrobiologie in den letzten Jahren, die uns immer bessere Einblicke in die Zusammensetzung mikrobieller Gemeinschaften ermöglicht haben, werden die technologischen Fortschritte in der Umweltmikrobiologie zukünftig immer mehr erlauben, die Funktionen, Aktivitäten und die Physiologie von Mikroorganismen in der Umwelt zu untersuchen.

Alle Fachgruppen der VAAM müssen regelmäßig ihre Fortführung beantragen, und die Umweltmikrobiologie ist 2015 an der Reihe. Im Rahmen des Minisymposiums in Marburg wird deshalb auch die Fachgruppensitzung abgehalten, auf der die Fortführung beantragt werden soll.

Sprecher:
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Stellvertretender Sprecher:
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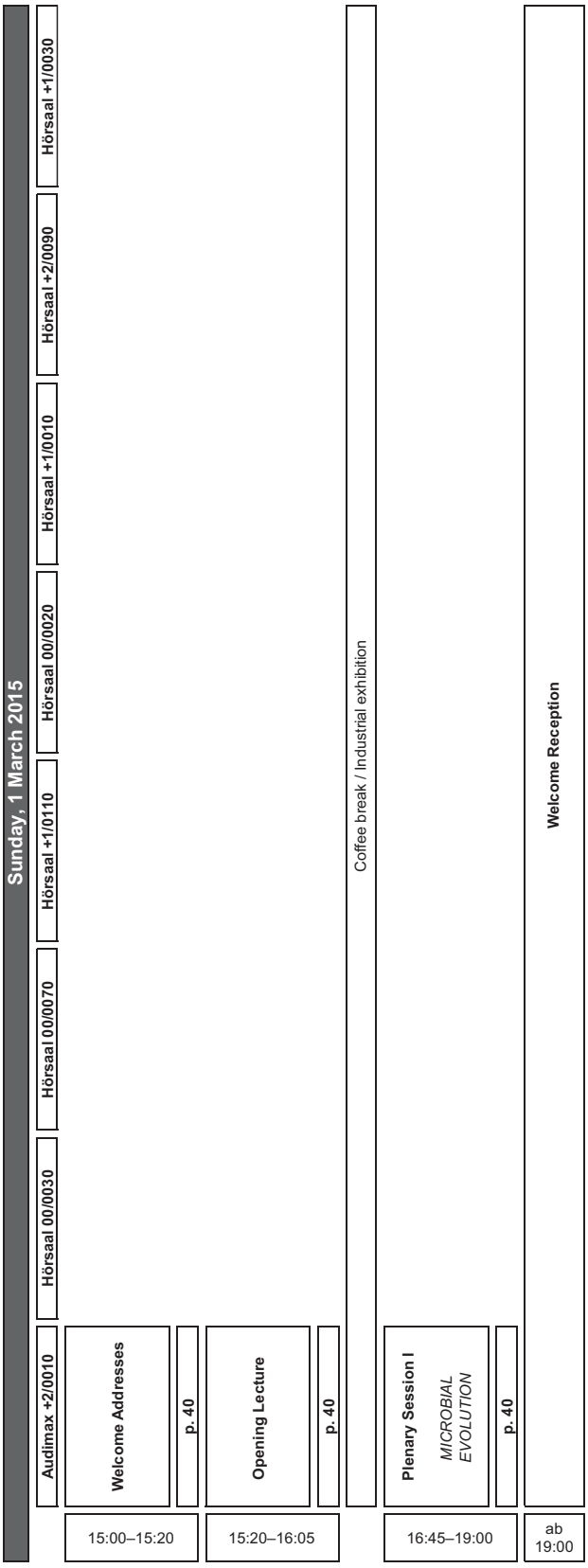
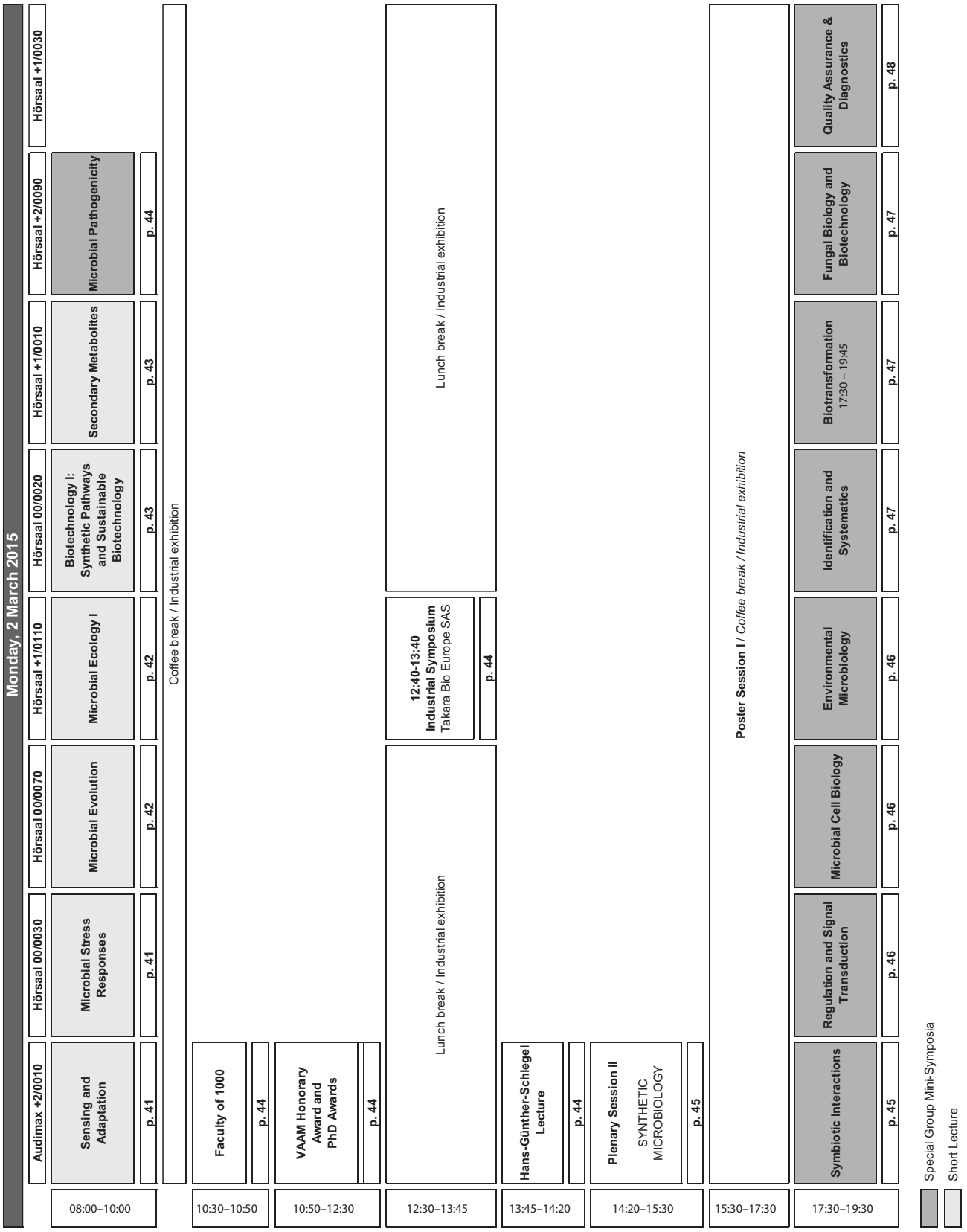
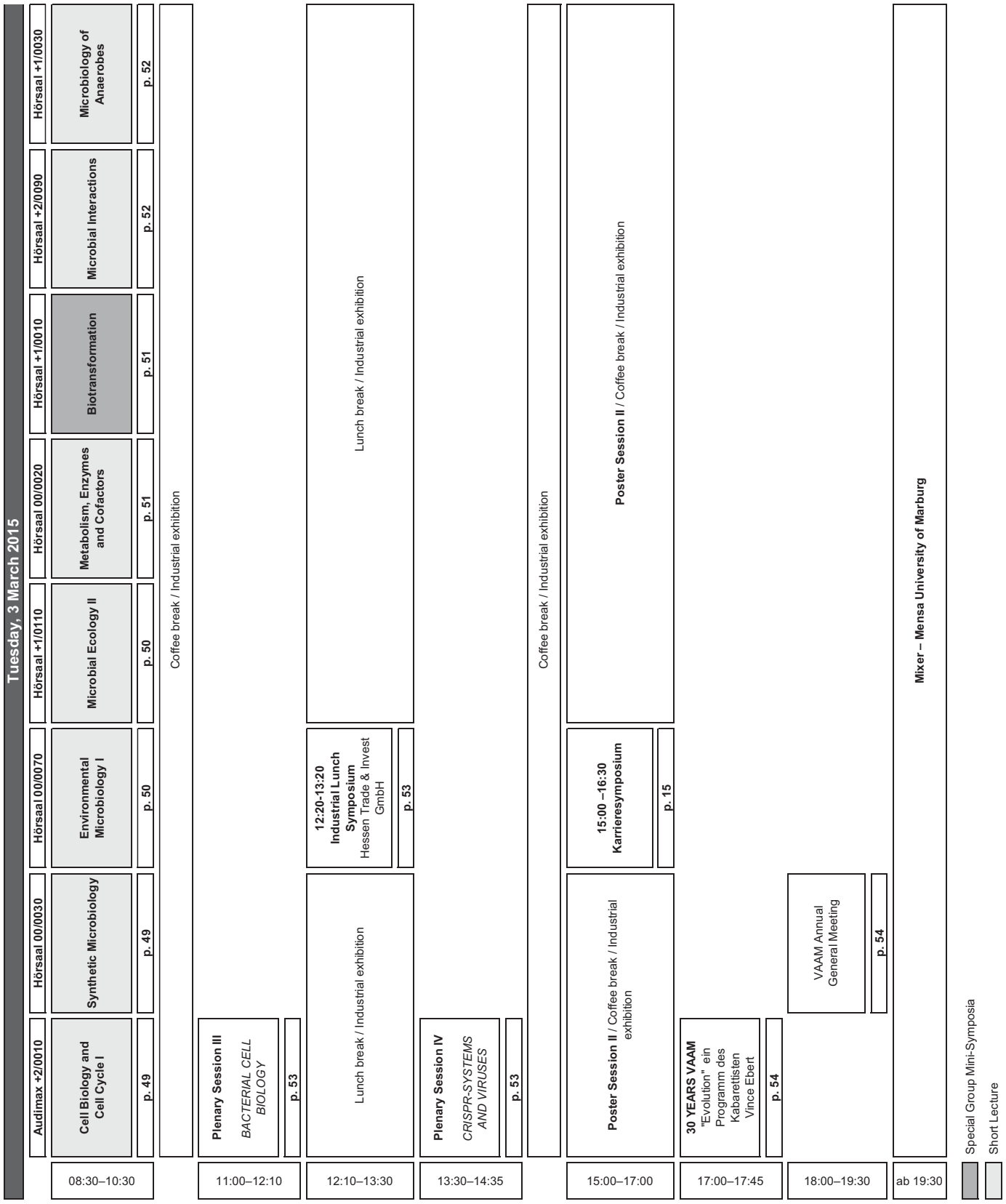
Programme Overview · Sunday · March 01, 2015



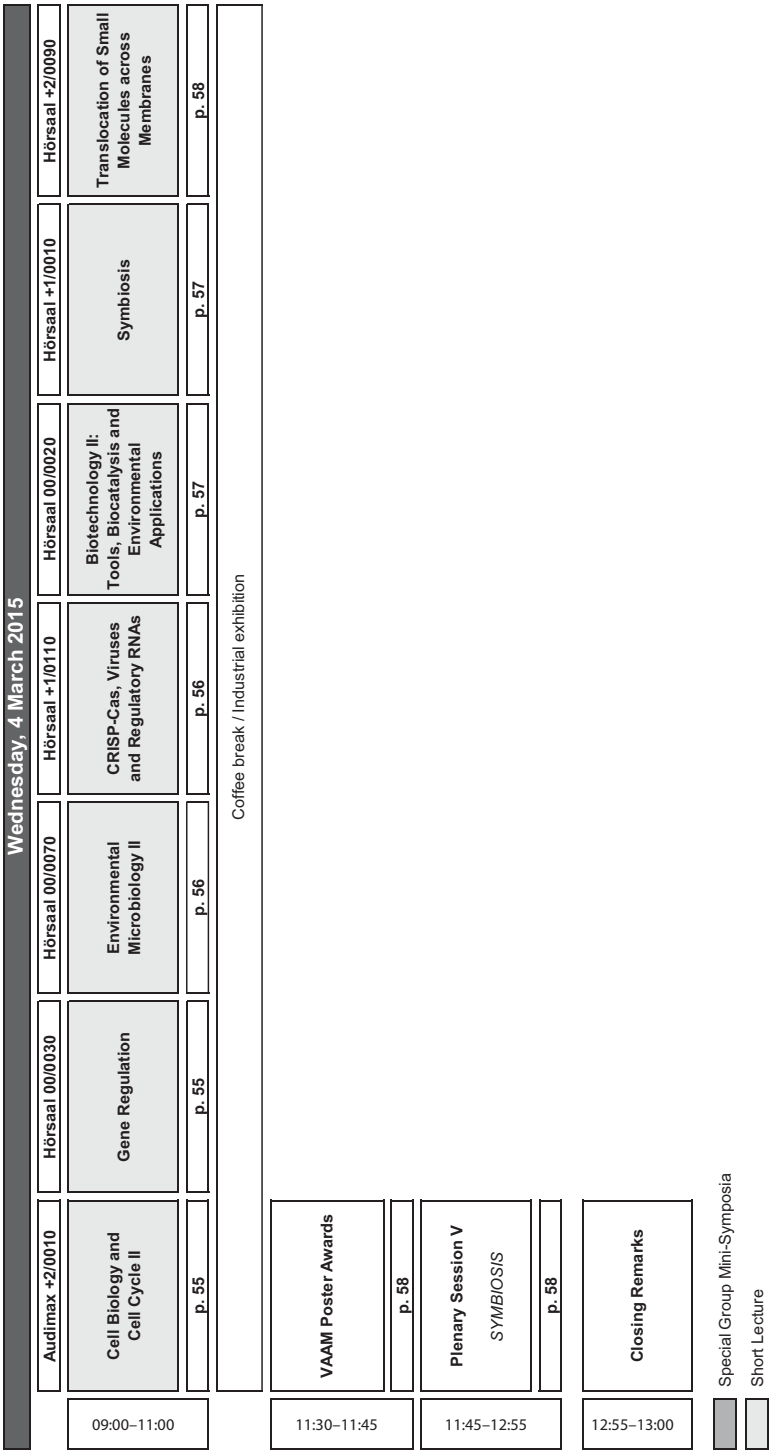
Programme Overview · Monday · March 02, 2015



Programme Overview · Tuesday · March 03, 2015



Programme Overview · Wednesday · March 04, 2015



CONFERENCE PROGRAMME

Annual Conference 2015 of the VAAM

Sunday, 1 March 2015

Room	Hörsaal +2/0010
15:00–15:20	**Welcome Addresses** D. Jahn (President of the VAAM) E. Bremer (Conference Chair)
15:20–16:05 ISV01	**Opening Lecture** Ancient pathogen genomics – what we learn from historical epidemics J. Krause (Jena, Tübingen/DE)
16:05–16:45	Coffee break/Industrial exhibition
16:45–19:00	**Plenary Session I – Microbial Evolution**
16:45 ISV02	Phenotypic and genomic evolution during a 60.000-generation experiment with *Escherichia coli* R. E. Lenski (Michigan/US)
17:20 ISV03	Genome dynamic and accessory elements in the plant pathogenic fungus *Zymoseptoria tritici* E. H. Stukenbrock (Kiel, Plön/DE)
17:55 ISV04	Disentangling the origins of eukaryotic cells M. Embley (Newcastle/UK)
18:30 ISV05	Origins of major archaeal clades correspond to gene acquisitions from bacteria W. F. Martin (Düsseldorf/DE)
19:00–21:00 Room	**Welcome Reception** Industrial Exhibition

Monday, 2 March 2015

08:00–10:00	**Short lectures** and **Mini-Symposia Special Group** (see page 41–44)
10:00–10:30	Coffee break/Industrial exhibition
10:30–10:50 Room	**Faculty of 1000** Hörsaal +2/0010
ISV06	F1000 – a new way of writing, discovering and sharing science M. Torkar (London/UK)
10:50–12:30	**VAAM Honorary Award and PhD Awards**
Room	Hörsaal +2/0010
10:50	**VAAM Honorary Award**
ISV16	Killing for DNA – the type VI secretion system of *Vibrio cholerae*, fosters horizontal gene transfer M. Blokesch (Lausanne/CH)
11:30	**PhD Awards**
12:40–13:40	**Industrial Symposia Takara Bio Europe SAS** (see page 44)/Coffee break/Industrial exhibition
13:45–14:20 Room	**Hans-Günter-Schlegel-Lecture** Hörsaal +2/0010
ISV07	Adaptation of hydrogen bacteria to an aerobic life style B. Friedrich (Berlin/DE)
14:20–15:30 Room	**Plenary Session II – Synthetic Microbiology** Hörsaal +2/0010
14:20 ISV08	Synthetic biology for the production of high-value chemicals E. Takano (Manchester/UK)
14:55 ISV09	Cyborgization of soil bacteria for smart degradation of environmental pollutants V. de Lorenzo (Madrid/ES)
15:30–17:30 Room	**Poster Session I** (see page 45)**/Industrial exhibition** Foyer
17:30–19:30	**Mini-Symposia Special Groups** (see page 45–48)

CONFERENCE PROGRAMME

Annual Conference 2015 of the VAAM

Tuesday, 3 March 2015

08:30–10:30	**Short lectures** and **Mini-Symposia Special Group** (see page 49–52)
10:30–11:00	Coffee break/Industrial exhibition
11:00–12:10	**Plenary Session III – Bacterial cell biology**
Room	Hörsaal +2/0010
11:00	Cell biology of a bacterial predator
ISV10	L. Sockett (Nottingham/UK)
11:35	How electron cryotomography is opening a new window into bacterial cell biology
ISV11	G. J. Jensen (Pasadena/US)
12:20–13:20	**Lunch Symposia Hessen Trade & Invest GmbH** (see page 53)/Coffee break/Industrial exhibition
	Auf dem Weg in die Bioökonomie – ohne Mikrobiologie geht es nicht!
Room	Hörsaal 00/0070
13:30–14:35	**Plenary Session IV – CRISPR-systems and viruses**
Room	Hörsaal +2/0010
13:30	CRISPR-Cas9 bacterial adaptive immunity – biology, mechanisms and evolution
ISV12	E. Charpentier (Umeå/SE, Braunschweig, Hanover/DE)
14:05	Revenge of the phages – defeating bacterial defences
ISV13	S. Moineau (Québec/CA)
15:00–17:00	**Postersession II** (see page 53)**/Industrial exhibition**
Room	Foyer
15:00–16:30	**Karrieresymposium**
Room	Hörsaal 00/0070
17:00–17:45	**30 YEARS VAAM** (see page 54)
Room	Hörsaal +2/0010
	… ein ungewöhnlicher Blick in die Mikrobenwelt …
	Wissenschaftskabarettist Vince Ebert
18:00–19:30	**VAAM Annual General Meeting**
Room	Hörsaal 00/0030
19:30–00:00	**Mixer**

Wednesday, 4 March 2015

09:00–11:00	**Short Lectures** (see page 55–58)
11:00–11:30	Coffee break/Industrial exhibition
11:30–11:45	**VAAM Poster Awards**
Room	Hörsaal +2/0010
11:45–12:55	**Plenary Session VI – Symbiosis**
Room	Hörsaal +2/0010
11:45	Are lipo-chitooligosaccharides solely symbiotic signals in plant-microbe interactions?
ISV14	G. Stacey (Columbia/US)
12:20	The dynamics of bacterial communities in guts of social bees
ISV15	N. A. Moran (Austin/US)
12:55–13:00	**Closing Remarks**
Room	Hörsaal +2/0010

SCIENTIFIC PROGRAMME, SUNDAY, 1 MARCH 2015

Annual Conference 2015 of the VAAM

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

Room Industrial Exhibition

15:00–15:20 **Welcome Addresses**

Room Hörsaal +2/0010

15:00 D. Jahn (President of the VAAM)

15:10 E. Bremer (Conference Chair)

15:20–16:05 **Opening Lecture**

Room Hörsaal +2/0010
Chair M. Bölker (Marburg/DE)

ISV01 Ancient pathogen genomics – what we learn from historical epidemics
J. Krause (Jena, Tübingen/DE)

16:05–16:45 Coffee break/Industrial exhibition

16:45–19:00 **Plenary Session I – Microbial Evolution**

Room Hörsaal +2/0010
Chairs R. Lill, H.-U. Mösch (Marburg/DE)

16:45
ISV02 Phenotypic and genomic evolution during a 60.000-generation experiment with *Escherichia coli*
R. E. Lenski (Michigan/US)

17:20
ISV03 Genome dynamic and accessory elements in the plant pathogenic fungus *Zymoseptoria tritici*
E. H. Stukenbrock (Kiel, Plön/DE)

17:55
ISV04 Disentangling the origins of eukaryotic cells
M. Embley (Newcastle/UK)

18:30
ISV05 Origins of major archeal clades correspond to gene acquisitions from bacteria
W. F. Martin (Düsseldorf/DE)

19:00–21:00 **Welcome Reception**

Room Industrial Exhibition

Annual Conference 2015 of the VAAM

08:00–10:00 Short Lecture – Sensing and Adaptation

Room	Hörsaal +2/0010
Chairs	V. Sourjik, A. Batschauer (Marburg/DE)
08:00 SAV01	IN and OUT, ON and OFF – an efficient concept of stimulus perception by a histidine kinase H. Schramke (Martinsried/Munich/DE)
08:15 SAV02	LuxR solos as central players in communication of *Photorhabdus* species S. Brameyer (Martinsried/Munich/DE)
08:30 SAV03	Need-based activation of antibiotic resistance by a flux-sensing mechanism G. Fritz (Marburg/DE)
08:45 SAV04	A phosphorylation-dependent network regulates motility of *Sulfolobus acidocaldarius* L. Hoffmann (Freiburg/DE)
09:00 SAV05	Comparative proteomic analysis reveals that the Tor pathway in *Aspergillus fumigates* is involved in iron regulation V. Valiante (Jena/DE)
09:15 SAV06	Structure and function of a phycobiliprotein lyase from a marine cyanophage J. Schwach (Bochum/DE)
09:30 SAV07	The bacteriochlorophyll biosynthesis of the marine bacterium *Dinoroseobacter shibae* and its lightdependent regulation S. Heyber (Braunschweig/DE)
09:45 SAV08	Distinct features of closely related *Natrialba* species suggest an adaptation towards different ecological habitats R. L. Hahnke (Braunschweig/DE)

08:00–10:00 Short Lecture – Microbial Stress Response

Room	Hörsaal 00/0030
Chairs	G. Bange, H.-U. Mösch (Marburg/DE)
08:00 MSV01	Long and short range heterogeneity of production of amyloid curli fibres and cellulose is essential for morphogenesis of *Escherichia coli* macrocolony biofilms D. O. Serra (Berlin/DE)
08:15 MSV02	Genes involved in the formation of multicellular aggregates in *Staphylococcus aureus* C. Wermser (Würzburg/DE)
08:30 MSV03	Regulation of thermotolerance development in *Bacillus subtilis* H. Schäfer (Hanover/DE)
08:45 MSV04	Structural basis for (p)ppGpp catalysis by an oligomeric alarmone synthetase and its allosteric regulation by pppGpp but not ppGpp W. Steinchen (Marburg/DE)
09:00 MSV05	Iron-, heme- and cobalamin-binding properties of the *Streptomyces* sensory protein HbpS involved in anti-oxidative stress response D. Ortiz de Orué Lucana (Osnabrück/DE)
09:15 MSV06	Fungoglobin – a novel member of the sensor globin family supports hypoxic growth of the pathogenic fungus *Aspergillus fumigatus* F. Hillmann (Jena/DE)
09:30 MSV07	The garlic sulfur compound Allicin causes protein sulfoallylation and disulfide stress in *Bacillus subtilis* and *Staphylococcus aureus* H. Antelmann (Greifswald/DE)
09:45 MSV08	Bacterial stress response to daptomycin M. Wenzel (Amsterdam/NL)

SCIENTIFIC PROGRAMME, MONDAY, 2 MARCH 2015

Annual Conference 2015 of the VAAM

08:00–10:00 Short Lecture – Microbial Evolution

Room: Hörsaal 00/0070
Chairs: T. Waldminghaus (Marburg/DE), S. Häußler (Braunschweig/DE)

08.00 MEvV01 — *Paracoccus denitrificans* and nitrite: 500 generations – problem solved
S. Müller (Bremen, Braunschweig/DE)

08:15 MEvV02 — Laboratory evolution of a fungal pathogen in macrophages restores virulence of a non-pathogenic mutant
S. Brunke (Jena/DE)

08:30 MEvV03 — Sociomicrobiology of *Bacillus subtilis* pellicle biofilms
A. T. Kovacs (Jena/DE)

08:45 MEvV04 — On the evolution of wrinkly spreaders, wrinkleality and fitness
A. Spiers (Dundee/UK)

09:00 MEvV05 — Evolutionary dynamics of *Salmonella* typhimurium cooperative virulence
M. R. J. Diard (Zürich/CH)

09:15 MEvV06 — Microevolution of *Pseudomonas aeruginosa* in cystic fibrosis lungs
J. Klockgether (Hanover/DE)

09:30 MEvV07 — The mtDNA sequence of the *Muco*r-related fusion parasite *Parasitella parasitica* – evidence for an unusually high number of intron-situated homing endonuclease genes
J. Wöstemeyer (Jena/DE)

09:45 MEvV08 — Proteome and carbon flux analysis of *P. aeruginosa* clinical isolates from different infection sites
C. Lassek (Greifswald/DE)

08:00–10:00 Short Lecture – Microbial Ecology I

Room: Hörsaal +1/0110
Chairs: S. Kolb (Jena/DE), J. A. Müller (Leipzig/DE)

08:00 MEcV01 — *Archaea* dominate the ammonia oxidizing communities in Savanna soils along a granitic and basaltic toposequence in Kruger National Park (South Africa)
M. Herrmann (Jena, Leipzig/DE)

08:15 MEcV02 — Effect of eCO_2 on microbial communities involved in N cycling in soils
K. Brenzinger (Marburg/Giessen/DE)

08:30 MEcV03 — Physiologic and genomic characterization of a novel *Nitrospira* species enriched under anaerobic, nitrate reducing conditions
S. Lücker (Nijmegen/NL)

08:45 MEcV04 — Plant polymers modulate the active microbial community in paddy soil
C.-E. Wegner (Marburg/DE)

09:00 MEcV05 — Chitin degradation by a complex microbial community in soil
A. S. Wieczorek (Bayreuth/DE)

09:15 MEcV06 — Comparative analysis of the root microbiome of *Verticillium longisporum* resistant and susceptible rapseed line
H. Haghighi (Giessen/DE)

09:30 MEcV07 — Functional redundancy of electroactive microbial biofilms fed by domestic wastewater
C. Koch (Leipzig/DE)

09:45 MEcV08 — Characterization of the active fraction of the chicken microbiota using metaproteomics
B. Tilocca (Stuttgart/DE)

Annual Conference 2015 of the VAAM

08:00–10:00 Short Lecture – Biotechnology I: Synthetic Pathways & Sustainable Biotechnology

Room	Hörsaal 00/0020
Chairs	T. J. Erb, J. Heider (Marburg/DE)
08:00 BTV01	Metabolic engineering of *Corynebacterium glutamicum* for production of L-leucine and 2-ketoisocaproate M. Vogt (Jülich/DE)
08:15 BTV02	Expanding the product scope of *Pseudomonas putida* J. Mi (Frankfurt am Main/DE)
08:30 BTV03	Synthetic pathways engineering in *Escherichia coli* strains for the production of serotonin and 5-hydroxytryptophan J. A. Mora-Villalobos (Hamburg/DE)
08:45 BTV04	Reprogramming nonribosomal peptide synthetases from *Xenorhabdus* and *Photorhabdus* K. A. J. Bozhüyük (Frankfurt am Main/DE)
09:00 BTV05	Biomass-degrading multifusion enzyme chimeras from thermophiles S. Elleuche (Hamburg/DE)
09:15 BTV06	Selective acetone production using acetogenic bacteria F. Bengelsdorf (Ulm/DE)
09:30 BTV07	From Sunlight and CO_2 to value-added products – establishing a stable co-culture between *Synechococcus elongates* and *Pseudomonas putida* H. Löwe (Garching/DE)
09:45 BTV08	LIPOMAR – Macroalgae as a promising feedstock for the production of high-value chemicals C. Schäfers (Hamburg/DE)

08:00–10:00 Short Lecture – Secondary Metabolites

Room	Hörsaal +1/0010
Chairs	M. Marahiel, S. Li (Marburg/DE)
08:00 SMeV01	The antibiotic roseoflavin from *Streptomyces davawensis* – mechanism of action and resistance M. Mack (Mannheim/DE)
08:15 SMeV02	Molecular probing as novel tool to dissect and control the stereochemistry of enoylthioester reductases: lessons from fatty acid biosynthesis for polyketide engineering B. Vögeli (Marburg/DE)
08:30 SMeV03	Recombinant production of the lipopeptide biosurfactant Serrawettin W1 S. Thies (Jülich/DE)
08:45 SMeV04	Insights into the structural diversity of bacterial lasso peptides J. Hegemann (Marburg/DE)
09:00 SMeV05	Glycolipid biosynthesis in *Ustilago maydis* B. Sandrock (Marburg/DE)
09:15 SMeV06	Comparison of the exometabolome composition of two members of the *Roseobacter* clade B. E. Noriega-Ortega (Oldenburg/DE)
09:30 SMeV07	Identification of activated secondary metabolite clusters on protein and metabolic level of *Aspergillus nidulans* after inducing low temperature stress B. Hanf (Jena/DE)
09:45 SMeV08	Unraveling the biosynthesis of secondary metabolite ‘Sodorifen’ of *S. plymuthica 4Rx13* D. Domik (Rostock/DE)

SCIENTIFIC PROGRAMME, MONDAY, 2 MARCH 2015

Annual Conference 2015 of the VAAM

08:00–10:00 **Mini-Symposia Special Group – Host-pathogen interactions of pathogenic bacteria FG Microbial Pathogenicity**

Room	Hörsaal +2/0090
Chairs	A. Schubert-Unkmeir (Würzburg/DE), S. Hammerschmidt (Greifswald/DE)
08:00 MPV-FG01	Unraveling the function and structure of a lipocalin lipoprotein affecting virulence in *Streptococcus pneumoniae* S. Hammerschmidt (Greifswald/DE)
08:20 MPV-FG02	On the role of autocleavage of the switch protein of bacterial type III secretion systems S. Wagner (Tübingen/DE)
08:40 MPV-FG03	A protein quality control mechanism might contribute to survival of world-wide distributed *Pseudomonas aeruginosa* clone C strains in environmental and clinical niches U. Römling (Stockholm/SE)
09:00 MPV-FG04	*Legionella pneumophila* outer membrane vesicles are potent pro-inflammatory stimulators A. L. Merkel (Marburg/DE)
09:20 MPV-FG05	The role of Fur in iron homeostasis in *Clostridium difficile* M. Berges (Braunschweig/DE)
09:40 MPV-FG06	A glycosphingolipid receptor activates Abl signaling for bacterial invasion S. Zheng (Freiburg/DE)

10:30–10:50 **Faculty of 1000**

Room	Hörsaal +2/0010
Chair	W. Buckel (Marburg/DE)
10:30 ISV06	F1000 – a new way of writing, discovering and sharing science M. Torkar (London/UK)

10:50–12:30 **VAAM Honorary Award and PhD Awards**

Room	Hörsaal +2/0010
10:50–11:30 Chairs	VAAM Honorary Award D. Jahn (Braunschweig/DE)
10:50 ISV16	Killing for DNA – the type VI secretion system of *Vibrio cholerae*, fosters horizontal gene transfer M. Blokesch (Lausanne/CH)
11:30–12:30 Chair	PhD Awards W. Buckel (Marburg/DE) Sponsored by BASF SE, Sanofi Aventis Deutschland GmbH, Bayer Schering Pharma und New England Biolabs GmbH.

12:40–13:40 **Industrial Symposia Takara Bio Europe SAS**

Room	Hörsaal +1/0110
Chair	S. Deppermann (Bochum/DE)
IMV01	Change the way you think about Cloning … Discover In-Fusion® M. Raman (Paris/FR)

13:45–14:20 **Hans-Günter-Schlegel-Lecture**

Room	Hörsaal +2/0010
Chair	R. Thauer (Marburg/DE)
13:45 ISV07	Adaptation of hydrogen bacteria to an aerobic life style B. Friedrich (Berlin/DE)

Annual Conference 2015 of the VAAM

14:20–15:30 Plenary Session II – Synthetic Microbiology

Room: Hörsaal +2/0010
Chairs: A. Becker, P. Graumann (Marburg/DE)

14:20 ISV08 — Synthetic biology for the production of high-value chemicals
E. Takano (Manchester/UK)

14:55 ISV09 — Cyborgization of soil bacteria for smart degradation of environmental pollutants
V. de Lorenzo (Madrid/ES)

15:30–17:30 Poster Session I

The posters should be switched by the authors in the time between Monday evening (19:30 h) and Tuesday morning (10:00 h).

Room: Foyer

Cell Biology and Cell Cycle (CCP01 – CCP49)
Environmental Microbiology (EMP01 – EMP53)
Free Topics (FTP01 – FTP06)
Microbial Ecology (MEcP01 – MEcP36)
Microbial Evolution (MEvP01 – MEvP19)
Microbial Interactions (MIP01 – MIP27)
Protein Folding and Degradation (PFP01)
Single Cell Analysis (SCP01 – SCP06)
Symbiosis (SYP01 – SYP24)
Toxin-/Anti-Toxins (TOP01)
Translocation of Large Molecules across Membranes and Protein Targeting (TLP01 – TLP25)
Translocation of Small Molecules across Membranes (TAP01 – TAP09)

17:30–19:30 Mini-Symposia Special Group – Insect-Microbe Symbioses FG Symbiotic Interactions

Room: Hörsaal +2/0010
Chairs: A. Brune (Marburg/DE), C. Welte (Nijmegen/NL)

17:30 SIV-FG01 — Metabolic coevolution between symbiotic bacteria and their insect hosts
A. E. Douglas (Ithaca/US)

18:00 SIV-FG02 — Diversity and functions of the ecto- and endosymbionts of cellulolytic protists in the gut of termites
Y. Hongoh (Tokyo/JP)

18:30 SIV-FG03 — The gut microbiome of phytopathogenic root fly larvae – insights into the detoxification of plant secondary metabolites by insect-associated microbes
C. Welte (Nijmegen/NL)

18:45 SIV-FG04 — A case of bacteria-mediated egg defense – the *Burkholderia*: Lagriid symbiosis
L. Florez (Jena/DE)

19:00 SIV-FG05 — Beggars can be choosers – host selection of gut microbiota in cockroaches
A. Mikaelyan (Marburg/DE)

19:15 SIV-FG06 — Vitamin supplementation by gut symbionts ensures metabolic homeostasis in an insect host
H. Salem (Jena/DE)

SCIENTIFIC PROGRAMME, MONDAY, 2 MARCH 2015

Annual Conference 2015 of the VAAM

17:30–19:30 **Mini-Symposia Special Group – Regulatory Diversity of Extracytoplasmic Function (ECF) Sigma Factors**
FG Regulation and Signal Transduction

Room	Hörsaal 00/0030
Chairs	J. Frunzke (Jülich/DE), T. Mascher (Martinsried/Munich/DE)
17:30 RSV-FG01	Extracytoplasmic function (ECF) σ factors – the third pillar of bacterial signal transduction T. Mascher (Martinsried/Munich/DE)
17:45 RSV-FG02	Physiological role and regulatory crosstalk of ECF sigma factors in *Bacillus subtilis* J. Helmann (Ithaca/US)
18:30 RSV-FG03	General stress response in alpha-proteobacteria A. Becker (Marburg/DE)
19:00 RSV-FG04	Metal sensing and transmembrane signal transduction by the CnrYXH complex from *Cupriavidus metallidurans* A. Maillard (Grenoble/FR)

17:30–19:30 **Mini-Symposia Special Group – Membrane Domains in Microbes**
FG Microbial Cell Biology

Room	Hörsaal 00/0070
Chair	H. Engelhardt (Martinsried/Munich/DE)
17:30 MCV-FG01	Actin homolog MreB has a conserved function in organizing bacterial cytoplasmic membranes H. Strahl (Newcastle upon Tyne/UK)
17:55 MCV-FG02	Spatial organization of the bacterial plasma membrane M. Bramkamp (Martinsried/Munich/DE)
18:20 MCV-FG03	Domain organization of membrane proteins in *Bacillus subtilis* P. Graumann (Marburg/DE)
18:45 MCV-FG04	The evolution of molecular order in the cell membrane J. Sáenz (Dresden/DE)

17:30–19:30 **Mini-Symposia Special Group – Applications of single cell techniques in environmental microbiology**
FG Environmental Microbiology

Room	Hörsaal +1/0110
Chairs	R. U. Meckenstock (Duisburg/DE), M. Kuypers (Bremen/DE)
17:30 EMV-FG01	Correlative imaging of microbial activity in Nature M. Kuypers (Bremen/DE)
18:00 EMV-FG02	Application of Raman activated cell sorting to single cell genomics W. Huang (Oxford/UK)
18:30 EMV-FG03	Tracking heavy water (D_2O) incorporation for identifying and sorting active microbial cells B. Berry (Vienna/AT)
18:45 EMV-FG04	Combined Epifluorescence and Kelvin Probe Force Microscopy (EFM-KPFM) for the study of bacterial attachment to ferrous surfaces C. Thyssen (Essen/DE)
19:00 EMV-FG05	A novel technology for single-cell manipulation and adhesion force quantification – fluidic force microscopy E. Potthoff (Zürich/CH)
19:15 EMV-FG06	Nitrite- and nitrate-dependent methanotrophs – environmental detection and relevance in freshwater Ecosystems K. Ettwig (Nijmegen/NL)

Annual Conference 2015 of the VAAM

17:30–19:30 **Mini-Symposia Special Group – The place of genomics in taxonomy – or vice versa?**
FG Identification and Systematics

Room Hörsaal 00/0020
Chairs H.-J. Busse (Vienna/AT), A. Lipski (Bonn/DE)

17:30 The impact of the genomic encyclopaedia of *Bacteria* and *Archaea* on microbial taxonomy
ISV-FG01 H.-P. Klenk (Newcastle upon Tyne/UK)

18:10 Bacterial species in the light of genomics and metagenomics
ISV-FG02 K. Konstantinidis (Atlanta/US)

18:50 Genome-based taxonomy of the genus *Corynebacterium*
ISV-FG03 J. Kalinowski (Bielefeld/DE)

17:30–19:45 **Mini-Symposia Special Group – Paving the way from Protein expression to Protein production "Recent developments in heterologous expression for technical enzymes"**
FG Biotransformation

Room Hörsaal +1/0010
Chair K.-E. Jäger (Jülich/DE)

17:30 Mechanism of protein secretion in *Escherichia coli*
A. Driessen (Groningen/DE)

18:15 Proteomic analyses of bacteria used in industrial fermentation processes
BTV-FG01 B. Voigt (Greifswald/DE)

18:45 *Escherichia coli* secretion technology – a holistic approach for the high-yield production of antibody fragments
G. Wich (Munich/DE)

19:15 Leveraging the perfect interplay of diverse expression tools for high level protein production with
BTV-FG02 *Pichia pastoris*
T. Purkarthofer (Grambach, Graz/DE)

17:30–19:30 **Mini-Symposia Special Group – Fungal Biology and Biotechnology**

Room Hörsaal +2/0090
Chairs V. Meyer (Berlin/DE), S. Pöggeler (Göttingen/DE), D. Troppens (Göttingen/DE)

Four European Marie Curie International Training Networks are currently running in the area of fungal biology and biotechnology: FungiBrain, ImResFun, QuantFung and YEASTCELL. About 50 young scientists participate in these networks and study different aspects of fungal biology including growth and morphogenesis, signaling networks, fungal virulence and secondary metabolism.
The Special Group has invited eight young scientists from all four networks to present their research projects in this Mini-Symposia.

SCIENTIFIC PROGRAMME, MONDAY, 2 MARCH 2015

Annual Conference 2015 of the VAAM

17:30–19:00 **Mini-Symposia Special Group – Control, Detection and prevention of micro-organisms during use in clinical and work environment**
FG Quality Assurance & Diagnostics

Room	Hörsaal +1/0030
Chairs	A. Seiffert-Störiko (Höchst/DE), S. Prowe (Berlin/DE)
17:30 QDV-FG01	Bioaerosols from agricultural and waste management facilities – measurement and assessment U. Jäckel (Berlin/DE)
17:48 QDV-FG02	Novel microfluidic biosensor for online monitoring of biofilm formation T. Schwartz (Eggenstein-Leopoldshafen/DE)
18:06 QDV-FG03	Solutions for overcoming testing challenges with disinfectant coupon studies J. Polarine (St. Louis/US)
18:24 QDV-FG04	Blood platelet transfusion relevant bacteria reference strains – suitability test of different *Morganella morganii* strains E. Spindler-Raffel (Langen/DE)
18:42 QDV-FG05	Regulatory aspects of microbial quality of cell based medicinal products U. Schurig (Langen/DE)

Annual Conference 2015 of the VAAM

08:30–10:30 Short Lecture – Cell Biology and Cell Cycle I

Room: Hörsaal +2/0010
Chairs: K. Jonas (Marburg/DE), M. Bramkamp (Martinsried/Munich/DE)

08:30 CCV01 — Pre- and postdivisional functions of *Listeria monocytogenes* DivIVA
S. Halbedel (Wernigerode/DE)

08:45 CCV02 — The PomXYZ proteins self-organize on the chromosome to localize at mid-cell by active, directed motion and position cell division
D. Schumacher (Marburg/DE)

09:00 CCV03 — The role of dynamin-like proteins in the developmental control of cell division in *Streptomyces*
S. Schlimpert (Norwich/UK)

09:15 CCV04 — Off the wall – from filamentous growth to primordial cells and back again
K. Ramijan Carmiol (Leiden/NL)

09:30 CCV05 — The polar peptidoglycan synthesis complex in *Caulobacter crescentus*
M. Billini (Marburg/DE)

09:45 CCV06 — Polar localization of chemotactic signaling arrays in polarly flagellated bacteria
S. Ringgaard (Marburg/DE)

10:00 CCV07 — Akinetes – resistant cells of filamentous cyanobacteria
I. Maldener (Tübingen/DE)

10:15 CCV08 — Overproduction of flotillin influences cell differentiation and shape in *Bacillus subtilis*
B. Mielich-Süß (Würzburg/DE)

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

Room: Hörsaal 00/0030
Chairs: T. Waldminghaus (Marburg/DE), F. Commichau (Göttingen/DE)

08:30 SMV01 — Construction and analysis of synthetic RNA modules derived from prokaryotic regulatory RNAs
J. Roßmanith (Bochum/DE)

08:45 SMV02 — Towards synthetic CO_2 fixation in *Methylobacterium extorquens* – establishing genetic tools for Alphaproteobacteria and realizing artificial CO_2-fixation *in vitro*
L. Schada von Borzyskowski (Zürich/CH, Marburg/DE)

09:00 SMV03 — A LOV2 domain-based optogenetic tool for synthetic regulation of protein stability in eukaryotic cells
C. Taxis (Marburg/DE)

09:15 SMV04 — MreB paralogs of *Bacillus subtilis* associate with a lipid bilayer and co-polymerize to a single curved filament *in vitro*
C. Reimold (Marburg/DE)

09:30 SMV05 — Synthetic secondary chromosomes in *Escherichia coli* based on the replication origin of chromosome II in *Vibrio cholerae*
S. Messerschmidt (Marburg/DE)

09:45 SMV06 — Global signal integration of bacterial two-component systems
C. Lahiri (Kolkata/IN)

10:00 SMV07 — Site-directed mutagenesis switching a dimethylallyl tryptophan synthase to a specific tyrosine C3-prenylating enzyme
A. Fan (Marburg/DE)

10:15 SMV08 — *Corynebacterium glutamicum* as a host for the production of GABA
J. Jorge (Bielefeld/DE)

SCIENTIFIC PROGRAMME, TUESDAY, 3 MARCH 2015

Annual Conference 2015 of the VAAM

08:30–10:30 Short Lecture – Environmental Microbiology I

Room: Hörsaal 00/0070
Chairs: M. Dumont (Marburg/DE), C. Lueke (Nijmegen/NL)

08:30 EMV01	Comparative analysis highlights genome reduction and the evolutionary split of *Acholeplasma* and '*Candidatus* Phytoplasma' M. Kube (Berlin/DE)
08:45 EMV02	The green impact – bacterioplankton response towards a phytoplankton spring bloom in the southern North Sea assessed by comparative metagenomic and metatranscriptomic approaches B. Wemheuer (Göttingen/DE)
09:00 EMV03	Meta-genomics of the Arabian Sea oxygen minimum zone C. Lüke (Nijmegen/NL)
09:15 EMV04	Comparative metagenomics and high-resolution binning reveal functional roles of the gut microbiota in higher termites K. Rossmassler (Marburg/DE)
09:30 EMV05	Reconstruction of the microbial phosphorus turnover in forest soils F. Bergkemper (Neuherberg/DE)
09:45 EMV06	Environmental controls on methanogenic communities in frozen-ground-affected soils on the northeastern Qinghai-Tibetan Plateau S. Yang (Potsdam/DE, Lanzhou/CN)
10:00 EMV07	Fungal microbiome analysis during biodegradation of biodiesel and diesel/biodiesel blends in laboratory soil microcosms G. Matos (Rio de Janeiro/BR, Louvain-la-Neuve/BE)
10:15 EMV08	The genome of *Rhodococcus opacus* 1CP – reconstruction of degradation pathways of aromatic compounds D. Tischler (Freiberg/DE)

08:30–10:30 Short Lecture – Microbial Ecology II

Room: Hörsaal +1/0110
Chairs: B. Engelen (Oldenburg/DE), A. Meyerdierks (Bremen/DE)

08:30 MEcV09	Different utilization of alginate among marine *Alteromonas macleodii* ecotypes indicate ecological speciation M. Wietz (Oldenburg/DE)
08:45 MEcV10	Genome and ecophysiology of a gammaproteobacterium of the genus *Reinekea* associated with North Sea spring phytoplankton blooms B. Avci (Bremen/DE)
09:00 MEcV11	The sixth element – a 102-kb plasmid of *Dinoroseobacter shibae* modulates chromosomal gene expression J. Petersen (Braunschweig/DE)
09:15 MEcV12	Free-living, particle-associated and benthic *Roseobacter* populations show distinctive distributions along the German/Scandinavian North Sea coast S. Kanukollu (Oldenburg/DE)
09:30 MEcV13	Polysaccharide hydrolysis and visualization of uptake across contrasting oceanic provinces in the Atlantic Ocean G. Reintjes (Bremen/DE)
09:45 MEcV14	Heterotrophic *Proteobacteria* with hydrocarbon degrading potential as major players in intermediate mixing zones at hydrothermal vents D. V. Meier (Bremen/DE)
10:00 MEcV15	Quantifying microbial communities of the methane cycle in two subsea permafrost deposits of the central Laptev sea M. Winkel (Potsdam/DE)
10:15 MEcV16	Characterization of continental deep-subsurface microbial communities in the Iberian Pyrite Belt (IPB) A. Arce-Rodriguez (Braunschweig/DE)

Annual Conference 2015 of the VAAM

08:30–10:30 Short Lecture – Metabolism, Enzymes and Cofactors

Room	Hörsaal 00/0020
Chairs	J. Heider, T. J. Erb (Marburg/DE)
08:30 MCV01	X-ray crystallography reveals new surprises in methanogenic enzymes T. Wagner (Marburg/DE)
08:45 MCV02	DNA-based investigations of plant production and bacterial consumption of chloromethane in the *Arabidopsis thaliana* phyllosphere S. Vuilleumier (Strasbourg/FR)
09:00 MCV03	Fungal propionyl-CoA degradation via methylcitrate cycle and citric acid cycle – evidence for a second active site in aconitase AcoA C. Maerker (Munich/DE)
09:15 MCV04	Dedicated maturation factors for the essential ribosome-associated iron-sulfur protein Rli1 V. Paul (Marburg/DE)
09:30 MCV05	Cell wall recycling in *Staphylococcus aureus* – role of the N-acetylmuramic acid 6 phosphate etherase M. Borisova (Tübingen/DE)
09:45 MCV06	Structure-function relationships of the rhodopin 3,4-desaturase (CrtD) of *Rhodospirillum rubrum* C. Autenrieth (Stuttgart/DE)
10:00 MCV07	Acetate assimilation via the methylaspartate cycle in haloarchaea F. Borjian Borujeni (Freiburg/DE)
10:15 MCV08	A novel function-based screen suited to seek RubisCOs from metagenomic libraries – improving our understanding of RubisCO regulation and activation mechanisms. S. Böhnke (Hamburg/DE)

08:30–10:30 Mini-Symposia Special Group – Paving the way from Protein expression to Protein production "Recent developments in heterologous expression for technical enzymes" FG Biotransformation

Room	Hörsaal +1/0010
Chair	K. Liebeton (Zwingenberg/DE)
08:30 BTV-FG03	Fungal strain development for improved protein production – protease mutant approaches and genome mining for novel enzymes discovery P. Punt (Leiden, Zeist/NL)
09:00	Expression of Enzymes for lignocellulosic ethanol (Bioethanol 2G) P. Gutiérrez Gomez (Salamanca/ES)
09:30	The Challenge of Technology Transfer from R&D to Industrial Scale G. Hofmann (Capua/IT)
10:00	Regulatory Aspects of Enzymes for Food, Feed and Technical Applications K.-H. Maurer (Darmstadt/DE)

SCIENTIFIC PROGRAMME, TUESDAY, 3 MARCH 2015

Annual Conference 2015 of the VAAM

08:30–10:30 Short Lecture – Microbial Interactions

Room: Hörsaal +2/0090
Chairs: U. Maier, R. Kahmann (Marburg/DE)

08:30 MIV01 — Hiding in plain sight, root endophytic community is the „true“ producer of maytansine in *Putterlickia plants*
S. Kusari (Dortmund/DE)

08:45 MIV02 — Influence of different phosphate sources on active bacterial microbiome in the rhizosphere and endorhiza of barley (*Hordeum vulgare* L.)
M. Cardinale (Giessen/DE)

09:00 MIV03 — Metabolic cross-feeding via inter-cellular nanotubes among bacteria
C. Kost (Jena/DE)

09:15 MIV04 — Iron adaptation mechanisms by the pathogenic yeast *Candida albicans* are responsive to a bacterial quorum sensing molecule
F. Hennicke (Frankfurt am Main/DE)

09:30 MIV05 — FIB/SEM and (serial) Electron Tomography of the enigmatic *Ignicoccus hospitalis/Nanoarchaeum equitans* Co-Culture
T. Heimerl (Regensburg, Marburg/DE)

09:45 MIV06 — Tin3 of *Ustilago maydis* – a dual function effector?
D. Lanver (Marburg/DE)

10:00 MIV07 — Role of PilY1 in adhesion to host cells and intracellular infection of *Legionella pneumophila*
J. Hoppe (Braunschweig/DE)

10:15 MIV08 — Inflammasome activation in *Salmonella*-infected primary human macrophages is dependent on flagellin
J. Kortmann (Stanford/US)

08:30–10:30 Short Lecture – Microbiology of Anaerobes

Room: Hörsaal +1/0030
Chairs: W. Buckel, S. Shima (Marburg/DE)

08:30 MAV01 — Lactate metabolism in the acetogenic bacterium *Acetobacterium woodii*
M. Weghoff (Frankfurt am Main/DE)

08:45 MAV02 — The structure of the NADH-dependent reduced ferredoxin-NADP oxidoreductase – Insights into flavin-based electron bifurcation
J. K. Demmer (Frankfurt am Main, Marburg/DE)

09:00 MAV03 — The electron transport chain of *Wolinella succinogenes* sulfite respiration
J. Simon (Darmstadt/DE)

09:15 MAV04 — An enzyme producing rocket fuel – Structure of hydrazine synthase from anammox bacteria
A. Dietl (Heidelberg/DE)

09:30 MAV05 — Mechanism of dearomatization/dehalogenation by ATP-dependent benzoyl-CoA reductases
O. Tiedt (Freiburg/DE)

09:45 MAV06 — Identification of tetrachloroethene respiratory chain components in *Sulfurospirillum multivorans*
J. Gadkari (Jena/DE)

10:00 MAV07 — Single gene insertion creates a synthetic pathway for bioalcohols in a hyperthermophilic archaeon
M. Basen (Athens/US, Frankfurt am Main/DE)

10:15 MAV08 — Comprehensive view on the thiol proteome of the obligate anaerobe *Clostridium difficile* and its *perR* mutant under infection-relevant conditions
S. Sievers (Greifswald/DE)

Annual Conference 2015 of the VAAM

11:00–12:10 Plenary Session III – Bacterial cell biology

Room: Hörsaal +2/0010
Chairs: L. Søgaard-Andersen, M. Thanbichler (Marburg/DE)

11:00 ISV10 — Cell biology of a bacterial predator
L. Sockett (Nottingham/UK)

11:35 ISV11 — How electron crytomography is opening a new window into bacterial cell biology
G. J. Jensen (Pasadena/US)

12:20–13:20 Lunchsymposium Hessen Trade & Invest GmbH

Auf dem Weg in die Bioökonomie – ohne Mikrobiologie geht es nicht!

Room: Hörsaal 00/0070

Schlüsseltechnologie einer sich wandelnden Wirtschaft

Moderation: Dr. Detlef Terzenbach (Hessen Trade & Invest GmbH/ Wiesbaden)

Dieses Symposium soll einen Einblick in ausgewählte Tätigkeitsfelder in der Wirtschaft geben. Nach einem kurzen Einführungsvortrag diskutieren Experten die Schlüsselrolle der Mikrobiologie für die wissensbasierte Bioökonomie.

Experten:
Dr. Peter Hammann (Sanofi-Aventis Deutschland GmbH, Frankfurt am Main/DE)
Dr. Stefan Bartoschek (Science4Life, Frankfurt am Main/DE)
Dr. Manfred Kircher (KADIB Advice in Bioeconomy, Frankfurt am Main/DE)

13:30–14:35 Plenary Session IV – CRISPR-systems and viruses

Room: Hörsaal +2/0010
Chairs: L. Randau, G. Bange (Marburg/DE)

13:30 ISV12 — CRISPR-Cas9 bacterial adaptive immunity – biology, mechanisms and evolution
E. Charpentier (Umeå/SE, Braunschweig, Hanover/DE)

14:05 ISV13 — Revenge of the phages – defeating bacterial defences
S. Moineau (Québec/CA)

15:00–17:00 Poster Session II

The posters can be put up by the authors in the time between Monday evening (19:30 h) and Tuesday morning (10:00 h).

Room: Foyer

Biotechnology (BTP01 – BTP53)
CRISPR-Cas, Viruses and Regulatory RNAs (CVP01 – CVP26)
Gene Regulation (GRP01 – GRP23)
Metabolism, Enzymes and Cofactors (MCP02 – MCP34)
Microbial Stress Responses (MSP01 – MSP30)
Microbiology of Anaerobes (MAP01 – MAP28)
Secondary Messengers (SMeP01 – SMeP05)
Secondary Metabolites (SMaP01 – SMaP15)
Sensing and Adaptation (SAP01 – SAP 27)
Synthetic Microbiology (SMP01 – SMP21)

SCIENTIFIC PROGRAMME, TUESDAY, 3 MARCH 2015

Annual Conference 2015 of the VAAM

15:00–16:30 **Karrieresymposium**

Room Hörsaal 00/0030
Vorstellung verschiedener Berufsbilder in den Biowissenschaften (siehe Seite 15).

17:00–17:45 **30 YEARS VAAM**

Room Hörsaal +2/0010
Chair A. Störiko

… ein ungewöhnlicher Blick in die Mikrobenwelt …
Vince Ebert

18:00–19:30 **VAAM Annual General Meeting**

Room Hörsaal 00/0030

19:30 Mixer (see page 18)

Annual Conference 2015 of the VAAM

09:00–11:00 Short Lecture – Cell Biology and Cell Cycle II

Room: Hörsaal +2/0010
Chairs: C. Jogler (Braunschweig/DE), K. Thormann (Giessen/DE)

09:00 CCV09 — Upgrade of flagellar motility in *Shewanella oneidensis* MR-1
S. Brenzinger (Giessen/DE)

09:15 CCV10 — Photoautotrophic PHB metabolism – identification and characterization of a cyanobacterial Phasin
W. Hauf (Tübingen/DE)

09:30 CCV11 — Peptidoglycan present in *Planctomycetes* after all? Insights from an anammox bacterium
M. van Teeseling (Nijmegen/NL)

09:45 CCV12 — Antituberculosis drugs affecting arabinogalactan synthesis selectively block elongation growth
K. Schubert (Martinsried/Munich/DE)

10:00 CCV13 — Molecular and proteomic analyses highlight the importance of the Cpx envelope stress system for acid stress and cell wall stability of *Escherichia coli*
E. Cudic (Osnabrück/DE)

10:15 CCV14 — Exploring cell wall recycling efficiency and autolysin function by specific radioactive labeling of peptidoglycan sugars in *Escherichia coli* and *Pseudomonas putida*
A. Schneider (Tübingen/DE)

10:30 CCV15 — A new route for the transport of β-barrel proteins through the periplasm in *Shewanella oneidensis* MR-1
S. Stephan (Karlsruhe/DE)

10:45 CCV16 — Energy conservation in members of the genus *Ignicoccus* and in *Nanoarchaeum equitans*
H. Huber (Regensburg/DE)

09:00–11:00 Short Lecture – Gene Regulation

Room: Hörsaal 00/0030
Chairs: H.-U. Mösch, G. Bange (Marburg/DE)

09:00 GRV01 — Blocking sporulation of *Bacillus subtilis* at high salinity – SigH is the culprit
N. Widderich (Marburg/DE)

09:15 GRV02 — Characterization of four homologous stress response regulators of the AraC/XylS family from the fire blight pathogen *Erwinia amylovora*
H. Weingart (Bremen/DE)

09:30 GRV03 — Defining the regulon of genes controlled by σ^E, a key regulator of cell envelope stress in *Streptomyces coelicolor*
X. Huang (Munich/DE)

09:45 GRV04 — States of emergency in *Bacillus subtilis* – the commander in chief σ^B rules a complex regulatory network
A. Reder (Greifswald/DE)

10:00 GRV05 — Regulation of *opuB* expression in *Bacillus subtilis* involves a SigB-dependent antisense RNA
U. Mäder (Greifswald/DE)

10:15 GRV06 — Denitrification associated transcriptome of the acid-tolerant denitrifier *Rhodanobacter denitrificans*
M. A. Horn (Bayreuth/DE)

10:30 GRV07 — RcsB – a versatile transcription factor in *Escherichia coli*
D. Pannen (Cologne/DE)

10:45 GRV08 — *Staphylococcus lugdunensis* – an amplifiable *isd* locus
S. Heilbronner (Tübingen/DE, Dublin/IE)

SCIENTIFIC PROGRAMME, WEDNESDAY, 4 MARCH 2015

Annual Conference 2015 of the VAAM

09:00–11:00 Short Lecture – Environmental Microbiology II

Room: Hörsaal 00/0070
Chairs: M. Kästner (Leipzig/DE), D. Wagner (Potsdam/DE)

09:00 EMV09 — Iron-dependent anaerobic methane oxidation in the Bothnian Sea sediment
O. Rasigraf (Nijmegen/NL)

09:15 EMV10 — Long distance electron transfer by cable bacteria in contaminated aquifers
H. Müller (Munich/DE)

09:30 EMV11 — Investigation of the degradation of ^{13}C-labeled fungal biomass in soil – fate of carbon in a soil bioreactor system
M. Schweigert (Leipzig/DE)

09:45 EMV12 — Microbial growth at subzero temperatures and anoxic conditions – the new limits for life on Earth and icy planetary bodies
A. Perfumo (Potsdam/DE, Noordwijk/NL)

10:00 EMV13 — „Hooking" – omics and imaging techniques: the lifestyle and ultrastructure of *Candidatus Altiarchaeum hamiconexum*
A. Perras (Graz/AT, Regensburg/DE)

10:15 EMV14 — What is coming out of a biogas plant? – an overview of microbiome studies and cultivation of antibiotic resistant bacteria from input and output samples of German biogas plants
S. P. Glaeser (Giessen/DE)

10:30 EMV15 — First biochemical, spectroscopic and X-ray crystallographic characterization of an actinobacterial „high affinity" [NiFe]-hydrogenase
C. Schäfer (Mülheim, Berlin/DE)

10:45 EMV16 — Identification of enzymes involved in degradation of the *Pseudomonas aeruginosa* quinolone signals in *Rhodococcus erythropolis* BG43
C. Müller (Münster/DE)

09:00–11:00 Short Lecture – CRISPR-Cas, Viruses and Regulatory RNAs

Room: Hörsaal +1/0110
Chairs: L. Randau, M. Bölker (Marburg/DE)

09:00 CVV01 — The immune defence system of *Haloarchaea*
A. Marchfelder (Ulm/DE)

09:15 CVV02 — Targeted gene-silencing through CRISPR-mediated RNA interference in the hyperthermophilic archaeon *Sulfolobus solfataricus*
Z. Zebec (Vienna/AT)

09:30 CVV03 — Localization of Cas9 protein in heterologous bacteria
L. Jakutyte-Giraitiene (Vilnius/LT)

09:45 CVV04 — Self-assembly of a pyramidal archaeal virion egress structure
T. Quax (Freiburg/DE, Paris/FR)

10:00 CVV05 — Differential RNA-seq of *Vibrio cholerae* identifies the VqmR sRNA as a regulator of collective behaviors
K. Papenfort (Princeton/US)

10:15 CVV06 — The sRNA NsiR4 is involved in controlling nitrogen assimilation in cyanobacteria by posttranscriptional regulation of glutamine synthetase inactivation factor IF7
S. Klähn (Freiburg/DE)

10:30 CVV07 — Functional analysis of small RNAs based on dRNA-seq of *Bradyrhizobium japonicum* in liquid culture and in nodules
S. Thalmann (Giessen/DE)

10:45 CVV08 — C/D box sRNA-guided 2′-O-methylation patterns of archaeal rRNA molecules
V. Tripp (Marburg/DE)

Annual Conference 2015 of the VAAM

09:00–11:00 Short Lecture – Biotechnology II: Tools, Biocatalysts and Environmental Applications

Room: Hörsaal 00/0020
Chairs: J. Heider, T. J. Erb (Marburg/DE)

09:00 BTV09 — A toolbox for the study and high-yield production of [NiFe]-hydrogenases along with their respective maturation circuits in *Escherichia coli*
J. Schiffels (Jülich/DE)

09:15 BTV10 — Bioreactor design considerations for the use of electrolytic oxohydrogen gas as a growth substrate in biotechnology
M. Krehenbrink (Münster/DE)

09:30 BTV11 — Protein evolution for *in vivo* synthetic catalysis in extremophiles
M. Maier (Eggenstein-Leopoldshafen/DE)

09:45 BTV12 — Biotechnological production of ω-hydroxy fatty acids with the help of metabolically engineered *Yarrowia lipolytica* strains
M. Gatter (Dresden/DE)

10:00 BTV13 — Back to nature – biosynthesis of indigoid dyes
T. Heine (Freiberg/DE)

10:15 BTV14 — Laccase-mediated transformation of various bisphenols for application in a bioreactor for wastewater treatment
M. Meister (Greifswald/DE)

10:30 BTV15 — Using an enzyme secreting, genetically modified *E. coli* strain to supply bilirubin oxidase to a biofuel cell cathode
J. Eipper (Freiburg/DE)

10:45 BTV16 — Dynamics of bacterial community structure during non-destructive biocatalytic desulfurization of various organosulfur compounds
W. Ismail (Manama/BH)

09:00–11:00 Short Lecture – Symbiosis

Room: Hörsaal +1/0010
Chairs: R. Kahmann, U. Maier (Marburg/DE)

09:00 SYV01 — Evolution from pathogen to commensalist – *Pseudomonas aeruginosa* obtains selfish fitness gains through virulence attenuation during in vivo adaptation to immunocompetent and immunocompromised metazoan hosts
G. Jansen (Kiel/DE)

09:15 SYV02 — *Endomicrobium proavitum*, a free-living relative of flagellate endosymbionts in termite guts – a real-time model of reductive genome evolution
H. Zheng (Marburg/DE)

09:30 SYV03 — An arsenal of toxin-related genes in the genomes of beneficial symbionts from deep-sea hydrothermal vent mussels
L. Sayavedra (Bremen/DE)

09:45 SYV04 — Interaction of non-seed plants with arbuscular mycorrhizal fungi
M. Ortega Pérez (Marburg/DE)

10:00 SYV05 — Bacterial symbionts as major players in the biology of the whitefly *Bemisia tabaci*
M. Ghanim (Bet Dagan/IL)

10:15 SYV06 — Towards exploring a novel symbiosis between *Planctomycetes* and macroalgae
M. Jogler (Braunschweig/DE)

10:30 SYV07 — Mutualistic root endophytism is not associated with the reduction of saprotrophic characters and requires a non-compromised plant innate immunity
A. Zuccaro (Cologne/DE)

10:45 SYV08 — Volatiles of symbiotic bacteria of the human skin microbiome
M. C. Lemfack (Rostock/DE)

SCIENTIFIC PROGRAMME, WEDNESDAY, 4 MARCH 2015

Annual Conference 2015 of the VAAM

09:00–11:00 Short Lecture – Translocation of Small Molecules across Membranes

Room: Hörsaal +2/0090
Chairs: T. Hoffmann, E. Bremer (Marburg/DE)

09:00 TAV01 — How does *Staphylococcus aureus* generate membrane potential?
S. Mayer (Tübingen/DE)

09:15 TAV02 — The crystal structure of the Na^+-translocating NADH ubiquinone oxidoreductase from *Vibrio cholerae*
J. Steubner (Stuttgart/DE)

09:30 TAV03 — ATP-dependent conformational changes trigger substrate capture and release in an ECF-type biotin transporter
F. Finkenwirth (Berlin/DE)

09:45 TAV04 — Transport meets systems biology – characterization of fructose efflux in *Corynebacterium glutamicum*
N. Brühl (Cologne/DE)

10:00 TAV05 — The influence of the N-terminal helix on formate translocation by the pentameric formate channel FocA
D. Hunger (Halle/Saale/DE)

10:15 TAV06 — Extracellular loop 4 acts as periplasmic gate of the proline transporter PutP
S. Bracher (Martinsried/Munich/DE)

10:30 TAV07 — Phosphate specific porins of *Pseudomonas aeruginosa* outer membrane – structure, dynamics and ion-selectivity
S. Ganguly (Bremen/DE)

10:45 TAV08 — Unlocking the secret of MOMP the major porin from *Campylobacter jejuni* – revealing binding from translocation
N. Dhanasekar (Bremen/DE)

11:30–11:45 VAAM Poster Awards

Room: Hörsaal +2/0010
Chair: O. Zelder (Ludwigshafen/DE)

11:45–12:55 Plenary Session VI – Symbiosis

Room: Hörsaal +2/0010
Chairs: A. Brune, R. Kahmann (Marburg/DE)

11:45 ISV14 — Are lipo-chitooligosaccharides solely symbiotic signals in plant-microbe interactions?
G. Stacey (Missouri/US)

12:20 ISV15 — The dynamics of bacterial communities in guts of social bees
N. A. Moran (Texas/US)

12:55–13:00 Closing Remarks

Room: Hörsaal +2/0010
O. Zelder (Ludwigshafen/DE)

Oral Presentations

Poster Presentations

ISV01
Ancient pathogen genomics – what we learn from historic epidemics
*J. Krause[1,2]
[1]*Max Planck Institute for the Science of Human History, Jena, Germany*
[2]*University of Tübingen, Department of Archeological Sciences, Tübingen, Germany*

Genome wide data from ancient microbes may help to understand mechanisms of pathogen evolution and adaptation for emerging and re-emerging infectious disease causing agents. Using high throughput DNA sequencing in combination with targeted DNA enrichment protocols we have reconstructed medieval bacterial genomes of *Yersinia pestis*, *Mycobacterium leprae* and *Mycobacterium tuberculosis* from ancient skeletal remains. Phylogenetic analysis indicate that the ancient *Y.pestis* strain from the Black Death pandemic is ancestral to most extant strains and falls very close to the ancestral node of human infectious *Y. pestis* bacteria. Temporal estimates suggest that the Black Death of 1346 - 1351 was the main historical event responsible for the introduction and worldwide dissemination of currently circulating *Y. pestis* strains pathogenic to humans, and further indicates that contemporary *Y. pestis* epidemics have their origins in the medieval era. In contrast the medieval *M. leprae* strains fall within the current genetic diversity and are found on at least two main branches in the phylogenetic tree of leprosy bacteria. The reconstructed *M.tuberculosis* genomes from 1000 year old Peruvian genomes, however, cluster together with *M.tuberculosis* strains found in modern mammalian species, suggesting a zoonotic origin for tuberculosis in the pre-columbian New World, likely introduced into human populations by contact to sea mammals. Dating analysis reveal a most recent common ancestor of *Y.pestis, M.leprae* and all *M.tuberculosis* strains within the last 6000 years, suggesting that all three human pathogens may have a recent Neolithic origin.

ISV02
Phenotypic and genomic evolution during a 60.000-generation experiment with *Escherichia coli*
*R. Lenski[1]
[1]*Michigan State University, Michigan, United States*

Evolution is an on-going process; therefore, it can be studied experimentally in organisms with suitably fast generations. We have propagated 12 populations of *E. coli* in a simple laboratory environment for over 25 years and 60 000 generations. Two goals of this experiment are to examine the repeatability of evolution and characterize the dynamics of evolution. At the phenotypic level, we have quantified the trajectory of adaptation by natural selection, identified many cases of parallel evolution, and observed the origin of a new function that transcends the usual definition of *E. coli* as a species. We have also sequenced hundreds of genomes to characterize the dynamics of genome evolution in these populations. The genomic data provide new insights into the coupling of phenotypic and genetic evolution, and into the role of complex mutations in the emergence of key innovations.

ISV03
Genome dynamic and accessory elements in the plant pathogenic fungus *Zymoseptoria tritici*
M. Moeller[1,2], K. Schotanus[1,2], J. L. Soyer[1,3,2], M. Freitag[4],
*E. H. Stukenbrock[1,2]
[1]*Christian-Albrechts University of Kiel, Environmental Genomics, Kiel, Germany*
[2]*Max Planck Institute for Evolutionary Biology, Plön, Germany*
[3]*INRA, UR 1290 BIOGER-CPP, Thiverval-Grignon, Germany*
[4]*Oregon State University, Department of Biochemistry and Biophysics, Corvallis, United States*

Accessory chromosomes are present in the genomes of many organisms. In fungi these chromosomes are present at different frequencies in populations and usually characterized by higher repetitive DNA content and lower gene density. In the fungal pathogen *Zymoseptoria tritici*, as many as eight discrete accessory chromosomes occur in different strains comprising up to 12% of the total genome. So far no functional role has been assigned to these chromosomes. Comparative genome analyses show that genes on accessory chromosomes accumulate considerably more mutations compared to genes on core chromosomes consistent with a relaxation of selective constraints. Yet, accessory chromosomes have existed as separate entities in the karyotypes of *Zymoseptoria* species over longer evolutionary time. We set out to investigate the properties of accessory chromosomes underlying their rapid evolution and sequence dynamics. We used ChIP-seq with antibodies against the centromere specific histone CenH3, as well as the euchromatic mark H3K4me2 and the heterochromatic marks H3K9me3 and H3K27me3 to identify centromeres and determine the distribution and proportion of euchromatin and heterochromatin in the *Z. tritici* genome. Next, we conducted a detailed computational analysis of subtelomeric repeats to compare telomeres of core and accessory chromosomes. We show that accessory chromosomes have centromeres and telomeres identical to those of core chromosomes. However, accessory chromosomes of *Z. tritici* have dramatically higher enrichment of heterochromatin, consistent with a higher proportion of repetitive DNA. To further investigate the importance of histone modifications on chromosome stability we deleted the two histone methyltransferases KMT1 and KMT6 that generate heterochromatin. *Z. tritici* mutants and wild type were propagated across 1000 cell divisions to study chromosome stability. Chromosome separation of progenitor and evolved lineages showed chromosome loss and structural rearrangements in the genome of kmt1 and kmt6 mutants. Together our results suggest a central role of chromatin modifications in the maintenance of chromosome stability in *Z. tritici*. Dynamic changes of chromatin structures thereby contribute to karyotypic variation and chromosome evolution in this important pathogen.

ISV04
Disentangling the origins of eukaryotic cells
*M. Embley[1]
[1]*Newcastle University, Institute for Cell and Molecular Biosciences, Newcastle, United Kingdom*

The classic three domains ribosomal RNA tree rooted on the bacterial branch is depicted in most textbooks, and has life divided into three domains with Eukaryotes the sister group of all Archaea. Recent work, however, suggests that an alternative hypothesis called the eocyte tree, whereby vital components of the eukaryotic ribosome were inherited from within the Archaea, is the better-supported hypothesis using current data and methods. This suggests that a bona-fide archaeon played a role in eukaryotic origins. It follows that eukaryotes are a derived group and not, as sometimes claimed, a primary domain of life. At the same time, it has been shown that eukaryotic genomes contain large numbers of genes of bacterial origin: some of these appear to have originated from the mitochondrial endosymbiosis - which is currently thought to be an ancestral event affecting all eukaryotes - but many others appear to have originated from different prokaryotic groups. In my talk I will critically discuss these data and their current interpretations - including where disagreements and challenges still lie, and their implications for how we currently view the origin and evolution of eukaryotes.
Embley, T. M. and Martin, W. (2006). Eukaryote evolution, changes and challenges. Nature, 440: 623-630.
Williams, T. A., Foster, P. G., Cox, C. and Embley T. M. (2013). An archaeal origin of eukaryotes supports only two primary domains of life. *Nature* 504, 231-236.

ISV05
Origins of major archaeal clades correspond to gene acquisitions from bacteria
*W. F. Martin[1], F. L. Sousa[1], S. Nelson-Sathi[1]
[1]*University of Düsseldorf, Institute for Molecular Evolution, Düsseldorf, Germany*

The mechanisms that underlie the origin of major prokaryotic groups are poorly understood. In principle, the origin of both species and higher taxa among prokaryotes should entail similar mechanisms—ecological interactions with the environment paired with natural genetic variation involving lineage-specific gene innovations and lineage-specific gene acquisitions. To investigate the origin of higher taxa in archaea, we have determined gene distributions and gene phylogenies for the 267,568 protein-coding genes of 134 sequenced archaeal genomes in the context of their homologues from 1,847 reference bacterial genomes. Archaeal-specific gene families define 13 traditionally recognized archaeal higher taxa in our sample. Unexpectedly, the origins of these 13 groups correspond to 2,264 group-specific gene acquisitions from bacteria. Interdomain gene transfer is highly asymmetric, transfers frombacteria to archaea aremore than fivefold more frequent than vice versa. Gene transfers identified at major evolutionary transitions among prokaryotes specifically implicate gene acquisitions for metabolic functions from bacteria as key innovations in the origin of higher archaeal taxa.

ISV06
F1000 – a new way of writing, discovering and sharing science
*M. Torkar[1]
[1]*F1000Research, Faculty of 1000, London, United Kingdom*

There has been much debate that traditional publishing processes and the limitations imposed by existing formats, all of which were developed for journals that were solely distributed in print and had restricted space for papers, hamper the progress of science in various ways:
(1) the scientific literature is incomplete as there is a bias towards 'positive' and 'interesting' results; (2) protracted peer-review processes mean that valuable new results can often only be shared with, and built upon, by others after significant delays; and (3) insufficient methodological details and lack of data in publications prevent effective reproducibility.
F1000Research is a relatively new Open Science publishing platform set up specifically to address these issues: it publishes research and debate across all life sciences, including microbiology, irrespective of the perceived level of interest and novelty, and it operates a post publication peer review model, offering very rapid publication without editorial bias and fully transparent peer review by invited experts. F1000Research also puts much focus on the inclusion of source data in easily accessible formats and on detailed descriptions of methods, making it easier for others to repeat the analysis presented in an article.
F1000Research is part of Faculty of 1000, which recently also launched a new authoring tool that will revolutionise the way that researchers write and collaborate. The tool incorporates over 150,000 article recommendations written by more than 5,000 eminent scientists and clinical researchers who have been systematically surveying the scientific literature over the past decade for the most relevant publications in their fields.

ISV07
Adaptation of hydrogen bacteria to an aerobic life style
*B. Friedrich[1]
[1]*Humboldt Universität zu Berlin, Institut für Biologie, Berlin, Germany*

Microbes have developed the capacity to use molecular hydrogen (H_2) as energy source several billions of years ago. Pioneering work by Hans Günter Schlegel focused on the physiology and biochemistry of aerobic H_2-consuming bacteria, the so-called Knallgas bacteria. Both biological H_2 oxidation as well as H_2 production is catalyzed by phylogenetically diverse groups of hydrogenases. As these metalloenzymes are usually exquisitely oxygen sensitive, H_2 metabolism under aerobic conditions, as it occurs in Knallgas bacteria, requires specific devices to allow catalysis in the presence O_2. Oxygen-tolerant H_2 cycling involves hydrogenases that have undergone structural and catalytic changes and that have developed an O_2-adapted biosynthetic machinery as well as specific regulatory governance.
I will present four examples of [NiFe]-hydrogenases to demonstrate how O_2 tolerance or even O_2 resistance can be acquired. This includes recent high-resolution crystal structure analyses of a particular subtype of [NiFe]-hydrogenase that is predominantly found in aerobic or facultative aerobic H_2-oxidizers. Then I will describe how we have used genetic analysis to identify and characterize the accessory components required for biogenesis of [NiFe]-hydrogenases in the presence of air. After discussing what is known about this complex metal center assembly process, I will end with a short survey on H_2 responsive transcriptional regulation.

ISV08
Synthetic biology for the production of high-value chemicals
*E. Takano[1]
[1]*University of Manchester, Manchester Centre for Synthetic Biology of Fine and Speciality Chemicals (SYNBIOCHEM), Manchester Institute of Biotechnology, Faculty of Life Sciences, Manchester, United Kingdom*

Our ability to readily sequence complete genomes and to manipulate/re-design them on a large scale enables the design and construction of organisms with new functionalities of unprecedented scope ("synthetic biology"). We explore these possibilities in the context of high-value chemical production. Many microorganisms already have the machinery to produce diverse bioactive molecules that can be used in health, agriculture and food. As a first step towards re-engineering these high-value chemical biosynthesis pathways for enhanced productivity and diversity, we aim to understand the transcriptional circuitry controlling the native biosynthetic gene clusters. As a result we can refactor the control systems using orthogonal transcription and translation mechanisms, based on promoter libraries, signalling molecule circuits and ncRNA, while maintaining the subtle relationships necessary for optimal function. In addition, we are expanding our collection of computational tools for the detection and analysis of secondary metabolite biosynthesis gene clusters, to enrich our library of parts and building blocks for pathway engineering. We combine this analysis with high-resolution mass spectrometry analysis, which we also employ for the debugging of the engineered systems. Furthermore, we are using computational modelling (constraint-based descriptions of bacterial metabolism) to identify suitable overproduction hosts and pinpoint biosynthetic bottlenecks to target for further cellular engineering in a synthetic biology strategy.

ISV09
Cyborgization of soil bacteria for smart degradation of environmental pollutants
*V. de Lorenzo[1]
[1]*National Center of Biotechnology, Systems Biology, Madrid, Spain*

Much of contemporary Synthetic Biology aims at re-programming entire microorganisms (rather than single genes) for enhancing existing functions and/or performing new-to-nature tasks. In the case of bacteria, key aspects to this end include the removal of undesired genomic segments, systems for production of directed mutants and allelic replacements, random mutant libraries to discover new functions, and means to stably implant larger genetic networks into the genome of specific hosts. The list of Gram-negative species that are appealing for such genetic refactoring operations is growingly expanding, but soil bacteria such as *Pseudomonas putida* are pre-endowed with the metabolic, physiological and stress-endurance traits that are demanded by current and future synthetic biology and biotechnological needs. A closer look at the metabolic properties of this bacterium helps to understand why it can host such a variety of biodegradation pathways. Glycolysis in *P. putida* has a number of unique features. Several of the oxidative steps are coupled to the reduction of NADP+ to NADPH, used to generate anabolic precursors needed for bacterial growth and exploited as antioxidant currency under exogenous and endogenous stressful conditions. We are in the midst of an effort to standardize molecular tools for easing a radical, deep engineering of the *P. putida* genome much further than our current abilities, which do not go at this time beyond the ~ 20 kb / ~20 gene range. We argue that adoption of standardized vectors can become a phenomenal shortcut to fill the gap between the existing power of DNA synthesis and the actual engineering of predictable and efficacious strains. Our current attempts to use these tools for [i] programming of *P. putida* KT2440 to switch towards an anaerobic metabolic regime and [ii] modifying lifestyle from planktonic to surface-attached for the sake of engineering catalytic biofilms will be presented.

Martínez-García & de Lorenzo, V. (2011) *Env Microbiol* 13: 2702-2716. Nikel & de Lorenzo (2012) *J. Biotechnol 163(2):143-54.* Nikel, P.I. Martínez-García, E. and de Lorenzo, V. (2014) *Nature Microbiol Revs.* 12: 368-379. Martínez-García *et al* (2014) *Nucleic Acids Res*. PMID: 25392407. Martínez-García *et al* (2014) *Front Bioeng Biotechnol* 2:46. Martínez-García *et al.* (2014) *Microb Cell Fact* 13:159.

ISV10
Cell Biology of a Bacterial Predator
*L. Sockett[1]
[1]*University of Nottingham, School of Life Sciences, Nottingham, United Kingdom*

Bdellovibrio bacteriovorus is a predatory delta-proteobacterium, discovered from soil in Germany by Hans Stolp. It has a 3.8Mb genome inside a vibroid cell only 1µm x 0.3µm. Locomotion can be by a single, membrane-sheathed flagellum or by gliding protein complexes. Locomotion delivers *Bdellovibrio* to encounters with other Gram-negative cells, which they bind and then invade. Growth of *Bdellovibrio* is then intraperiplasmic, in a sessile phase inside the, now-dead, but intact, prey. The nutrients available from the prey are finite and dependent on cell volume. This fact brings cell biology demands on the *Bdellovibrio* and filamentous cell division forms odd or even numbers of progeny predators. *Bdellovibrio* motility resumes at the end of predator replication to escape the exhausted prey cell by flagellar or gliding motility. The non flagellar pole is specialised for prey-interaction and this involves Type IV pili and associated regulatory proteins, functions for which are becoming understood. This presentation will review the cell biology of these predatory bacteria and compare proteins involved in their cellular organisation to those known in non predatory bacteria.

ISV11
How electron cryotomography is opening a new window into microbial cell biology
*G. Jensen[1]
[1]*Caltech, HHMI, Pasadena, United States*

In the last ten years electron cryotomography has made it possible to visualize large macromolecular assemblies inside intact cells in a near-native, "frozen-hydrated" state in 3-D to a few nanometers resolution. Increasingly, atomic models of individual proteins and smaller complexes obtained by X-ray crystallography, NMR spectroscopy, or other methods can be fit into cryotomograms to reveal how the various pieces work together inside cells. A few good pictures is therefore sometimes all that is really needed to distinguish between competing models. To illustrate these points, I will examples from our recent work in bacterial and archaeal cell biology, including as time permits new images and mechanistic insights into the bacterial cytoskeleton, cell wall, chemoreceptor arrays, flagellar motors, and secretion systems.

ISV12
CRISPR-Cas9 bacterial adaptive immunity – biology, mechanisms and evolution
*E. Charpentier[1,2,3]
[1]*Helmholtz Centre for Infection Research, Department of Regulation in Infection Biology, Braunschweig, Germany*
[2]*Umeå University, The Laboratory for Molecular Infection Medicine Sweden (MIMS), Umeå Centre for Microbial Research (UCMR), Department of Molecular Biology, Umeå, Germany*
[3]*Hanover Medical School, Hanover, Germany*

The RNA-programmable CRISPR-Cas9 system has recently emerged as a transforming technology in biological sciences, allowing rapid and efficient targeted genome editing, chromosomal marking and gene regulation. In this system, the endonuclease Cas9 or catalytically inactive Cas9 variants are programmed with single guide RNAs (sgRNAs) to target site-specifically any DNA sequence of interest given the presence of a short sequence (Protospacer Adjacent Motif, PAM) juxtaposed to the complementary region between the sgRNA and target DNA. The system is efficient, versatile and easily programmable.
Originally, CRISPR-Cas is an RNA-mediated adaptive immune system that protects procaryotes from invading mobile genetic elements (phages, plasmids). Short CRISPR RNA (crRNA) molecules containing unique genome-targeting spacers commonly guide Cas protein(s) to the invading cognate nucleic acids to affect their maintenance. CRISPR-Cas9 originates from the type II CRISPR-Cas system that has evolved unique mechanisms for the maturation of crRNAs and targeting of invading DNA, identified in *Streptococcus pyogenes*. On the basis of the discovery of the DNA targeting mechanism, we proposed that RNA-programmable Cas9 could offer considerable potential for genome editing in cells of the three kingdoms of life for biotechnological and biomedical purposes. As demonstrated by a large number of studies published in the last 18 months, DNA targeting by CRISPR-Cas9 has quickly been adopted by the scientific community to edit and silence genomes in a large variety of cells and organisms. I will discuss the biological roles, mechanisms and evolution of the type II CRISPR-Cas9 system in bacteria and the applications of the system as a novel genome engineering technology.

ISV13
Revenge of the phages – defeating bacterial defences
*S. Moineau[1]
[1]*Université Laval, Département de Biochimie, Microbiologie et Bio-Informatique, Faculté des Sciences et de Génie, Québec City, Canada*

Bacteriophages (phages) are viruses that specifically infect bacteria, which eventually lead to cell lysis and the release of several new virions ready to infect related bacterial cells. Phages are now recognized as the most abundant biological entities on our planet and they outnumber bacteria by an estimated tenfold. As such, they play major role in the ecological balance of the microbial life and the recycling of nutrients. Phages are also extremely diversified. This diversity is mostly driven by their dynamic adaptation when facing, among others, selective pressure such as phage resistance mechanisms, which are widespread in bacterial hosts. Indeed in order to proliferate in phage-rich ecosystems, bacteria have developed an impressive arsenal of defense mechanisms, including the inhibition of phage adsorption and genome entry, restriction-modification (R-M) systems, abortive infection mechanisms, and CRISPR-Cas systems.
In response, phages have evolved multiple tactics to avoid, circumvent or subvert these antiviral mechanisms in order to thrive in most environments. These findings have enhanced our knowledge of phage biology, evolution and phage-host interactions. These studies have also practical applications such as in the control of phage populations (development of phage-resistant bacteria for the fermentation and biotechnology industries) or in the selection of virulent phages (for use in biocontrol/biosanitation purposes). Here, I will highlight the most important counter-attacks used by phages to evade natural antiphage systems, with a focus on R-M and CRISPR-Cas systems.

ISV14
Are lipo-chitooligosaccharides solely symbiotic signals in plant-microbe interactions?
*G. Stacey[1]
[1]*University of Missouri, Divisions of Biochemistry and Plant Science, Columbia, United States*

Lipo-chitooligosaccharies (LCO) and chito-oligosaccharides (CO) have emerged as key, microbial signals recognized by plant hosts to either trigger symbiotic interactions or defense against pathogens. CO recognition appears to be an ancient trait, shared by plants and animals, although the mechanism of host recognition differs. In plants, these molecules are recognized by lysin-motif, receptor-like kinases (LysM RLK). Work in our laboratory has focused on two aspects: one, the structure and function of the CO/LCO receptor complex and two, the biological activity of LCO/CO relevant to either symbiosis or pathogen defense. It now seems well established that CO/LCO receptors consist of a heterodimer of a LysM RLK with an active kinase domain and a LysM-motif co-receptor lacking kinase activity. At least in Arabidopsis and rice, it is this co-receptor that primarily mediates CO recognition. The critical role of LCO signaling in the rhizobial symbiosis led to the idea that specificity for this signal determines the ability of the host to support the rhizobial symbiosis. However, our data show that many, if not all, non-legumes recognize LCO signals. This is perhaps not surprising since LCO signals are also involved in the mycorrhizal symbiosis, which is widespread in plants. However, even non-mycorrhizal plants recognize LCO, which in this case results in suppression of plant pathogen defense. Indeed, we hypothesize that LCO production to reduce plant immunity may have evolved first to support a pathogenic lifestyle of rhizobia, which later attenuated and then took on a key role in the induction of symbiotic development. These results have direct relevance to on-going efforts to engineer non-legumes to support a nitrogen-fixing rhizobial symbiosis. The results suggest that it is not a lack of LCO recognition that prevents such an interaction but, instead, LCO recognition in non-legumes is not coupled to the developmental pathways needed to support rhizobial infection.

ISV15
The dynamics of bacterial communities in guts of social bees
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A central role of microbial partners, particularly gut bacteria, in animal ecology and evolution is increasingly evident. Honey bees and bumble bees have characteristic bacterial species in their guts most of which are absent from most other bees or other insects. Unlike gut communities in many insects, these communities are composed of specialists, not found in other environments. In honey bees, gut communities are dominated by 6 to 8 bacterial species, each consisting of numerous strains that differ in gene repertoires. Three of these species are Gram negative bacteria that have recently been described as *Gilliamella apicola*, *Snodgrassella alvi*, and *Frischella perrara*; these dominate in the ileum region of the hindgut. Experiments show that these bacteria are transmitted through social interactions, and that each honey bee worker is fully colonized before leaving the hive for the first time. Related bacteria occur in bumble bees, in which they have been shown to confer protection against trypanosome parasites. Another similarity to the human gut microbiota is the existence of extensive strain diversity within particular species in the community: analyses both from metagenomic sequencing and from single cell genomics indicate that a single honey bee colony can harbor numerous divergent strains within *S. alvi* and within *G. apicola*. One gene category for which gene sets differ extensively is that associated with sugar and carbohydrate metabolism by *G. apicola.* Since honey bees encounter a wide variety of plant-produced carbohydrates, these differences among symbiont capabilities may impact the host nutrition and ability to use or detoxify different dietary components. Genomic analyses and

experimental assays indicate that a subset of strains of *G. apicola* is able to digest pectin, suggesting a possible role in pollen wall digestion. Newly emerged adult bees lack the microbiota and can be inoculated experimentally with known strains, enabling further experiments on their effects on host biology and potentially enabling the development of probiotics for honey bees. The full extent of favorable or harmful effects of these bacteria is not yet known.

ISV16

Killing for DNA – the type VI secretion system of *Vibrio cholerae* fosters horizontal gene transfer

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Vibrio cholerae, the causative agent of cholera, is considered an important model organism for studying the role that climate has on infectious diseases and for elucidating virulence regulation. In the latter context, the involvement of quorum sensing (QS) and the type VI secretion system (T6SS) have been extensively studied even though the T6SS is been considered to be "silent" in pandemic *V. cholerae* strains. Much less is known about the bacterium's lifestyle in its natural environment, where it often associates with chitinous surfaces of small crustaceans and their molts. Upon growth on chitin, *V. cholerae* enters the state of natural competence for transformation [1], which enables the bacterium to take up free DNA from the environment. We initially investigated the regulatory network driving natural competence and transformation [2-4] and more recently also the mechanistic aspects of the DNA uptake machinery [5-7]. Here, we show that the chitin and QS-dependent competence regulon of diverse pandemic and epidemic *V. cholerae* strains includes the T6SS-encoding gene cluster and that the T6SS contributes to enhanced horizontal gene transfer by means of natural transformation. Moreover, we used live cell imaging to visualize the competence-induced and T6SS-mediated killing of prey cells and the subsequent uptake of their DNA by the competent predator cell. Our results indicate that the competence-mediated induction of the T6SS enhances horizontal gene transfer by deliberate killing of neighboring non-immune cells and taking up their DNA [8].

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BTV01

Metabolic engineering of *Corynebacterium glutamicum* for production of L-leucine and 2-ketoisocaproate

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Introduction: Microorganisms such as *Corynebacterium glutamicum* are used for biotechnological production approaches. The branched-chain amino acid L-leucine and its keto acid precursor 2-ketoisocaproate (KIC) represent interesting products due to their diverse applications in food, feed and pharmaceutical industry.
Objectives: Rational design of *C. glutamicum* for efficient production of L-leucine and KIC.
Methods: Production strains were designed using genetic engineering and their production performance was characterized in shake-flask and bioreactor cultivations. HPLC methods were employed for product quantification.
Results: Genetically modifications to convert *C. glutamicum* wild type into a L-leucine producer involved: I) genomic integration of three copies of a gene for a feedback-resistant 2-isopropylmalate synthase under control of a strong promoter, II) integration of a gene for a feedback-resistant acetohydroxyacid synthase, III) deletions of *ltbR* and *iolR* encoding transcriptional regulators and IV) reduction of citrate synthase activity. In fed-batch bioreactor cultivations, the best producer accumulated L-leucine to levels exceeding the solubility limit of 24 g/l, with a molar product yield of 0.30 mol L-leucine per mol glucose and a volumetric productivity of 4.3 mmol/(l*h) [1]. The deletion of the gene encoding the transaminase IlvE in L-leucine producers yielded strains for the production of KIC, accumulating up to 6.1 g/l KIC in shake-flask cultivations [2].
Conclusions: The best producer showed highest values for yield, productivity and titer reported for a rationally constructed bacterial L-leucine production strain so far. Precipitation of L-leucine simplifies downstream processing. The absence of auxotrophies, heterologous genes and plasmids prevents conflicts with regulatory restrictions as well as the use of supplements or antibiotics. Therefore, the strain is highly interesting for the industrial large scale production of L-leucine. Our experiments show that the engineered L-leucine production strain can also serve as basis for the construction of KIC producers.

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BTV02

Expanding the product scope of *Pseudomonas putida*

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Monoterpenoids are interesting biomolecules due to their application as aroma and flavor compounds and as antimicrobial, antifungal and anticarcinogenic agents, respectively. Since chemical synthesis is often hampered by complex chiral structures and extraction from natural sources is often non-economic due to low natural availability, biotechnological production of monoterpenoids constitutes a promising alternative strategy. One prerequisite using microbes for those frequently antimicrobial monoterpenoids is their terpenoid resilience. Therefore, *Pseudomonas putida*, known to be highly tolerant to many solvents and aromatic hydrocarbons, represents an attractive production host.
In our work, we present *P. putida* DSM 12264 as an efficient microbial host not only as a natural biocatalyst but also as a recombinant strain and even as a microbial cell factory for monoterpenoid production. *P. putida* DSM 12264 that naturally converts high amounts of limonene and geraniol to perillic acid and geranic acid, respectively, was tested as a biocatalyst for non-natural monoterpenoic substrates using model monooxygenase $P450_{cin}$ (CYP176A1) and its native redox partner cindoxin CinC of *Citrobacter braakii*. The resulting recombinant strain was able to produce up to 5 g/L 2b-hydroxy-1,8-cineole in 3 days in the fed-batch bioreactor. Furthermore, the application of this strain as a microbial cell factory for *de novo* production of monoterpenoid was demonstrated by introduction of the geraniol synthase of *Ocimum basilicum* and the mevalonate pathway of *Myxococcus xanthus*. The resulting strain was able to produce ~ 190 mg/L geranic acid in 2 days in the bioreactor. To the best of our knowledge, this is the first example of *de novo* monoterpenoic acid production with an engineered microbe so far.

BTV03

Synthetic pathways engineering in *Escherichia coli* strains for the production of serotonin and 5-hydroxytryptophan

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Introduction: 5-Hydroxytryptophan (5-HT) is a natural non-proteinogenic aromatic amino acid with therapeutic effect over various conditions (*e.g*. depression, insomnia, chronic headaches, binge eating associated with obesity); serotonin is widely distributed amine involved in a variety of functions in animals as well as in plants. Currently they are chemically produced, or extracted from seeds of the African plant *Griffonia simplicifolia* [1]. Biotechnological production provides a promising alternative, unfortunately requires pathways that are not present in prokaryotic systems. To this end, synthetic biology and protein engineering are helpful to produce these compounds with high yield and titer in *E. coli*.
Objectives: We aim to incorporate a synthetic pathway in *E. coli* strains so that they are capable to convert sugars into 5-HT and serotonin through the tryptophan biosynthesis.

Materials & Methods: For 5-HT production, tryptophan hydroxylase related enzymes were first analyzed *in silico*. Phenylalanine hydroxylase (PAH) and a tryptamine 5-hydroxylase (T5H) were chosen as candidates for protein engineering. Modeling and docking analyses enable us to predict residues related to substrate preference. With Iterative Saturation Mutagenesis we aim to change substrate preference. Heterologous expression of a hydroxylase coupled with tryptophan decarboxylase (TDC) yields into serotonin.
Results: PAH and T5H present low activity toward tryptophan, this is our starting point to route the 5-HT production. Three residues from PAH were identified to be involved in the substrate preference, two of these homologous residues from other PAH have been already associated with substrate preference [2]; the third one is a novel site. We were able to produce serotonin from tryptophan coexpressing TDC and T5H.
Conclusion: We were able to produce 5HT and serotonin en *E. coli*. To get beyond the proof of the concept, further protein engineering, optimization and coordination between the expressions of the different enzymes is required. Also, production can be linked to the central metabolism of the bacteria using an *E. coli* tryptophan producer strains as the chassis wherein to express our enzymes.

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BTV04

Reprogramming nonribosomal peptide Synthetases from *Xenorhabdus* and *Photorhabdus*

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Nonribosomal peptide synthetases (NRPS) are multifunctional enzymes with a modular organization. Each module is responsible for the incorporation of one specific amino acid into the growing peptide chain.[1] Since 1995, when Marahiel *et al.* were able to show that it is possible to recombine NRPS through exchanging adenylation-thiolation didomains, NRPS research came into focus.[2] Since then, many groups have investigated NRPSs exhaustively and tried to reprogram them with little success.
Our research is based on NRPSs expressed by entomopathogenic bacteria of the genera *Xenorhabdus* and *Photorhabdus*.[3] These Gram-negative bacteria live in symbiosis with nematodes of the genera *Steinernema* and *Heterorhabditis*, respectively, and produce a broad range of nonribosomaly synthesized peptides. This diverse pool of biosynthetic gene clusters enabled us to investigate NRPSs *in silico* as well as *in vitro* and *in vivo*.
As a result of preliminary *in silico* studies (homology modelling and docking) of several adenylation domains we were able to alter substrate specificities of two domains *in vitro*.[4] However, our primary research goal is to understand how artificial NRPS can be designed from scratch. Recently, we have been able to make unique NRPS module combinations, as well as replace a thioesterase domain with a terminal condensation domain. This led to the biosynthesis of novel cyclic and linear peptides. These results, along with phylogenetic reconstructions have allowed us to develop easy-to-follow rules for designing artificial NRPS from scratch.
We will describe our strategy for the successful design, cloning and expression of new artificial peptides.

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BTV05

Biomass-degrading multifusion enzyme chimeras from thermophiles

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Introduction: Lignocellulose is the major component of plant cell walls and an important source of fixed carbon. Utilization of lignocellulose-derived polysaccharides is of tremendous importance for versatile applications in industry including bioenergy production. Therefore, there is a certain need of stable and functional (hemi-)cellulose-degrading enzymes that are able to withstand harsh conditions present in industrial processes [1].
Objectives: Enzymatic hydrolysis of (hemi-)cellulose requires the concerted action of multiple glycoside hydrolases often working in synergy. Nature's invention to form such modular multifunctional enzyme complexes can be mimicked by gene fusion methods [2].
Methods: The LE (*Lgu*I/*Eco*81I)-cloning strategy was developed to enable the continuous step-wise ligation of DNA-fragments into a vector system with a pair of restriction enzymes that produce identical non-palindromic overhangs. Thereby, this cloning system is not limited by restrictions sites in the MCS and allows flexible orders of multiple fusion partners [3].
Results: A set of recombinant fusion proteins consisting of two to five partners were produced. A dual affinity chromatography approach using a N-terminal HIS-tag and C-terminal STREP-tag fusions facilitated easy purification. Details on production and purification efficiency including biochemical properties of bi- and multifunctional fusion proteins composed of heat-stable endoglucanase, endoxylanase, beta-glucosidases and cellulose binding module will be presented [4].
Conclusion: Fusion of genes to produce multifunctional enzymes is an interesting tool for industrial application, because of synergistic effects and due to lower production costs. Thereby, artificial chimeric enzymes can be created that are superior over monofunctional biocatalysts.

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BTV06

Selective acetone production using acetogenic bacteria

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Introduction: Gas fermentation (H_2 + CO_2 or H_2 + CO as substrate) is a microbial process performed by autotrophic acetogenic bacteria that use the Wood-Ljungdahl pathway. Using these biocatalysts (wild types and recombinant strains) in the fermentation process, biofuels such as ethanol or butanol, as well as biocommodities such as acetate, lactate, butyrate, 2,3-butanediol, isopropanol, and acetone can be produced.Acetone for instance, is an essential industrial bulk chemical which is nowadays mainly produced from fossil resources at a global capacity of 6.7 million tons per year (2011).
Objektives: Since some products cannot be naturally produced by acetogenic bacteria, selected strains were metabolically reengineered for the production of acetone.
Material and Methods: Different vectors containing the identical synthetic acetone synthesis operon, were transformed in various autotrophic acetogenic bacteria strains. The operon used contained 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 the P*thlA* promoter (promoter of the thiolase gene)), all of them originating from solventogenic *Clostridium acetobutylicum*. Respective recombinant strains were cultivated as flask batch cultures and characterized concerning growth as well as acetone production.
Results: Acetone production using recombinant acetogenic strains was confirmed under heterotrophic as well as autotrophic growth conditions. Under autotrophic conditions with H_2 + CO_2, the recombinant strains produced up to 15 mM acetone within 700 h. Currently, the productivity is optimized in up-scaled reactor cultivations. In addition, a formation of acetone/isopropanol mixture could be verified in recombinant acetogenic bacteria that possess a primary/secondary alcohol dehydrogenase.
Conclusion: Acetogenic bacteria were successfully reengineered to autotrophically produce acetone and isopropanol, respectively. The use of cheap and abundant carbon sources offers a great potential for further investigations and reduction of greenhouse gas emissions.

BTV07
From Sunlight and CO_2 to value-added products – establishing a stable co-culture between *Synechococcus elongates* and *Pseudomonas putida*
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Genetically modified cyanobacteria are capable of producing large amounts of carbohydrates, even exceeding the areal productivity of the traditional crop-based production of sucrose [1, 2, 3]. This new approach therefore constitutes a viable and very promising alternative for the production of carbohydrates using less land and resources.
Our aim is to investigate the potential of cyanobacterial carbohydrates as a feedstock for industrial biotechnology. To this end, we established a stable mixed culture of the photosynthetically active bacterium *Synechococcus elongatus* and *Pseudomonas putida,* a model organism that can produce various valuable products.
S. elongatus cscB and *S. elongatus invA*, *glf*, *galU* produce and excrete sucrose or a mixture of glucose and fructose, respectively, upon salt shock [1, 2]. These exported carbohydrates serve as substrates for *P. putida*.
In a first step, the medium composition was adjusted to join the requirements of both organisms and analytical tools for the discrimination between them were developed. By this, we could successfully establish a stable mixed culture in shaking flasks and a labscale 1.8-L photobioreactor. We analyzed in detail the influence of various key parameters, as light, pH, salt concentration and gas transfer for the production of sugar by *S. elongatus*. Bacterial growth and integrity was followed by (fluoro-) spectrometry and flow cytometry. The results suggest that light density and salt concentration are the crucial factors for sugar production and thereby growth of *P. putida* in the mixed culture.
Taken together, the data presented here shows the potential of a bacterial mixed culture using cyanobacterial carbohydrates as a feedstock for a second production strain. *P. putida*, as a natural polyhydroxyalkanoate producer, is well suited to demonstrate this approach for the production of bulk products. Possible processes for future applications are proposed.

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BTV08
LIPOMAR - Macroalgae as a promising feedstock for the production of high-value chemicals
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Introduction: In the light of current environmental challenges, such as climate change and the depletion of fossil fuel reservoirs, a global switch to biodegradable and renewable products is highly demanded. Thus, different concepts have been developed in the last years for the utilization of plant biomass based on starch or lignocellulose. Recently, degradation of marine biomass has also become more attractive. Especially macroalgae represent a promising alternative feedstock due to their abundance and high content of carbohydrates [1]. One major advantage that comes along with the use of macroalgae instead of terrestrial biomass is the lack of lignin, leading to facilitated processing during industrial applications.
Objectives: The efficient hydrolysis of macroalgae biomass is a challenging task and requires efficient enzymes for the bioconversion of the highly complex substrate, which consists of polysaccharides like laminarin, carrageenan and alginate.
Results: Here we present results of the project LIPOMAR, "lipids and surface active molecules from marine biomass", that was established by a consortium of academic and industrial partners. Especially, the enzymatic bioconversion of beach-stranded macroalgae e.g. brown algae or seaweed and the identification and characterization of corresponding enzymes are of great interest.
Conclusion: We report on the successful identification of various macroalgae-degrading enzymes by sequence-based screening approaches of metagenomic datasets from extreme environments. Furthermore, we present biochemical characteristics of some of these genes that were heterologously expressed in *Escherichia coli*.

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BTV09
A toolbox for the study and high-yield production of [NiFe]-hydrogenases along with their respective maturation circuits in *Escherichia coli*
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Introduction: [NiFe]-hydrogenases are evolutionarily ancient enzymes catalyzing the reversible cleavage of molecular hydrogen at metal-containing centers. Activation of these sophisticated biocatalysts requires a set of specific maturases, a fact that contributes substantially to the complexity surrounding their recombinant production.
Objectives: We aimed at the development of a toolbox to study maturation circuits and, finally, to overcome common hurdles related to high-yield hydrogenase production and activation in a heterologous host.
Methods: A T7-polymerase based system for multiple gene expression in *Escherichia coli* was designed, which places each gene under control of individual promoters and terminators. Following modular conception, assembly of gene cassettes and subsequent distribution among a set of compatible plasmids was arbitrary. This allowed the combination of different maturase sets, which were functionally tested for hydrogenase activation. A lactose-based autoinduction strategy was applied and further optimized by stepwise modification of carbon source proportions, iron and nickel supplementation as well as aeration [1].
Results: The soluble [NiFe]-hydrogenase (SH) from *Cupriavidus necator* (*Cn*) was successfully produced and matured in *E. coli* by expression of 12-14 *Cn* genes. Activity of the heterologous maturation machinery peaked in late stationary growth phase and was, unlike the SH itself, substantially increased under microaerobic conditions. Activation of the enzyme was further achieved by combining SH- and maturase-containing extracts *in vitro*. Recombinant SH was purified by affinity chromatography, yielding the most active preparations of the enzyme obtained so far [1].
Conclusion: A combinatorial cloning and expression system was developed, which enables the high-yield production of multi-subunit enzymes requiring post-translational maturation for activation.

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BTV10
Bioreactor design considerations for the use of electrolytic oxohydrogen gas as a growth substrate in biotechnology
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Introduction: Molecular hydrogen is abundantly produced in the environment, and a large number of bacterial species are able to use this element as an energy source. The oxidation of hydrogen yields a large amount of energy, and some of the fastest bacterial growth rates have been measured during growth on hydrogen. An oxygen/hydrogen mixture (oxyhydrogen) can be easily and efficiently produced by electrolysis of water, and the possibility of production by direct electrolysis of mineral growth media was already noted by Schlegel and Lafferty in 1965 [1].
Objectives: This study aims to identify the technical, biological, and safety considerations in the design of an economically feasible electrolytic oxohydrogen bioreactor.
Methods: Various aerobic hydrogenotroph bacteria were assessed for their potential as production strains and tested for growth using different electrode materials and configurations, voltages, and media. Various bioreactors were designed to address the issues of carbon dioxide dosing, pH control, hydrogen and oxygen sensing, efficient excess oxygen removal, and safeguards against the risk of explosion of oxohydrogen.
Results: *Ralstonia eutropha* was identified as the most suitable production strain due to its good oxygen-tolerance and biotechnological versatility. Good growth could be achieved at hydrogen concentrations below the explosion limit, thereby very significantly reducing the risk of explosion. The calculated electricity cost required for growth compared favourably to those of organic substrates commonly used in biotechnology.
Conclusion: Electrolytic oxohydrogen bioreactors appear to be an economically feasible option for biotechnology. Advantages include the negligible transport and handling costs for electricity, the option of using electricity from sustainable energy sources, and the inherent carbon dioxide sequestration of hydrogenotrophy. Remaining challenges include carbon dioxide dosing and the general explosion risk associated with oxohydrogen.

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BTV11

Protein evolution for *in vivo* synthetic catalysis in Extremophiles

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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 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. These enzymes can also be transferred to extremophile host systems to enable the use of whole cell catalysts based on extremophile host organisms. 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. To achieve thermostabilization we used a bioinformatics approach, screening every sequence position with all 20 proteinogenic amino acids identifying single mutations which should enhance stability. The activities of all KIVD variants were subsequently characterized using a coupled assay at different temperatures ranging from 45°C to 65°C. The assay was based the consumption of NADH thus correlating the activity of the particular KIVD variants with an optical readout. In order to verify the temperature vs. activity profile of the enzyme variants in a more direct fashion, HPLC analysis was performed on the most promising candidates quantifying the production of isobutyraldehyde at defined temperatures. In summary, we were able to determine mutations that contributed markedly to stability, increasing the thermostability by several °C without compromising the enzymatic activity. The *in vivo* analysis of these engineered KIVD variants is currently underway.

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BTV12

Biotechnological production of ω-hydroxy fatty acids with the help of metabolically engineered *Yarrowia lipolytica* strains

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It is known that the destruction of β-oxidation enables ω-oxidation of fatty acids in the non-conventional yeast *Yarrowia lipolytica*. Therefore strains lacking β-oxidation can be used for the biotechnological production of long chain α,ω-dicarboxylic acids [1]. Besides the production of dicarboxylic acids, the biotechnological production of ω-hydroxy fatty acids is of special economic interest since the chemical synthesis of both is not able without the formation of unwanted byproducts. ω-Hydroxy fatty acids can be used as monomers for the production of biobased plastics and as valuable components in lubricants, adhesives, cosmetic ingredients and anticancer therapeutics [2].

To enable the biotechnological production of ω-hydroxy fatty acids with the yeast *Y. lipolytica*, their degradation during ω-oxidation has to be stopped. Therefore eight relevant (fatty) alcohol dehydrogenase genes (*FADH*, *ADH1-7*) and one alcohol oxidase gene (*FAO1*) were identified in *Y. lipolytica* by comparative sequence analysis. All relevant genes were deleted in *Y. lipolytica* H222ΔP, a strain lacking β-oxidation by the deletion of the acyl-CoA oxidase genes (*POX1-6*). Hereby the deletion of the fatty alcohol oxidase gene, which has not been described yet in *Y. lipolytica*, exhibited the highest effect.

Our results indicate that both (fatty) alcohol dehydrogenases and an alcohol oxidase are involved in ω-oxidation of long chain fatty acids whereby latter plays the major role. The *POX*, *ADH* and *FAO* deleted strain *Y. lipolytica* H222ΔPΔAΔF is able to convert *n*-alkanes to ω-hydroxy fatty acids in large quantities. The overexpression of *FAO1* can be further used to improve existing strains for the production of dicarboxylic acids.

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BTV13

Back to nature – biosynthesis of indigoid dyes

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Introduction: Indigoid dyes are used by humans since ancient times via extraction from organisms. Beyond that they maintain a value as colorant in textile and food industry by today. Research effort is directed towards the use in photovoltaics and pharmaceutical industry. The annual production of indigo dyes amounts to 80,000 tons [1]. Therefore it was necessary to switch to chemical syntheses which were accompanied by negative effects, e.g. need for toxic reactants or high temperatures. To evade such problems several attempts have been made to establish a biotechnological way using oxygenases for indigo production. But, indirubin is formed as a by-product in such processes [2,3]. Recently a novel flavoprotein monooxygenase type was found to form indigo. The so called styrene monooxygenases (SMOs; EC 1.14.14.11) catalyzes the epoxidation of styrene into (S)-styrene oxide. SMOs are composed of a epoxidase (StyA) and a reductase component (StyB). The latter delivers FADH2 to StyA on the expense of NADH [4].

Objectives: Herein we describe a novel approach for the bioproduction of (high-value) dyes.

Methods: An artificial fusion between *styA* and *styB* was constructed to obtain a self-sufficient enzyme (StyALxB) for catalysis. Further, we created and screened a set of different SMOs for activity and substrate specificity. Different growth media were used according to the desired dye production. In addition we checked methods for a viable extraction of the dyes from cell slurry.

Results: All SMOs assayed were able to catalyze indigo formation whereas the fusion proteins seem to be more efficient compared to single epoxidases. Interestingly, we observed almost no by-products. We were able to synthesize substituted indigo dyes by the use of different substrates and growth media.

Conclusion: A highly specific catalyst for the biosynthesis of indigoid dyes and a purification strategy were generated.

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BTV14

Laccase-mediated transformation of various bisphenols for application in a bioreactor for wastewater treatment

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Introduction: Xenoestrogens such as bisphenols are insufficiently eliminated by wastewater treatment plants [1]. Thus, these substances persist and become problematic for aquatic environment. In addition, their low concentrations may cause toxic effects e.g. an inhibition of gonad growth in fish [2]. The phenolic compounds are potential substrates for the enzyme laccase [E.C. 1.10.3.2.], which oxidizes hydroxylated aromatic compounds to reactive radicals that can undergo either coupling reactions e.g. to polymers or cleavage reactions [3].

Objectives: The aim was to reduce the xenoestrogen concentration using a fungal laccase and to identify possible products. The results of these experiments could be used for the development of a bioreactor for use in wastewater treatment plants for the degradation of recalcitrant compounds.

Methods: The laccase C from the ligninolytic fungus *Trametes* spec. (TsL) was used as a catalyst for potential degradation of bisphenols A, B, C, E, F and Z. The removal of substrates as well as product formation was determined by HPLC analyses. The structural characterization of products was performed by GC/MS and LC/MS.

Results: The TsL was able to degrade all tested bisphenols. Bisphenol F (bis-(4-hydroxyphenyl)-methane) was used as a model substance for product elucidation. The elimination rate was dependent on the bisphenol

F concentration used and on the amount of enzyme. Low molecular weight products resulting from cleavage reactions of the C-C bond between the two aromatic rings of the bisphenol F were identified, and the formation of precipitates was also observed.
Conclusion: The successful degradation of bisphenols verifies the great potential of the laccase for wastewater treatment. In the near future, we will determine the ideal conditions for a rapid elimination of endocrine disruptors using enzyme-based bioreactors.

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BTV15
Using an enzyme secreting, genetically modified *E. coli* strain to supply Bilirubin Oxidase to a biofuel cell cathode
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Enzymes are promising electrode catalysts for biofuel cell cathodes, due to their high catalytic activity and low overpotential regarding the reduction of oxygen. However, since they show only limited stability over time, new ways to extend the lifetime of enzymatic cathodes need to be found.
To overcome this limitation we aim to use the supernatant of enzyme-secreting microorganisms to continuously supply fresh redox-active enzymes to the cathode. Besides microorganisms that secrete these enzymes naturally, such as the white-rot fungus *Trametes versicolor* [1], genetically modified organisms are under consideration [2].
In the present work, an *Escherichia coli* strain was genetically modified towards secretion of the enzyme bilirubin oxidase (BOD), which can catalyze the cathodic reduction of oxygen. Thereto, the gene for BOD, a spore coat protein of *Bacillus pumilus*, was linked to a part of the gene encoding for pullulanase (*pulA*) secretion. Secretion of the fusion protein (BOD_pulA) was managed using the type-II secretion system from *Klebsiella oxytoca* as described elsewhere [3].
During cultivation in LB medium, bacterial growth and enzyme activity towards the redox indicator ABTS in the supernatant were monitored. After 30 h, enzyme activity reached a maximum. To relate the measured activity to the fusion protein, SDS-PAGE followed by MS-analysis was conducted. Electrochemical experiments were performed by supplying the supernatant of the modified *E. coli* culture (containing the enzyme BOD_pulA) to a carbon nanotube cathode. The current densities at 0.2 V vs. NHE and pH 5 yielded in 62 µA/cm^2 for the purified enzyme (1 U/ml towards ABTS) and 29 ± 12 µA/cm^2 for the supernatant containing the enzyme (0.015 U/ml).
In summary, we have achieved genetical modification of an *E. coli* strain towards secretion of the fusion protein BOD_pulA. The enzyme was detectable in the supernatant and showed electrocatalytic activity for oxygen reduction at a biofuel cell cathode. To improve the obtained current densities, the influence of electrode material on electrocatalytic activity will be investigated in future experiments.

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BTV16
Dynamics of bacterial community structure during non-destructive biocatalytic desulfurization of various organosulfur compounds
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Introduction: A commercially viable diesel biodesulfurization technology has not been developed yet. This is due to very low catalytic activity of the relevant microbes, among other factors. The majority of the biodesulfurization research has adopted pure microbial cultures [1].
Objective: Investigation of the biodesulfurization potential of a microbial consortium.
Methods: A mixed culture was retrieved from an enrichment using soil polluted with petroleum hydrocarbons as a source of bacteria. The enrichment culture contained dibenzothiophene (DBT) as a sole sulfur source. Substrate utilization was checked with HPLC. PCR-DGGE was applied to study the change in community structure.
Results: A mixed culture, AK6, utilized several organosulfur compounds that are commonly found in diesel and crude oil as a sole sulfur source in the presence of glucose as a carbon source. The tested organosulfur compounds included DBT, 4-methyldibenzothiophene (4-MDBT), benzothiophene (BT), 4,6 dimethyldibenzothiophene (4,6-DM-DBT). AK6 could not utilize these organosulfur compounds or dibenzylsulfide (DBS) as a carbon and sulfur source. Biodesulfurization of DBT proceeded via the non-destructive 4S pathway and PCR confirmed the presence of the 4S genes. In resting cell assays, AK6 transformed DBT to 2-hydroxybiphenyl with a specific activity of 8 µmol g dry cell weight^{-1} L^{-1}. This activity was 2-fold higher (4 µmol g dry cell weight^{-1} L^{-1}) than that of the reference strain *Rhodococcus erythropolis* IGTS8. The structure of the AK6 consortium changed according to the provided sulfur source. The major DGGE bands represented members of the genera *Sphingobacterium*, *Klebsiella*, *Pseudomonas*, *Stenotrophomonas*, *Cellulosimicrobium, Mycobacterium*, and *Rhodococcus*. The diversity index and OTUs richness were the highest in the 4,6-DM-DBT culture, whereas the DBT only (no glucose) cultures revealed the lowest diversity index and OTUs richness.
Conclusion: Mixed cultures can be more efficient than axenic cultures for the development of a biodesulfurization technology.

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CCV01
Pre- and postdivisional functions of *Listeria monocytogenes* DivIVA
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Introduction: DivIVA is a highly conserved cell division protein found in many Gram-positives. It binds to curved membranes at the septa and the cell poles and recruits other proteins to these sites. *Bacillus subtilis* DivIVA controls division site selection via the MinCDJ system. In contrast, DivIVA from the closely related species *Listeria monocytogenes* affects autolysin secretion via the SecA2 secretion route, thus explaining the massive cell chaining of a listerial Δ*divIVA* strain (1). Interestingly, no classical *min* phenotype, *i. e.* filamentation and minicell production, was observed with a listerial Δ*divIVA* mutant.
Objectives: The aim of this study was to analyze DivIVA-dependency of the *L. monocytogenes* division site selection system.
Methods: Two-hybrid and pull-down assays were used to analyze protein-protein interactions among the listerial MinCDJ proteins. Mutants lacking *minC*, *minD* and *minJ* were constructed and their phenotypes were compared with those of Δ*divIVA* and Δ*secA2* strains. Localisation patterns of Min-GFP fusions were documented in different strain backgrounds and linkage of DivIVA with the Min system was tested in epistasis experiments.
Results: DivIVA was shown to be required for septal recruitment of MinCD, but not for septal localization of MinJ. DivIVA interacted directly with MinJ and MinD, suggesting that the linear model of interactions described for the *B. subtilis* system (DivIVA→MinJ→MinD→MinC) cannot be applied to *L. monocytogenes*. Analysis of cell size distributions showed that deletion of *minCD* but not of *minJ* resulted in a division phenotype. A hitherto unknown division defect for the Δ*divIVA* mutant was uncovered by comparing cell lengths of Δ*divIVA* and Δ*secA2* strains.
Conclusion: In addition to its post-divisional function in autolysin secretion, we describe a second function of *L. monocytogenes* DivIVA (2). This function is to control septal recruitment of MinCD, an event prior to septum formation. Differences in the architecture of the Min system between *B. subtilis* and *L. monocytogenes* show that this system is far away from being understood and hint towards a more specific role of MinJ.

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CCV02

The PomXYZ proteins self-organize on the chromosome to localize at midcell by active, directed motion and position cell division

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Accurate positioning of the division site is essential for generating appropriately-sized daughter cells with a correct chromosome number. *Escherichia coli*, *Bacillus subtilis* and *Caulobacter crescentus* use the Min- or the MipZ-system to position the (Fts)Z-ring and, therefore, the division site. *Myxococcus xanthus* lacks both of these systems. Instead we recently reported that formation and positioning of the Z-ring and cell division at midcell depends on the ParA-like ATPase PomZ[1].
Here, we report that PomX and PomY, in addition to PomZ, are important for formation and positioning of the Z-ring. PomXY colocalize with PomZ in a dynamically localized cluster and depend on each other for correct localization and formation of this cluster. Early during the cell cycle the PomXYZ cluster localize on the nucleoid and away from midcell. Subsequently, it relocates in a directed manner to midcell and localizes here before the end of replication as well as before and in the absence of FtsZ. PomXYZ interact in all three pair-wise combinations *in vitro* and *in vivo*. *In vivo* PomZ recruits the PomXY complex to the nucleoid and stimulates the movement of the PomXYZ cluster to midcell in an ATP hydrolysis-dependent manner. *In vitro* ATPase activity of PomZ is stimulated by PomXY in the presence of DNA. Additionally, PomY and PomZ separately inhibit FtsZ filament formation *in vitro* and PomX stimulates FtsZ to form helical filaments. These data demonstrate that *M. xanthus* uses a novel system to precisely mark midcell for Z-ring formation and cell division. We propose a model in which Z-ring formation is inhibited throughout cells by PomY and PomZ. At midcell, PomX interacts with PomYZ and FtsZ to establish a zone that is permissive to Z-ring formation either by inhibition of PomYZ function and/or by direct stimulation of Z-ring formation.

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CCV03

The role of dynamin-like proteins in the developmental control of cell division in *Streptomyces*

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The *Streptomyces* developmental program involves mycelial growth and differentiation into dormant uni-genomic spores. During the reproductive phase, a massive and synchronous cell division event leads to the deposition of 50 or more regularly spaced FtsZ-rings along the sporogenic hyphae within a short time. Among the regulatory genes identified to be central to developmentally-controlled cell division is the transcription factor *whiH*. Exploiting the ability of *S. venezuelae* to sporulate in liquid culture, we have characterized the WhiH regulon. Through this route, a WhiH target promoter was identified that controls an operon of two genes encoding dynamin-like proteins (DynAB).
Dynamin-like proteins are large GTPases that play critical roles in diverse cellular processes in eukaryotes that require membrane fusion or fission. Although bacterial dynamin-like proteins have been partially characterized, their precise function in bacteria has remained poorly understood. Interestingly, in *Streptomyces*, the disruption of DynAB leads to the creation of long compartments containing more than one copy of the chromosome, indicating that the dynamins are required for normal sporulation septation. Using time-lapse fluorescence imaging, we found that DynAB co-localize with cytokinetic FtsZ-rings and are important for the stabilization of these rings. We are currently studying the molecular mechanism underlying the observed effect of DynAB on the regular assembly of the cell division machinery and the successful completion of normal sporulation septation.

CCV04

Off the wall – from filamentous growth to primordial cells and back again

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Streptomycetes are filamentous bacteria that grow by apical tip extension. This process is orchestrated by the tropomyosin-like protein DivIVA, which is present at hyphal tips. DivIVA interacts with various proteins, among which the cellulose synthase-like protein CslA. This protein synthesizes a β-(1,4)-glycan, which is thought to protect growing apices that are continuously being remodeled. To obtain further insight in the role of DivIVA and CslA in polar growth and morphogenesis, we have recently generated so-called *Streptomyces* L-forms that can grow without peptidoglycan. As a consequence, such cells are round and lack any obvious form of polarity. L-form cells have recently been suggested to resemble primordial cell, based on the observation that their growth and proliferation do not require the canonical cytoskeletal or cell division proteins. Instead, their proliferation can merely be explained by physical processes. However, our work on *Streptomyces* L-forms suggests that these cells require glycans, such as those formed by CslA, for their growth. Such glycans might have served for protection of early life forms, before the modern cell wall was invented. We have recently isolated an L-form mutant strain, which readily switches back and forth between mycelial and L-form growth. This mutant with the capability to re-synthesize peptidoglycan is crucial to understand which genes play an essential role in proliferation of L-forms, but also to unravel the mechanism underlying filamentous growth.

CCV05

The polar peptidoglycan synthesis complex in *Caulobacter crescentus*

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Introduction: The stalk is the morphological hallmark of the sessile and reproductively active state of *Caulobacter crescentus* cells. Surprisingly, under phosphate starvation *Caulobacter* cells elongate extensively their stalk and, to lesser extent, their cell body, as they gradually enter a dormant state.
Objectives: In this study, we attempt to identify the mechanisms that *Caulobacter* employs in order to form and elongate the stalk in the presence and the absence of phosphate.
Methods: A systematic analysis with genetic approaches and localization studies of all cytoskeletal and peptidoglycan remodeling elements of *Caulobacter* revealed that certain components of both the septal and elongation-specific peptidoglycan biosynthetic machinery localize to the stalked pole and participate in stalk elongation. A statistical analysis of the corresponding mutants identified certain components that appeared to specifically affect stalk elongation but not cell body growth. Finally, measurements of peptidoglycan incorporation in phosphate-starved wild-type and mutant cells showed that the dynamics of peptidoglycan synthesis between the cell body and the stalk are different.
Results: Our data point to the presence of an additional peptidoglycan biosynthetic complex in *Caulobacter* that forms at the stalked pole and is responsible for the formation and elongation of the stalk. Interestingly, the composition and dynamics of this polar complex are different from those of the septal and elongation-specific complexes, which typically affect growth of the cell body.
Conclusions: *Caulobacter*, in contrast to other rod shaped bacteria, forms three different peptidoglycan biosynthetic complexes, namely a septal, elongation-specific, and polar complex. Under phosphate starvation, the polar complex becomes gradually the main source of peptidoglycan biosynthesis, and this transition seems to be governed by the intracellular phosphate concentration *per se*.

CCV06
Polar localization of chemotactic signaling arrays in polarly flagellated bacteria
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Bacterial chemotaxis proteins are organized into ordered arrays. In peritrichous organisms, such as *Escherichia coli*, stochastic assembly processes are thought to account for the placement of chemo- taxis arrays, which are nonuniformly distributed. We found that chemotactic signaling arrays in polarly flagellated vibrios are uniformly polar and that array localization is dependent on the ParA-like ATPase ParC and partner protein ParP. ParP interacts with ParC and is integral to array localization. ParC's principal contribution to chemotaxis appears to be via positioning of ParP. Once recruited to the pole by ParC, ParP sequesters arrays at this site by capturing and preventing the dissociation of chemotactic signaling proteins. Notably, ParP also stabilizes chemotactic protein complexes in the absence of ParC, indicating that some of its activity is independent of this interaction partner. ParP interacts with CheA via CheA's localization and inheritance domain, a region found only in polarly flagellated organisms that encode ParP, ParC, and CheA. In addition we have found that ParP interacts with the signaling domain of multiple methyl-accepting-chemotaxis proteins (MCPs). Disruption of this interaction results in mislocalization of signaling arrays. As a consequence signaling arrays are no longer properly positioned at the cell pole and not stably inherited by daughter cells at cell division. The interaction of ParP with MCPs is mediated via ParP's C-terminal CheW-like domain - a region distinct from its interaction domains for ParC and CheA. This important interaction network, centered around ParP, enables the polar localization and sequestration of chemotaxis arrays in polarly flagellated organisms.

CCV07
Akinetes – resistant cells of filamentous cyanobacteria
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Introduction: Akinetes are spore-like non-motile cells that differentiate from vegetative cells of filamentous cyanobacteria from members of the *Nostocales*. They enable survival under changing environmental conditions like cold and nutrient deficiency. Various environmental factors trigger the differentiation of akinetes including light intensity, temperature and starvation. Akinetes are larger, have a thick envelope and contain huge amounts of reserve materials.
Objectives: We want to describe the metabolic and morphological changes that take place in the maturation process of akinetes and germination in 2 different strains, *N. punctiforme* and *A. variabilis*, in more detail.
Methods: Morphological changes were investigated by light microscopy (LM) and transmission electron microscopy (TEM) of ultra thin sections. The cyanophycin concentration was measured *in vitro* and different types of granules were specifically stained *in vivo*. Fluorescent dyes were used to detect DNA and lipids by epi-fluorescent microscopy. Photosynthesis was measured by PAM fluorometry, O_2 production and respiration by Clark electrode.
Results: The best conditions to induce akinetes varied clearly between both strains. High amounts of large cyanophycin granules accumulated in akinetes induced by phosphorous starvation. By dim light induction, polyphosphate granules were identified in immature akinetes. Photosynthesis and respiration decreased, hence the thylakoid membranes rearranged, but DNA accumulated. Akinetes from *N. punctiforme* showed great variety, ranging from unicellular spores to filaments, with different granules, membrane organization and akinete envelope. In the case of *A. variabilis* the akinetes were always unicellular with a thick envelope composed of different layers, which contained a lipid layer. During the fast germination process small filaments of vegetative cells emerged from the akinete envelope.
Conclusion: The related strains show clear differences in akinete formation. However, they share in common the nature of reserve granules and general metabolic and physiological changes during akinete differentiation and germination. Whilst akinete formation takes many days, the germination process occurs in few hours, way faster than the normal generation time of the strains.

CCV08
Overproduction of Flotillin Influences Cell Differentiation and Shape in *Bacillus subtilis*
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Similar to eukaryotic cells, bacteria have a sophisticated membrane organization and focus membrane-related signaling processes in functional membrane microdomains that are structurally and functionally similar to eukaryotic lipid rafts (1-3). The flotillin proteins have been traditionally used as a marker for lipid rafts, since they localize exclusively within these membrane microdomains. Flotillins are suggested to work as a chaperons that recruit proteins to lipid rafts and facilitate the interaction of lipid raft proteins (4, 5). Overproduction of flotillin in eukaryotic cells has often been observed in combination with severe diseases with impaired signaling, but a molecular link between these phenomena has not been demonstrated. We use the tractable model organism *Bacillus subtilis* to draw a link between physiological alterations that occur by an artificial overproduction of flotillin proteins. Interestingly, we discovered that cell differentiation and cell division programs are severely affected by an overexpression of flotillin proteins, which is in part due to an unusual stabilization of the raft-associated protease FtsH. Our work shows that *B. subtilis* can be used as a working model to address intricate questions related to the functionality of lipid rafts (6).

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CCV09
Upgrade of flagellar motility in *Shewanella oneidensis* MR-1
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Introduction: In contrast do many well characterized bacteria that harbor a single flagellar system powered by one complementary stator set, *Shewanella oneidensis* MR-1 (MR-1) possesses the proton-dependent MotAB as well as the sodium ion-driven PomAB complex along with a polar flagellar system. The number of each stator found in the motor is dependent on the environmental sodium-ion concentration with a higher ratio of MotAB:PomAB under low salt concentrations. Thus, MR-1 may form a hybrid motor that is simultaneously using H^+ and Na^+ gradients. Our group has previously shown that both stators are solely sufficient to power motility in liquid environments. However, cells only encoding the MotAB stator do not spread on soft agar plates and quickly cease to swim in planktonic cultures presumably upon dropping oxygen concentrations.
Objective: This study explores the evolution and functional dynamics in multiprotein complexes.
Methods: The characterization of the stators occurred via a combined approach of FRAP and fluorescence microscopy in concert with physiological characterizations.
Results: Several spontaneous small mutations in the so-called 'plug-domain' of MotB were found to bypass the requirement for high oxygen concentrations although the overall speed of the cells decreases. This 'plug-domain' is a small amphipathic alpha-helix close to the transmembrane domain that is thought to prevent ion-leakage in inactive stators. Mutations that either add charge to the hydrophobic face of the plug-domain or break the helix also caused the up-motile phenotype. FRAP analysis demonstrated that, rather unexpectedly, the mutated stator is present in the motor at lower numbers and has a significantly higher turnover than wild-type MotAB. Together, the findings confirmed that the amphipathic characteristics of the helix is required for wild-type interactions of the stator with the motor.
Conclusion: Apparently, MR-1 has evolved a stator that exhibits a more stable motor - stator interaction but is less efficient in promoting a robust motility at low oxygen concentrations. Thus, the suggested hybrid motor of MR-1 may not only respond to changing Na^+ -concentrations but also adjust motility in dependence to oxygen availability.

CCV10

Photoautotrophic PHB metabolism – Identification and characterization of a cyanobacterial Phasin

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Introduction: Cyanobacteria are photoautotrophic microorganisms which are able to fix atmospheric carbon dioxide utilizing Rubisco thus providing carbon backbones for primary metabolism. Carbon can also be stored intracellularly as glycogen and in some strains like ***Synechocystis sp.*** PCC 6803, polyhydroxybutyrate (PHB) accumulates when major nutrients like phosphorus or nitrogen are absent.
Objectives: So far only three enzymes, which participate in PHB metabolism, have been identified in ***Synechocystis,*** namely PhaA, PhaB and the heterodimeric PHB synthase PhaEC. Information on other proteins involved in PHB metabolism is missing.
Methods: Fluorescence microscopy and biochemical approaches were performed to characterize the putative phasin.
Results: In this work we describe the cyanobacterial phasin PhaP encoded by ***ssl2501***. Translational fusion of Ssl2501 with eGfp showed a clear co-localization to PHB granules. A deletion of ***ssl2501*** reduced the number of PHB granules per cell and the mean PHB granule size was increased as expected for a typical phasin. Deletion of ***ssl2501*** had no effect on the amount of PHB accumulated within cells; however, the biosynthetic activity of PHB synthase was negatively affected.
Conclusion: The results support the idea that Ssl2501 encodes a cyanobacterial phasin PhaP, which regulates the surface to volume ratio of PHB granules and can modulate PHB synthase activity.

CCV11

Peptidoglycan present in Planctomycetes after all? Insights from an anammox bacterium

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Planctomycetes are among the few bacteria proposed to lack peptidoglycan, a cell wall structure crucial for cell shape and integrity. Therefore, the planctomycetal cell envelope was considered to be neither Gram-positive nor Gram-negative. In addition to their exceptional cell envelope, their cell plan was found to have a unique degree of compartmentalization. Anaerobic ammonium-oxidizing (anammox) Planctomycetes are key players in the global nitrogen cycle that release fixed nitrogen back to the atmosphere as N_2. We set out to investigate the proposed lack of peptidoglycan in an anammox Planctomycete in order to better understand the cell envelope of this intriguing bacterial phylum. To this end, we used 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. Our results suggest that Planctomycetes could best be understood as Gram-negative bacteria.

CCV12

Antituberculosis drugs affecting arabinogalactan synthesis selectively block elongation growth

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Important pathogens like *Mycobacterium tuberculosis*, *M. leprae* or *Corynebycterium diphtheriae* belong to the CMN-branch (*Corynebacterineae*) of Actinobacteria. CMN-group bacteria are characterized by apical growth and a complex cell envelope consisting of covalently bound layers of peptidoglycan, arabinogalactan, and a mycomembrane. The mycomembrane is composed of covalently bound mycolic acids and free trehalose mycolates and is additionally surrounded by a mycolate capsule.
This cell envelope is the target of some first-line antibiotics for tuberculosis treatment. They include ethambutol (EMB) and benzothiazinone (BTZ), which inhibit the synthesis of arabinogalactan at different steps and thus alter the cell surface.
Many cells survive that treatment with antituberculosis drugs. Therefore we are interested in the molecular mechanisms that underlie cell survival on a single cell level focused on cell wall synthesis and cell division.
We use the nonpathogenic *C. glutamicum* and *M. phlei* as model organisms for apical growth, processes involved in cell division and asymmetry of growth velocity of the daughter cells.
Our methods comprise bio-orthogonal chemistry for labeling nascent peptidoglycan, fluorescence and electron microscopy, microfluidics and statistical methods for evaluation of large data sets.
Cells treated with EMB and BTZ exhibit an altered cell shape resembling stationary phase cells where peptidoglycan synthesis is greatly enhanced at the septum. This switch from apical to septal peptidoglycan synthesis leads to a complete arrest of apical growth. BTZ additionally affects cell division which in some cases leads to cell branching.
In *C. glutamicum,* in the case of EMB that switch is accompanied by a loss of the mycomembrane at the sites of new peptidoglycan synthesis. Whereas BTZ treated cells are still covered completely by the mycomembrane, its integrity is severely weakened.
Antibiotic treatment aiming at the arabinogalactan layer in CMN-group bacteria leads to a remarkable shift from apical to septal cell wall growth, similar to the situation in stationary growth phase. Our results explain how the bacteria survive drug treatment and we speculate that this effect can be exploited to render bacteria more susceptible to drugs affecting peptidoglycan synthesis.

CCV13

Molecular and proteomic analyses highlight the importance of the Cpx envelope stress system for acid stress and cell wall stability of *Escherichia coli*

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The integrity of the cell wall is essential for bacteria since it has to fulfill plenty of important tasks such as protecting the cell from adverse environmental conditions or phages and harbouring several transporters that are essential for cell's survival. The Cpx envelope stress system is well known to respond to stresses that impair the integrity of the cell envelope using a phosphorylation cascade for the transduction of signals from the outside to the inside of the cell [1]. It is established that the response regulator CpxR modulates the expression of folding factors and proteases that help to stabilize the integrity of the cell envelope [2]. However, the impact of the Cpx-system on the proteomic composition of *Escherichia coli* is unknown.
We performed shotgun analysis with *E. coli* wild type cells grown under Cpx-inducing or non-stress conditions. To verify Cpx-dependent targets we compared these data with the same experimental set up for a *cpxRA* deletion strain. We observed decreased levels of the acid stress response proteins HdeA, HdeB, HdeD and CadA after induction of the Cpx-system in *E. coli* wild type cells. We verified this proteomic data by analyzing the survival rate after extreme pH-shock (pH 2). The *cpxRA* deletion strain has a substantial increased survival rate under extreme pH stress indicating that the Cpx stress response is important for the survival under alkaline stress. Furthermore, our results clearly demonstrate the involvement of the active Cpx-system in cell wall stability. Activation of the Cpx-system leads to increased amounts of peptidoglycan-related proteins. In particular, we observed a 30fold increased protein level of YcfS, which is essential for cross-linking Braun's lipoprotein in peptidoglycan, after induction of the Cpx-system. Together, our results highlight the importance of the Cpx system for the integrity of the cell wall response.

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CCV14

Exploring cell wall recycling efficiency and autolysin function by specific radioactive labeling of peptidoglycan sugars in *Escherichia coli* and *Pseudomonas putida*

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Peptidoglycan surrounds almost every bacterial cell and is essential for viability and shape. It consists of glycan strands composed of the alternating sugars *N*-acetylglucosamine (GlcNAc) and *N*-acetylmuramic acid (MurNAc) and peptides. During turnover of the bacterial cell wall various peptidoglycan fragments are generated by the action of muramidases which can be of the lysozyme-type, generating MurNAc-containing fragments, or of the lytic transglycosylase-type, generating the 1,6-anhydro form of MurNAc. Using LC-MS and radioactive assay

methods we are able to identify the products and to search for differences in the accumulation of cell wall recycling products in bacterial strains or mutants of the same species. With this assay we recently identified a new peptidoglycan rescue pathway in pseudomonades, different to that of *E. coli*(1). Pseudomonads for example lack the main enzyme of the *E. coli* cell wall recycling pathway MurNAc-6-phosphate etherase MurQ, but possess recycling enzymes that bypass the cell wall de novo synthesis. Currently, we are investigating the efficiency of these two cell wall recycling pathways. To quantify the amount of recycling products and to compare the efficiency of the recycling, we applied a radioactive assay that allows the detection of cell wall sugars and peptidoglycan fragments in sub fmolar amounts.

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CCV15

A new route for the transport of β-barrel proteins through the periplasm in *Shewanella oneidensis* MR-1

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The facultative anaerobic bacterium *Shewanella oneidensis* can reduce insoluble electron acceptors like Fe(III)-oxides. The reduction of these insoluble metal oxides demands the activity of protein complexes that transfer respiratory electrons across the outer membrane to the surface of the cells. The MtrABC complex of *S. oneidensis* consists of the periplasmic *c*-type cytochrome MtrA, the β-barrel protein MtrB and the cell surface localized decaheme *c*-type cytochrome MtrC which catalyzed the terminal electron transfer reaction. Usually, β-barrel proteins are transported through the periplasm by aid of the two chaperons SurA and Skp. Thereafter, they are integrated in the outer membrane via the activity of the BAM-complex. Misfolded β-barrel proteins are degraded by the protease DegP (1). In previous work we could show that either instead or besides SurA or Skp the decaheme cytochrome MtrA is necessery to prevent DegP catalyzed degradation of MtrB (2). Here, we will present data that corroborates the hypothesis that MtrA has (besides its function in electron transfer) also a chaperone-like function for MtrB.
Western blot analyses revealed that MtrB has a different stability in a Δ*degP* mutant in the presence or absence of MtrA. Experiments with *skp* or *surA* deletion mutants showed that the folding of MtrB apparently does not depend. Further evidence for the hypothesis of a chaperon function of MtrA could also be gained by in vitro MtrB expression experiments. In order to conduct these assays we purified the BAM-complex and integrated it into liposomes. We could analyze the integration of MtrB into these liposomes after the addition of purified MtrA or SurA. The results of these assays also suggest that the β-barrel protein MtrB is integrated and folded by its own chaperone MtrA.

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CCV16

Energy conservation in members of the genus *Ignicoccus* and in *Nanoarchaeum equitans*

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Introduction: The crenarchaeal genus *Ignicoccus* harbours chemolithoautotrophs producing H_2S from elemental sulfur and H_2. All species exhibit a unique cell architecture for Archaea: their cell envelope consists of an inner membrane (IM) and an outer cellular membrane (OMC) which enclose a huge inter-membrane compartment (IMC) [1]. Surprisingly, in *I. hospitalis* the OMC contains the H_2:sulfur oxidoreductase and the ATP synthase. Thus, *I. hospitalis* is the first organism with an energized outer membrane and ATP synthesis within the IMC. Therefore, energy conservation is separated from information processing and protein biosynthesis in the cytoplasm [2]. In addition, *I. hospitalis* and *Nanoarchaeum equitans* (phylum Nanoarchaeota) form a unique archaeal association, in which *I. hospitalis* provides essential nutrients, lipids, and amino acids for the associated *N. equitans* [3].
Objectives: We investigated the composition and localization of the ATP synthase complex in all *Ignicoccus* species and in *N. equitans.*
Methods: Purification and characterization of the ATP synthases by solubilization of the membrane proteins followed by anion exchange columns; production of antibodies and immuno-localization by electron microscopy.
Results/Discussion: The purified ATP synthase complex from *I. hospitalis* exhibits all subunits, annotated in its genpome genome (A, B, C, D, E, F, I [a], and K [c]) and postulated for an archaeal A_0A_1 type enzyme. However, the enzyme complex falls apart very easily into the A_1 (440 kDa) and A_0 subcomplex (660 kDa). The ATP synthases of the other *Ignicoccus* species exhibit a quite similar composition, although their apparent molecular weights in native gels differ significantly from the *I. hospitalis* enzyme. In *N. equitans* all five subunits of the ATP synthase annotated in its genome were detected in proteome analyses and by biochemical purification. However, this composition is most probably not enough for ATP synthesis indicating that *N. equitans* might be an "energy predator" for its host.

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CVV01

The immune defence system of Haloarchaea

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The CRISPR-Cas system is a prokaryotic defense mechanism that protects cells against foreign invaders. Although approximately 90% of all sequenced Archaea encode CRISPR-Cas systems, only few species have been investigated to elucidate the precise mechanisms for the defence of viruses or plasmids (1). We are investigating the CRISPR-Cas system in *Haloferax volcanii*, that encodes a type I-B CRISPR-Cas system with eight Cas proteins and three CRISPR loci (2,3). To determine the requirements for a successful interference reaction we established a plasmid based invader system in *Hfx. volcanii* (4). We could determine the PAM sequences for *Haloferax,* that is now the second archaeon for which the PAM sequences could be identified *in vivo*. We could show that the system recognises six different PAM sequences in the defence reaction. Furthermore, we demonstrated that the interaction of the crRNA with the invader DNA requires a 10-nucleotide-long seed sequence (5).
To investigate the essential requirements of the crRNA for an effective interference we developed Cas6 independent expression of crRNAs *in vivo*. Using this system we could show that Cas6 is not required for the interference reaction and that the independently expressed crRNA is incorporated into Cascade without the help of Cas6. Different variants of the crRNA were generated using this system and analysed for their efficiency in the interference reaction. We also want to use this kind of crRNA to develop a gene knock down system (CRISPRi) for *Haloferax*.

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CVV02

Targeted gene -silencing through CRISPR-mediated RNA interference in the hyperthermophilic archaeon *Sulfolobus solfataricus*

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Small RNA molecules can silence gene expression *in vivo* in eukaryotes through the RNA interference (RNAi) system by targeting RNA. The recently discovered adaptive immune response that is widespread in bacteria and archaea is very distinct from this system but also acts through small RNAs. These small crRNAs of the clustered regularly interspaced short palindromic repeat (CRISPR) loci have been demonstrated to cause cleavage of complementary invading DNA in conjunction with associated

protein complexes in both Bacteria and Archaea. In addition, *in vitro* RNA cleavage was also shown for the type-III CRISPR complexes in hyperthermophilic archaea and bacteria, but their activity *in vivo* had remained obscure. Here we show CRISPR-mediated cleavage of mRNA of an invading virus in the hyperthermophilic archaeon *Sulfolobus solfataricus*, which produced complementary crRNA. Efficiency of mRNA cleavage was quantified by qPCR and was verified by Northern analyses and RACE. Using the same strategies, an artificial mini-CRISPR locus with a spacer targeting a chromosomal gene was constructed to knock down mRNA and protein production from a selected target (reporter-) gene in the organism. Even with a single complementary spacer a 50% reduction of the targeted mRNA and of corresponding protein activity was achieved when this mini-CRISPR locus was introduced into the organism. More recently we have explored possibilities to increase the silencing effect even higher, in order to be able to use the system for efficient gene-silencing in *Sulfolobus*.
Our results demonstrate that RNA can be cleaved in a process formally reminiscient of (eukaryotic) RNAi *in vivo* in and that the CRISPR system can be exploited to knock down genes of interest in archaea, and probably also in bacteria.

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CVV03
Localization of Cas9 protein in heterologous bacteria
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Introduction: Clustered regularly interspaced short palindromic repeats (CRISPR) together with CRISPR associated (*cas*) genes form an adaptive prokaryotic immune system which provides acquired resistance against viruses and plasmids. CRISPR consists of arrays of short conserved repeat sequences interspaced by unique DNA sequences called spacers. The CRISPR-Cas system functions by acquiring short pieces of foreign DNA as new spacers and subsequently uses them as templates to generate specific small RNA molecules (crRNA) which combined with Cas proteins into effector complexes that trigger degradation of foreign nucleic acid. In type II CRISPR-Cas systems, the silencing complex consists of a single Cas9 protein, which binds to crRNA/tracrRNA duplex to mediate sequence-specific cleavage of invasive double-stranded DNA. Cas9 functions as an RNA-guided DNA endonuclease, where cleavage specificity is directed by the crRNA, while cleavage is mediated by two active sites within Cas9 protein.
Objectives: To determine how Cas9-RNA complex finds specific cleavage sites of foreign DNA molecules we investigate the subcellular localization of the Cas9 protein from *Streptococcus thermophilus* in heterologous *Bacillus subtilis* host before and after bacteriophage infection.
Methods: We engineered a heterologous host by cloning a GFP fusion to Cas9 and a spacer from bacteriophage SPP1 into the chromosome of the gram-positive *B. subtilis*, which does not harbour CRISPR-Cas systems.
Using epifluorescence microscopy we analyzed the localization of Cas9-GFP in live *B. subtilis* cells.
Results: We found that the heterologous CRISPR-Cas system is functionally active in *B. subtilis* and provides resistance against bacteriophage SPP1 infection. Microscopy experiments showed that Cas9 displays largely diffuse localization in the cytoplasm of *B. subtilis* and possibly accumulates at the nucleoid. No differences in Cas9 localization were observed in SPP1-infected and uninfected *B. subtilis* cells carrying an active heterologous CRISPR3 system against SPP1. We also found that SPP1 phage DNA replication is inhibited in *B. subtilis* carrying CRISPR-Cas system.
Conclusion: In the heterologous *B. subtilis* host Cas9 protein provides fast degradation of foreign DNA and is distributed throughout the cytoplasm.

CVV04
Self-assembly of a pyramidal archaeal virion egress structure
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Introduction: Viruses have developed several strategies to escape from the host cells in which they replicate. The archaeal virus SIRV2 (*Sulfolobus islandicus* Rod-shaped Virus 2) makes use of pyramidal egress structures with sevenfold rotational symmetry (1). These Virus-associated pyramids (VAPs) assemble in the host cell membrane from the virus-encoded protein PVAP and open at the end of the infection cycle (2).
Objectives: We aimed to characterize the supramolecular organization and assembly of this unusual pyramidal structure.
Methods: We applied a combination of genetic, biochemical, and electron microscopic techniques to study formation of VAPs induced by infection of *S.islandicus* by SIRV2. In addition we tested the ability of PVAP to form pyramidal structures in the bacterium *Escherichia coli* and the eukaryote *Sacheromyces cerevisiae* (3).
Results: By whole-cell electron cryotomography, we monitored morphological changes in virus-infected host cells. Subtomogram averaging reveals the VAP structure, which consists of at least two layers. By heterologous expression of PVAP in cells from all three domains of life, it was demonstrated that the protein integrates indiscriminately into virtually any biological membrane, where it forms sevenfold pyramids. We identified protein domains essential for VAP formation in PVAP truncation mutants by assessing their ability to remodel the cell membrane.
Conclusion: Self-assembly of PVAP into pyramids requires at least two different, in-plane and out-of-plane, protein interactions. Our findings allow us to propose a model describing how PVAP arranges to form sevenfold pyramids and suggest how this small, robust protein may be used as a general membrane-remodeling system.

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CVV05
Differential RNA-seq of *Vibrio cholerae* identifies the VqmR sRNA as a regulator of collective behaviors
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Quorum sensing (QS) is a process of cell-cell communication that enables bacteria to transition between individual and collective lifestyles. QS controls virulence and biofilm formation in *Vibrio cholerae*, the causative agent of cholera disease. Differential RNA-sequencing of wild-type *V. cholerae* and a locked low-cell-density QS-mutant strain identified 7240 transcriptional start sites with ~47% initiated in the antisense direction. 107 of the transcripts do not appear to encode proteins suggesting they specify regulatory RNAs. We focused on one such transcript that we name VqmR. *vqmR* is located upstream of the *vqmA* gene encoding a DNA-binding transcription factor. Mutagenesis and microarray analyses demonstrate that VqmA activates *vqmR* transcription, that *vqmR* encodes a regulatory RNA, and VqmR directly controls at least eight mRNA targets including the *rtx* toxin genes and the *vpsT* transcriptional regulator of biofilm production. We show that VqmR inhibits biofilm formation through repression of *vpsT*. Together, these data provide the first global annotation of the *V. cholerae* transcription landscape and highlight the importance of post-transcriptional regulation for collective behaviors in this human pathogen.

CVV06

The sRNA NsiR4 is involved in controlling nitrogen assimilation in cyanobacteria by posttranscriptional regulation of glutamine synthetase inactivation factor IF7

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Nitrogen is an important macronutrient and in many habitats its availability is a limiting factor for the growth of microorganisms such as cyanobacteria. The global control of nitrogen metabolism is complex and shares common components with the specific responses to nitrogen depletion, including metabolic (2-oxoglutarate) as well as molecular signals (PII, NtcA). Recently, a substantial number of antisense RNAs and potentially trans-acting small RNAs (sRNAs) were identified in the unicellular, non-diazotrophic cyanobacterium *Synechocystis* sp. PCC 6803 and in the only distantly related, filamentous, nitrogen-fixing strain *Anabaena* sp. PCC 7120. Many of these sRNAs are differentially expressed, indicating their functional relevance under particular conditions. The sRNA NsiR4 (Nitrogen stress induced RNA 4) is strongly induced in both strains under nitrogen depletion and is controlled by NtcA, a global transcriptional regulator of genes that are involved in nitrogen metabolism. In addition, NsiR4 appears broadly conserved among cyanobacteria, supporting the idea of a regulatory function. Advanced target prediction suggested an interaction of NsiR4 with the *gifA* mRNA, which encodes an inhibitory protein for glutamine synthetase (GS) activity, an enzyme that plays a major role in the assimilation of nitrogen. Indeed, microarrays which were performed for NsiR4 knockout- as well as overexpressor strains of *Synechocystis* revealed an impact on the abundance of the *gifA* mRNA. Compared to wild-type, the level of *gifA* mRNA decreased upon NsiR4 overexpression, but was higher in an NsiR4 knockout strain. A corresponding inverse relationship was also observed for the GifA protein. An interaction with its predicted target region, covering the part of the 5'UTR which is probably involved in translation initiation, was verified in a heterologous reporter system, indicating a direct effect on the initiation of GifA translation. Our results point towards a pivotal role of NsiR4 in the regulatory network of nitrogen metabolism in cyanobacteria.

CVV07

Functional analysis of small RNAs based on dRNA-seq of *Bradyrhizobium japonicum* in liquid culture and in nodules

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We performed dRNA-seq analysis of *B. japonicum* USDA 110 in liquid culture and in soybean nodules (refs. 1, 2) leading to global detection of novel transcripts and transcriptional start sites (TSSs). To map TSSs on the bacterial genome of 9.1 Mb, a semi-automated TSS recognition procedure based on machine learning was developed. Using our TSS map we performed *de novo* prediction and mapping of promoter motifs of genes expressed either mainly in nodules or under both conditions. The results confirmed the major role of RpoN during symbiosis and revealed four motifs probably recognized by the housekeeping RpoD. We will show how these data enabled the analysis of an antisense RNA, which is complementary to an mRNA specifically induced in nodules. Furthermore, we will present a conserved small RNA, which leads to slower bacterial growth upon overproduction, shows higher expression in nodules than in free-living bacteria and is associated with the ribosomes.

CVV08

C/D box sRNA-guided 2′-*O*-methylation patterns of archaeal rRNA molecules

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Introduction: Archaea contain small non-coding RNAs, C/D box sRNAs, that guide 2'-*O*-methylation of their target RNAs, mostly ribosomal RNAs (rRNAs) [1,2]. A C/D box sRNA contains two guide sequences that identify their targets via base complementarity [3]. 2'-*O*-methylations play important roles in folding and stabilizing of rRNA molecules [4].
Objectives: We analyzed the production of C/D box sRNAs in seven archaeal species and predicted their methylation target sites. The identification of conserved methylation sites provides insights into the evolution of guide RNAs and rRNA folding.
Methods: Illumina RNA-Seq methodology was used to sequence all small RNA molecules of seven archaeal species. The guide regions of the identified C/D box sRNAs were extracted to allow deducing potential targets.
Results: 491 C/D box sRNAs were identified and possible interactions with rRNAs were found for 723 guide regions. Conserved methylation sites and regions were identified in the rRNAs, e.g close to the peptidyl transferase center. Furthermore, target sites were found that could be linked by the two guide regions of C/D box sRNAs. These include target sites that are distant in the rRNA sequence but are required to converge during folding.
Conclusion: These analyses reveal pan-archaeal 2'-*O*-methylation patterns of ribosomal RNAs. Hotspots for modifications are located in conserved functional core regions of the ribosome. Furthermore, the analysis of the two 2'-*O*-methylation targets of a single C/D box sRNA indicates that the two guide regions are often linked. Thus, C/D box sRNAs could act as RNA chaperones during ribosome biogenesis.

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EMV01

Comparative analysis highlights genome reduction and the evolutionary split of *Acholeplasma* and '*Candidatus* Phytoplasma'

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Introduction: *Acholeplasmataceae* comprises the genera *Acholeplasma* and '*Candidatus* Phytoplasma'. Acholeplasmas are described as saprophytic bacteria in general, while phytoplasma strains are characterized as obligate parasites of the plant phloem associated to diseases in more than 1,000 plant species. Changes in the plant phenotype are responsible for economical losses of more than 100 million €/year in the European Union. Genome research provides diagnostic markers, enabled the identification of effector proteins and reconstruction of the metabolism. The complete genome sequences of five phytoplasma strains and of four *Acholeplasma* spp. have been published so far (1,2).
Objectives: Comparative genome analyses provide insights into the evolutionary split of these two genera and the obligate parasitism of phytoplasmas.
Methods: Complete genomes determination differed between the projects ranging from clone-based Sanger sequencing, pyro-sequencing, sequencing by synthesis to single molecule real time sequencing. Genome annotation included functional reconstruction and comparative analyses accomplished by gene expression studies.
Results: Majority of the conserved gene core of phytoplasmas is also encoded by the analysed four acholeplasma genomes. Phytoplasmas are separated by a particular carboxylic acid metabolism, membrane proteins involved in host interaction and virulence factors.
Conclusion: Particularities of the phytoplasmas such as the symporter for the uptake of carboxylic acids and their conversion to pyruvate should be interpreted with respect to the Gram+ origin in contrast to genes encoding effectors, which may be derived from horizontal gene transfers.

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EMV02

The green impact: bacterioplankton response towards a phytoplankton spring bloom in the southern North Sea assessed by comparative metagenomic and metatranscriptomic approaches

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Phytoplankton blooms upend nutrient availabilities and other environmental factors and, thus, exhibit a severe impact on ecosystem functioning of microbial communities. However, the response of the bacterioplankton community to such blooms is still largely unexplored. The aim of the current study was to investigate the impact of a phytoplankton spring bloom on the ambient bacterial community structure in the southern North Sea.
For this purpose, water samples were collected inside of an algal spring bloom at different daytimes. Reference samples were taken outside the bloom. Structural changes of the community were assessed by pyrotag sequencing of 16S rRNA genes and transcripts generated from environmental DNA andRNA, respectively. Functional changes were assessed by direct sequencing of environmental DNA and mRNA. The corresponding datasets comprisedmore than 500 millionsequences across all samples. The recovered sequences were mapped on reference genomes of abundant marine groups and assembled metagenomic and metatranscriptomic datasets. Additionally, functional profiles were predicted from 16S rRNA data.
Several marine groups, including the marine *Roseobacter* clade, significantly responded towards bloom presence. The abundance of these groups was either increased or decreased indicating a stimulation or inhibition induced by bloom presence. The analysis of the mRNA datasets revealed differences in gene expression profiles between non-bloom and bloom samples. The genome-wide gene expression level of *Planktomarina temperata*, an abundant member of the *Roseobacter* clade, was increased inside the bloom. Genes that were differently expressed included heat shock protein-encoding genes, which showed higher expression levels inside the bloom.
The results provide a yet unique insight into structural and functional variations of marine bacterioplankton communities as response to a phytoplankton bloom. Therefore, they contribute to a deeper understanding of the complex impact of phytoplankton blooms on diversity, function and composition of these ambient communities.

EMV03

Meta-genomics of the Arabian Sea oxygen minimum zone

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Oxygen minimum zones (OMZs) play a major role in marine biogeochemical cycling. They are characterized by a high microbial diversity adapted to life along an oxic-anoxic gradient. In particular anammox and/or denitrification are dominant processes in these zones accounting for significant loss of nitrogen from the ocean. However, other processes within the nitrogen and also carbon cycling have not been explored well. Here, we studied the meta-genomes of filtered ocean water recovered at two different depths of the Arabian Sea OMZ, the upper zone (170m deep, PA2) and the central zone (600m, PA5). We systematically screened for marker genes indicative for key nitrogen and carbon cycling processes by blasting the meta-genomes data against manually curated functional gene databases. Both zones are characterized by a large fraction of alpha, gamma and delta-proteobacteria. In PA5, anammox contributed a significant amount to the overall diversity (about 8%) whereas Thaumarchaea were present in higher numbers in the upper zone (about 9%). The predominance of nitrifying archaea over bacteria could also be confirmed by *amoA* diversity. No bacterial *amoA* was detected in the entire dataset, and only very few sequences related nitrogen fixation were recovered. Furthermore, no canonical methane oxidizers could be found. However, *pmoA* sequences only distantly related to known diversity are present and could be linked to a published single cell genome, indicating the possibility of a cryptic methane cycle in the Arabian sea OMZ.

EMV04

Comparative metagenomics and high-resolution binning reveal functional roles of the gut microbiota in higher termites

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Introduction: The complex microbiota in the highly compartmentalized hindgut of higher termites is involved in the breakdown of lignocellulose and humic substances. However, the individual contributions of the mostly uncultured bacteria in wood- and soil-feeding termites are poorly understood.
Objectives: Our aim was to characterize the functional potential of the hindgut microbiota in termites from different feeding guilds and to assign the genes putatively involved in polysaccharide and peptide degradation to individual bacterial lineages.
Methods: We sequenced the metagenomes of the major hindgut compartments in six higher termites. Functional genes were classified using BLAST and HMMER 3. The normalized abundance of genes encoding major glycosyl hydrolase and peptidase families was compared between samples. Taxonomic assignment was improved by combining compositional binning and differential coverage of the assembled contigs.
Results: The dominant members of the bacterial communities in the individual hindgut compartments differed between feeding guilds. In addition, the abundance of glycosyl hydrolases varied significantly, with more cellulases, hemicellulases, and starch-degrading enzymes in the metagenomes of wood and grass feeders than of interface and soil feeders. However, strong discrepancies between 16S rRNA gene-based community structure and taxonomic classification of protein-coding genes indicated that the phylogenetic assignment according to BLAST hits was problematic due to the lack of reference genomes. *De novo* binning of contigs into "population genomes" allowed assigning the majority of cellulases in wood-feeding termites to members of Fibrobacteres and the TG3 phylum, whose members are specifically associated with wood fibers in termite guts.
Conclusions: The combination of comparative metagenomics and high-resolution binning techniques allows new insights into the functional potential of individual microbial lineages. The microbiota in the hindgut of higher termites show striking similarities between homologous compartments but differ strongly between feeding guilds, indicating that dietary changes during termite evolution were accompanied by corresponding shifts in the microbial communities.

EMV05

Reconstruction of the microbial phosphorus turnover in forest soils

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Many soils throughout the world are deficient in free and thus plant available Phosphorus (P), since the high reactivity of P leads to precipitation. However various microbes are known to be effective in mineralizing organically bound P as well as precipitated forms of inorganic P [1]. With this study we want to deepen our knowledge on the microbial P cycle. Our main focus is on the detection of enzymes related to solubilization and mineralization processes of soil P. Therefore, we analyzed samples from two contrasting forest soils containing high or low amounts of P. We assume that in a P lacking soil the recycling of organic bound P is dominant whereas in a P rich soil the solubilization of inorganic P prevails.
Metagenomic data was derived from whole genome shotgun sequencing using 454 technology. Data analysis was performed implying MEGAN5.
Functional annotation of sequencing data using KEGG database and HMMER detected all major enzymes of the microbial P cycle. Two types of acid and alkaline phosphomonoesterases were identified. The latter one showed 17 Hits in the P-rich soil compared to 6 Hits in the P-depleted soil. Furthermore enzymes degrading more specific forms of organic bound P like phytases and phosphotriesterases were found with similar abundances in both soils. However a phosphonatase showed twice as many positive reads in the P-depleted soil compared to the P-rich soil. Several microbial P uptake systems like the highly efficient Pst- or the constitutively expressed Pit-transporter could be detected. Interestingly both uptake systems show relatively high abundances. Twice as many reads compared to an acid phosphatase were identified for both systems. This demonstrates the importance of effective microbial P transporters to succeed in the struggle for P with plants. The PQQ-dependent glucose dehydrogenase, performing a crucial step in the solubilization of inorganic P, showed

significantly more annotated reads in the P rich soil. This is in accordance with our hypothesis that in a P rich soil the solubilization of inorganic P plays a major role. Whereas in a P depleted soil recycling processes of organic bound P are key drivers of the microbial P cycle.

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EMV06

Environmental controls on methanogenic communities in frozen-ground-affected soils on the northeastern Qinghai-Tibetan Plateau

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Introduction: The soil organic carbon (SOC) in alpine ecosystems on the Qinghai-Tibetan Plateau (QTP) could substantially feedback to the climate change. Biogenic methane produced by methanogenic archaea under anaerobic condition is our scientific concern. So far, little is known about the methanogens residing in the frozen-ground-affected plateau ecosystems, especially those at higher elevations (> 4000 m).
Objectives: The major objective is to characterize the methanogenic diversity and compositional heterogeneity across different sites in relation to the soil ecological properties.
Material and Methods: Samples were taken from 4 different sites on the northeastern QTP. The methanogenic community was compared via multiplexed/barcoded 454-pyrosequencing of PCR-amplified *mcrA* gene fragments. Raw data were processed on MOTHUR platform. Valid sequences were clustered into OTU at cutoff of 84% identity of *mcrA* genes. The beta diversity was compared and statistically analyzed by *R* packages.
Results: A high diversity of methanogens covering seven genera was explored. 85% reads on average over all libraries could be assigned to 25 abundant OTUs (relative abundance > 1%). The remaining 115 OTUs comprise the "rare biosphere". Generally, hydrogenotrophic methanogens are common in all libraries. Based on soil properties, all samples from the 4 research sites can be accordingly grouped into bog- and fen- like ecosystems. The acidic bog-like ecosystems are mainly composed of hydrogenotrophes such as *Methanoregula* and *Methanocellacaea*, whereas the latter was co-dominated by hydrogenotrophic and acetoclastic methanogenic communities. The community structure positively correlates with soil pH, calcium carbonate contents and salinity in the fen ecosystems, and with TOC and TN in the bog ecosystems.
Conclusion: Diverse methanogenic communities were present in wetlands on the NE QTP. The community composition, both the abundant taxa and the rare biosphere, vary in different types of wetlands. The hydrogenotrophic methanogens occur more commonly in the studied soils. Moreover, the communities are correlated with environmental variables of soil carbon and nitrogen in the Tibetan bogs and with proxies for soil maturity ($CaCO_3$, salinity, pH and sand content) in the minerotrophic fens.

EMV07

Fungal Microbiome Analysis during Biodegradation of Biodiesel and Diesel/Biodiesel Blends in Laboratory Soil Microcosms

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Introduction: Biodiesel consumption is ever-increasing worldwide through its use as pure product (B100) or its blending with petroleum diesel (e.g., B20 (20% biodiesel) and B5 (5% biodiesel) formulations). However, it is still necessary to define the ecotoxicological-safety endpoints and to decipher the mechanisms of biodegradation in sites contaminated by biodiesel and its blends.
Objectives: This work assessed the contribution of native soil fungal communities in B5- and B100-contaminated laboratory soil microcosms using reactor columns.
Materials & Methods: Closed reactor columns containing clay loam soil contaminated with biodiesel (EXPB100) and a low-level blend (EXPB5) (10% w/v) were incubated at 25-29 °C for 60 days and samples were collected at regular interval times at different column depths. Ecotoxicological tests using *Eisenia fetida*, quantification of culturable fungi by direct plate counting and morphological and phylogenetic characterization of fungal isolates have been carried out.
Results: Thirty-six species were identified in the initial pristine soil sample, mainly from *Aspergillus* sp., *Fusarium* sp., *Talaromyces* sp., *Cladorsporium* sp., *Lecanicillum* sp., *Penicillium* sp. genera. After 60 days of incubation, the fungal biomass concentration on the top of both reactors was very similar compared to the pristine soil, although a decrease in the amount of morphotypes was observed. At the end of incubation, the ecotoxicity of EXPB100 samples showed a decrease from 63% to 0% while samples from the top of EXPB5 reactor presented an ecotoxicity decrease from 100% to 53%.
Conclusion: These findings suggest that fungal populations, in association with bacteria, could contribute to the intrinsic biodegradation of biodiesel and its blends, mainly in aerobic environments. GC-FID analyses and targeted sequencing of ITS genes using illumina platforms will provide new insights into the biodegradation profiles of B100 and B5 and fungal microbial community structure and dynamics.

EMV08

The genome of *Rhodococcus opacus* 1CP – reconstruction of degradation pathways of aromatic compounds

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Introduction: *Rhodococcus opacus* 1CP was isolated with 2,4-dichlorophenol as sole source of carbon in Russia [1]. Meanwhile it served as a model organism to study degradation of aromatic compounds and the production of biosurfactants. Most interestingly, strain 1CP harbors a modified *ortho*-cleavage pathway to facilitate the degradation of 3-chlorocatechol [2]. Some of the metabolic activities were already linked to an earlier identified megaplasmid and were now reconstructed. The lack of *meta*-cleavage activities is still enigmatic.
Objectives: By a genomic approach the metabolic capabilities of strain 1CP should be uncovered.
Methods: Strain 1CP was cultivated on benzoate as sole source of carbon and energy, genomic DNA was isolated, purified, and passed to next generation sequencing as well as to scaffolding using paired-end libraries. The data obtained were used for assembly, annotation, and comparison. The genome was *in silico* screened for different degradation pathways of aromatic compounds and results were mapped onto growth experiments as well as to enzyme studies.
Results: The uncovered genome of strain 1CP is composed of a linear chromosome (7.7 Mb, about 7000 coding sequences, 61 RNAs, 67% GC) and three linear plasmids (p1CPa: 885 Kb; p1CPb: 348 Kb; p1CPc: 64 Kb). At first glance the 1CP-genome was similar to that of related

rhodococci. But, with a detailed analysis with respect to functional annotation some interesting features were determined. Thus exemplary genetic and bioactivity information on the degradation of (amino,-hydroxyl-)benzoates, (chloro-, methyl-)phenols, vanillate, and styrene were determined.
Conclusion: Strain 1CP is a potent degrader of (chloro)aromatic compounds which are funneled via three major intermediates (catechol, protocatechuate, phenylacetic acid) into central metabolism. Surprisingly many isoenzymes were determined for corresponding peripheral routes. Only in case of (chloro)phenol hydroxylases a metabolic activity was determined which means many potential activities seem to be silent.

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EMV09
Iron-dependent anaerobic methane oxidation in the Bothnian Sea sediment
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Anaerobic oxidation of methane (AOM) is the major biological methane sink in marine sediments. So far, most AOM research has focused on sulfate-dependent AOM, a process which has been recognized as a major methane removal mechanism in the sulfate transition zone where sulfate and methane are co-occurring. However, the potential of solid-phase electron acceptors, such as iron oxides, for methane oxidation has not been fully explored.
Here we provide geochemical evidence for iron-dependent AOM in the Bothnian Sea sediment below a shallow sulfate-transition zone. This sediment layer is characterized by non-sulfidic conditions, methane availability and high amounts of reactive iron oxides. Batch incubations with and without ^{13}C-labeled methane and nanoparticulate iron hydroxide were performed with sediment slurries from different depths. The incubations from the zone where iron oxides and methane co-occur showed significant production of $^{13}CO_2$, indicating the potential for iron-mediated AOM. DNA from both the original sediment and the active incubations was sequenced by using Ion Torrent technology. Phylogenetic comparison of 16S rRNA genes derived from metagenomes obtained from the original sediment and from active sediment slurries showed a significant increase in reads related to new uncultured Archaea and Clostridia after the 3 month incubation period. Functional gene analysis based on known enzymes for methane activation and iron reduction will be presented and discussed in relation to observed methane oxidation activity. Together, our results indicate that iron-dependent AOM may play an important role in the biogeochemical cycling of iron in sulfate-poor iron-rich marine sediments.

EMV10
Long distance electron transfer by cable bacteria in contaminated aquifers
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Introduction: In contaminated aquifers, biodegradation is in many cases restricted to a narrow zone at the plume fringe where steep gradients of electron donors and acceptors encounter [1]. There, a long distance electron transfer (LDET) by cable bacteria [2] could increase overall electron fluxes and might lead to enhanced contaminant degradation by recycling of electron acceptors.
Objectives: Within this study we aimed to detect LDET by cable bacteria in contaminated aquifers and evaluated its impacts on contaminant degradation.
Methods: We set up batch experiments with natural sediments amended with FeS as electron donor and regularly monitored the pore water chemistry in high resolution. Microbial communities were analyzed by T-RFLP, 454 pyrosequencing and fluorescence in situ hybridization. We also took intact sediment cores from the capillary fringe of a tar oil contaminated aquifer at 7 meters depth and analyzed the microbial communities at cm resolution.
Results: After 2-3 months, the pore water of batch cores showed pH, oxygen, and sulfide gradients characteristic for a LDET of 4-20 mm. Alkalinity fluxes and oxygen consumption suggested a current density of 10 mA/m^2 and thereby anodic recycling of 1.12 mmol/m^{2}d of electron acceptor sulfate. This process was most likely mediated by a novel group of filamentous *Desulfobulbaceae*, exhibiting only 88% similarity to marine cable bacteria in 16S gene. These groundwater cable bacteria were not only found in batch experiments but also *in situ* at the upper plume fringe.
Conclusion: LDET by novel groundwater cable bacteria naturally occurs in contaminated aquifers. This process increases the overall electron fluxes at the plume fringe and might enhance biodegradation by recycling of electron donor sulfate.

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EMV11
Investigation of the degradation of ^{13}C-labeled fungal biomass in soil: fate of carbon in a soil bioreactor system
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Nutrient balances and degradation processes in boreal forests are mainly influenced by interactions of plant roots and ectomycorrhizal fungi (EMF). Plants benefit from nitrogen compounds provided by their symbiotic interaction partner. In return EMF are provided by large amounts of carbon from the plants which is used for the synthesis of hyphal networks in soil and for metabolic activity for nutrient uptake.
Therefore, EMF play a major role in ecosystems of boreal forests and are consequently an important sink for carbon by building large amount of mycelia.
Recently, it has been shown that microbial biomass residues contribute significantly to soil organic matter formation. This suggests that also residues of EMF may be an important source for soil organic matter formation in forest soils where these fungi are abundant. However, the fate of EMF biomass residues in soils is unknown.
We therefore investigated the fate of EMF biomass in soil in a soil bioreactor system to quantify the contribution of this material to soil organic matter formation. As a model organism, we selected *Laccaria bicolor*, which was labelled by growing the fungus on ^{13}C glucose. The stable isotope-labeled biomass was then homogenized and incubated in a podzol from a typical forest site in Central Germany. The fate of the labeled biomass was traced by analyzing the amount of ^{13}C mineralized and the amount remaining in the soil. The fungal biomass carbon was mineralized rather rapidly during the first 50 days. Then the mineralization rate slowed down, but mineralization continued until the end of the experiment, when approximately 40% of the ^{13}C was mineralized and 60% remained in soil.
In addition, we analyzed biomolecules such as fatty acids to trace the incorporation of the *L. bicolor*-derived biomass carbon into other microorganisms and to identify potential primary consumers of fungal biomass. By these analyses, we found a significant incorporation of *L. bicolor*-derived carbon into a wide variety of different bacterial taxa, indicating the relevance of fungal biomass residues for soil bacteria as a carbon source, providing a comprehensive view of the role of EMF and their residues on soil carbon cycling.

EMV12
Microbial growth at subzero temperatures and anoxic conditions – the new limits for life on Earth and icy planetary bodies
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Introduction: Little is known about the capability of anaerobic microorganisms to adapt and grow at subzero temperatures. However, this type of extremophiles is of special importance with respect to the functioning of cold ecosystems on Earth and also to the search of life on extraterrestrial icy bodies and planets. This is the first study where the lowest limits of temperature sustaining cell replication and activity are assessed for anaerobic microorganisms [1].

Objectives: Main objectives were to determine the lowest temperature at which microbial cell replication can be measured, and the even lower temperature limits at which metabolic activity continues. In parallel, we set out to identify cold-adaptive strategies specific for anaerobic organisms.
Methods: The Antarctic bacterium *Clostridium psychrophilum* was used as model organism. Cell enumeration experiments using fluorescent dyes were combined with stable isotope probing techniques. Metabolic activity was assessed in terms of assimilation of ^{13}C-labelled carbon substrate both in the bulk biomass (EA-IRMS) and in single cells using nanoSIMS. Metabolic products were also identified and quantified by SPME-GCMS. To identify temperature-related morphological changes, electron microscopy was performed in parallel.
Results: The lowest temperature at which cell replication could be measured directly at the population level was -5 °C. At -10 and -15 °C the cell population became highly heterogeneous, differentiating in subpopulations characterised by different strategies to respond to the freeze-stress. Some cells could be imaged while undergoing division, some showed filamentous growth without dividing, and some others were found accumulating the carbon source in intracellular storage granules. In general, the metabolic level, and thus the growth rates, was strongly reduced. Cell replication could be therefore inferred from the assimilation of the ^{13}C-labelled carbon source.
Conclusion: Anaerobic microorganisms were demonstrated to be active at temperatures as low as -15 °C. Under these extreme conditions, cell heterogeneity was the predominant phenomenon in order to evolve a variety of adaptive strategies.

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EMV13
"Hooking" – omics and imaging techniques: The lifestyle and ultrastructure of *Candidatus* Altiarchaeum hamiconexum
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Introduction: The majority of microbial life on Earth thrives in the subsurface and remains largely unexplored with regard to diversity, life style, cellular organization and ultrastructure due to the limited access to these environments. Deep groundwater springs, however, provide excellent windows into the depth, transporting microbial communities from the depth to the accessible surface. Such model systems can help to reveal how microorganisms can cope with subsurface conditions, such as restricted sunlight and anoxia.
Objectives: Multifarious aspects of the biology of *Candidatus* Altiarchaeum hamiconexum (formerly known as "SM1 Euryarchaeon"[1],), a widespread, uncultivated subsurface Euryarchaeon, which is washed up in a highly active stage from the deep in almost pure, well-organized biofilms, were studied in detail.
Material methods: Various "omics" surveys (metagenomics, transcriptomics, proteomics) were combined with microscopy techniques to enlighten novel details concerning the ultrastructure and the life style.
Results: The coccoid, anaerobic *Can.* A. hamiconexum cells exhibit unique traits, such as an unusual autotrophic metabolism, a double-membrane layer and hundred well-organized unique cell surface appendages ("hami"[2]).
Interestingly, these filamentous structures are synthesized by one major protein species, assembling into a barbed wire region and a nano-grappling hook. Being highly stable and biodegradable, the threads are supposed to be useful tools for nano-biotechnological application. The encoding gene sequence could be identified and is now the basis for recombinant expression.
Conclusion: This study applied different techniques to reveal hidden traits of the exceptional *Can.* A. hamiconexum, which might be the underlying key for its environmental success.
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EMV14
What is coming out of a biogas plant? – an overview of microbiome studies and cultivation of antibiotic resistant bacteria from input and output samples of German biogas plants
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Input and output samples of 14 mesophilic and one thermophilic German biogas plants were investigated with respect to the overall bacterial community composition and the abundance of antibiotic resistant bacteria including beta-lactamase producing *Enterobacteriaceae* (ESBLs), Carbapemenase-producing *Enterobacteriaceae* (CPE), Methicillin-resistant *Staphylococcus aureus* (MRSA) and Vancomycin resistant Enterococci (VRE). The aim of the study was to investigate the risk of the release of potential pathogenic and antibiotic resistant bacteria originating from livestock husbandry after manure processing in biogas plants.
Bacterial communities were investigated by the generation of 16S rRNA gene amplicon 454 Pyrosequencing libraries. The abundance of ESBL, CPE, MRSA and VRE was investigated by a cultivation-dependent approach using specific pre-enrichment procedures and selective CHROMagar media, combined with a resistance gene screening using multiplex-PCR systems and phylogenetic identification by 16S rRNA gene sequencing.
At the phylum level, the bacterial communities of input samples were dominated by *Firmicutes* (22-97%), followed by *Actinobacteria* (up to 59%), *Proteobacteria* (up to 26%), and *Bacteroidetes* (up to 20%); those of output samples by *Firmicutes* (35-89%), followed by *Bacteroides* (up to 27%) and *Chloroflexi* (up to 18%). In contrast, *Actinobacteria* and *Proteobacteria* accounted for only 5 to 6% in the output samples. Most sequences of the output samples represented a so far uncultured bacterium which occurred in high abundance in biogas plants which were fed with conventional, but not in a biogas plant fed with organic farming manure. ESBL and VRE were detected in both, input and output samples. MRSA were not detected, but other *mecA* carrying staphylococci were cultured from input, but not from output samples. CPE were not detected at all but carbapeneme resistant *Acinetobacter* and *Pseudomonas* were isolated.
In summary, nothing is so far known about the high proportion of uncultured bacteria which are highly abundant in biogas plant output material. Compared to manure which was used as input material, the abundance of antibiotic resistant bacteria is lower in output samples of biogas plants, but resistant bacteria are not eliminated and hence can be released into the environment.

EMV15
First biochemical, spectroscopic and X-ray crystallographic characterization of an actinobacterial "high affinity" [NiFe]-hydrogenase
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Introduction: Though only a trace gas (ca. 0.5 ppmv), molecular hydrogen [H_2] has an impact on the oxidative capacity of the atmosphere. Therefore, the biogeochemical H_2 cycle stands in the focus of current research [1]. Upland soils have already been identified as the main sink for H_2. Only recently it became clear that the corresponding high-affinity H_2 uptake is predominantly mediated by Actinobacteria. These species harbor specialized [NiFe]-hydrogenases that have been affiliated to the novel group 5, based on phylogenetic and physiological properties [2,3]. It is proposed that these organisms can utilize trace H_2 to maintain a basic metabolism under challenging conditions, e. g. in spores or other dormant forms [3].
Objectives: We present the first detailed biochemical, spectroscopic and x-ray crystallographic characterization of a group 5 enzyme, using the actinobacterial hydrogenase (AH) recently discovered in the H_2 oxidizer *Ralstonia eutropha* H16 [4].
Methods: Spectrophotometric and amperometric activity assays, FTIR spectroscopy, X-ray crystallography.

Results: The AH consists of a large subunit carrying the active site and an electron-transferring small subunit, harboring three iron-sulfur clusters. Unlike most other [NiFe]-hydrogenases, all three clusters are of the [4Fe-4S] type, whereby the cluster proximal to the NiFe site shows an unusual coordination by three cysteines and one aspartate. Two functional units are electronically connected in a stable dimer. Unexpectedly, the AH doesn't show high affinity towards H_2 and is therefore not capable in the uptake of atmospheric H_2. However, the enzyme displays extraordinary thermostability and retains full H_2 oxidation activity in presence of atmospheric O_2 [4]. Accordingly, infrared spectroscopic investigations on the AH showed no O_2-inactivated states of the NiFe site.
Conclusion: Our data provide important insights in the structure and function of group 5 [NiFe]-hydrogenases and support their proposed role in energy generation under environmentally challenging conditions.

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EMV16
Identification of enzymes involved in degradation of the *Pseudomonas aeruginosa* quinolone signals in *Rhodococcus erythropolis* BG43
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Introduction: The *Pseudomonas* quinolone signal (PQS, 2-heptyl-3-hydroxy-4(1*H*)-quinolone) and its biosynthetic precursor 2-heptyl-4(1*H*)-quinolone (HHQ) as well as *N*-acylhomoserine lactones are employed as quorum sensing (QS) signal molecules by the opportunistic pathogen *P. aeruginosa*. QS is involved in regulating biofilm maturation and the expression of various virulence factors [1]. Considering the need for new antibacterial drugs, enzymes degrading signal molecules and hence reducing virulence of pathogens are promising molecular tools.
Objective: Isolation and characterisation of alkylquinolone (AQ) converting enzymes in the AQ degrading strain *Rhodococcus erythropolis* BG43.
Methods: The draft genome of *R. erythropolis* BG43 was analysed for genes encoding enzymes possibly involved in AQ degradation. Candidate genes were heterologously expressed and enzyme activities were characterised.
Results: *R. erythropolis* BG43 is able to degrade the signal molecules PQS and HHQ to anthranilate [2]. Results from *in vitro* assays suggested the involvement of a dioxygenase related to 2-methyl-3-hydroxy-4(1*H*)-quinolone 2,4 dioxygenase (Hod) from *Arthrobacter* sp. Rue61a in PQS degradation by strain BG43. Hod catalyses the cleavage of PQS to *N*-octanoylanthranilic acid and carbon monoxide, but its activity towards PQS is very low and fortuitous [3]. Remarkably, two *hod*-like genes, each organised in a cluster with genes encoding a monooxygenase and a hydrolase, were found in the genome of strain BG43. Heterologous expression of the gene clusters and *in vitro* assays indicated an NADH-dependent hydroxylation of HHQ to PQS catalysed by one of the monooxygenases. Both Hod-like enzymes converted PQS to *N*-octanoylanthranilic acid, and both hydrolases mediated release of anthranilic acid. One of the rhodococcal PQS-cleaving dioxygenases, purified from recombinant *Escherichia coli*, showed a specific activity of 30 U/mg.
Conclusion: Identification and isolation of new AQ degrading enzymes open up new perspectives to interfere with QS and virulence.

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GRV01
Blocking sporulation of *Bacillus subtilis* at high salinity – SigH is the culprit
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Introduction: The soil bacterium *Bacillus subtilis* is well known for its ability to form highly desiccation-resistant endospores. Since it frequently encounters increasing osmolarity and salt-stress in this habitat, it seems reasonable that sporulation would be an osmostress escape system when the salt content of the soil becomes so hostile that vegetative growth can no longer occur. Surprisingly, this is not the case since sporulation is severely inhibited in high salinity growth medium.
Objectives: Our goal was to identify the point at which sporulation is inhibited by high salinity and the factors that contribute to this block.
Methods: We constructed a set of stage-specific reporters in the sporulation pathway to determine when the developmental program is blocked at high salinity. We assessed the abundance of stage-specific gene products and sigma factors during cellular differentiation. We also purified RNA-polymerase to determine its association with different sigma factors during sporulation at high salinity. By genetic enrichment and whole genome sequencing, we identified suppressors capable of partially bypassing the salt-dependent block in sporulation.
Results: Fluorescence microscopy revealed that sporulation is blocked at high salinity early on in the differentiation process, since gene expression controlled by one of the earliest acting sporulation-specific sigma factors (σ^H) was strongly reduced. However, immunoblot analysis demonstrated that SigH protein levels were unaffected, indicating that regulation is post-translational. Preliminary data suggest that SigH inefficiently associates with core-RNAP in high salinity challenged *B. subtilis* cells. Suppressors that weakly bypass the salt-induced block in sporulation, map either in the promoter region of *kinA*, encoding the major sporulation specific histidine kinase, or in the coding region of *sigH*. We show that overexpression of *kinA* can bypass the SigH-dependent block in entry into the sporulation pathway at high salt. However and importantly, this *kinA* overexpression only partially suppresses spore formation.
Conclusion: Collectively, we conclude from our studies, that the improper functioning of SigH prevents salinity challenged cells from entering the sporulation process.

GRV02
Characterization of four homologous stress response regulators of the AraC/XylS family from the fire blight pathogen *Erwinia amylovora*
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The Gram-negative bacterium *Erwinia amylovora* is the causal agent of fire blight, a destructive disease in rosaceous plants such as apple and pear. In order to colonize its host, *E. amylovora* must be able to adapt to various environmental stresses. Transcriptional regulators of the AraC/XylS family are involved in antibiotic resistance, tolerance to organic solvents, oxidative stress, and virulence. We identified and characterized four homologous regulators of the AraC/XylS family from *E. amylovora* Ea1189. Two regulators were homologous to either Rob or SoxS, whereas the other two proteins, PliA and OpiA, did not show significant similarity to characterized members of the AraC/XylS family.
The AraC/XylS regulators Rob and SoxS from *Escherichia coli* are known to mediate antibiotic resistance, primarily by upregulating the multidrug efflux system AcrAB. However, none of the four AraC/XylS regulators from *E. amylovora* was able to induce multidrug resistance in the fire blight pathogen. Nevertheless, overexpression of *rob* led to a 2-fold increased expression of the *acrA* gene. However, the strain overexpressing *rob* showed increased resistance to only a limited number of antibiotics.
We could show that SoxS from *E. amylovora* is involved in superoxide resistance. A *soxS*-deficient mutant was not able to grow in the presence of paraquat, a superoxide-generating agent. Furthermore, the expression of the *soxS* gene was induced by redox-cycling agents.
PliA, an uncharacterized member of the AraC/XylS family, was highly upregulated during the early infection phase in apple rootstock and immature pear fruits. The expression of the *pliA* gene was induced by multiple compounds including apple leaf extracts, phenolic compounds, redox-cycling agents, heavy metals, and decanoate.
The second uncharacterized AraC/XylS regulator, OpiA, was shown to play a role in the regulation of osmotic and alkaline pH stress responses.

GRV03

Defining the regulon of genes controlled by σ^E, a key regulator of cell envelope stress in *Streptomyces coelicolor*

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Introduction: To respond to environmental stresses, bacteria employ different signaling cascades to initiate the transcription of responsive genes. In *Streptomyces coelicolor*, the σ^E-dependent signal transduction system mediates the response to cell envelope stress and ensures maintaining cell envelope integrity [1,2]. The *sigE* gene encodes an extracytoplasmic function (ECF) sigma factor. But so far, very little was known about the target genes that are under control of σ^E.
Objectives: The aim of this work is to define the regulon of genes controlled by σ^E.
Methods: To define the σ^E regulon, ChIP-seq, DNA microarray and bioinformatics analysis were performed. Additionally, S1 mapping and *in vitro* transcription experiments were applied to identify the σ^E binding consensus promoter sequence.
Results: More than 200 σ^E-target regions were identified by ChIP-seq experiments. The ECF σ^E recognizes a conserved promoter motif that is characterized by an "AAC" motif in the -35 region and a "TC" signature in the -10 region. Amongst them, a number of genes that are predicted to be involved in cell envelope-related functions were identified as σ^E targets.
Conclusions: Our work identified the σ^E regulon of *S. coelicolor* by a combination of global analysis together with detailed follow-up studies. Many σ^E targets are predicted to have cell envelope-related function, which supports and explains the known and important role of σ^E in orchestrating cell envelope stress response.

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GRV04

States of emergency in *Bacillus subtilis* – the commander in chief -σ^B rules a complex regulatory network

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Introduction: In order to protect themselves from destruction almost all organisms have evolved a so called general or environmental stress response that becomes initiated as a consequence of diverse stress signals. In *B. subtilis* the general stress response is under primary control of the alternative sigma factor σ^B. Although the basic mechanisms of σ^B activity control, transcriptional regulation and the physiological importance of the general stress proteins is well understood, we neither comprehensively understand the decision making processes and structure of the regulatory network, nor the distinct biochemical functions of about 60% of this physiologically highly important group of proteins.
Objectives: Our studies focused on the identification of new regulatory mechanisms and intersections connecting the σ^B regulon to so far separate regulatory units within the adaptational gene expression network of *B. subtilis.*
Materials and Methods: We performed transcriptomics (Tiling-Arrays, RT-PCR, Northern-blot); and proteomics (2D-gel electrophoresis, radioactive pulse chase labeling and immunoprecipitation, Western-blot) experiments.
Results: Although σ^B is activated in response to many diverse conditions, the precise expression level of many genes is specific to the features of the primary stress stimulus. We could show that secondary regulators such as Spx and MgsR come into play to integrate secondary signals beyond the primary decision of σ^B activation. Furthermore, we could demonstrate that σ^B is not only necessary for the induction of the general stress regulon, but also responsible to coordinately shut off expression of hundreds of genes in the face of harsh environmental stress. Two major examples for this observation are the direct activity control of the master regulators of sporulation (Spo0A) and competence development (ComK) by σ^B.
Conclusion: We provide the basis for an elaborate model of a hierarchically ordered regulatory pathway with σ^B as the primary master regulator enabling both, (*i*) the downstream-integration of secondary stress stimuli for a finely tuned target gene expression as well as (*ii*) its function as an "emergency brake system" to silence alternative developmental programs whose expression would be inappropriate under severe σ^B inducing stress conditions.

GRV05

Regulation of *opuB* expression in *Bacillus subtilis* involves a SigB-dependent antisense RNA

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Introduction: The soil bacterium *B. subtilis* uses compatible solutes such as glycine betaine to cope with hyperosmotic stress. Glycine betaine is taken up from the environment or can be synthesized from choline that is imported via OpuB and OpuC, two closely related ABC transporters. Because of their strikingly different substrate specificity [1], distinct physiological roles of OpuB and OpuC in stress adaptation can be assumed.
Objectives: In a tiling array transcriptome study of *B. subtilis* exposed to a wide range of environmental conditions [2], we noted differential induction of the *opuB* and *opuC* operons in response to either suddenly imposed or sustained increase in salinity. The tiling array data also revealed an antisense RNA (S1290) covering the *opuB* operon; S1290 was induced by osmotic stress as part of the SigB-mediated general stress response. Therefore, the role of SigB in modulating the osmotic control of *opuB* expression was investigated.
Methods: Transcript levels of *opuC*, *opuB*, and S1290 in response to osmotic upshifts (0.4 and 1 M NaCl, respectively) were analyzed in time-course experiments. Northern Blot analyses of *B. subtilis* wild type, *sigB* deletion and S1290 promoter mutant strains were performed to probe the effect of the antisense RNA on the observed differential expression of *opuB* and *opuC*.
Results: In line with the transient nature of the SigB-controlled general stress response, S1290 levels peaked at 10 or 30 min after osmotic upshift, depending on the NaCl concentration used; *opuB* expression started only at subsequent time points. This time-delayed induction of *opuB* was not observed in a mutant lacking SigB. In contrast to *opuB*, strong induction of *opuC* occurred at early time points after the addition of NaCl and returned to unstressed levels in cells adapted to growth in high-salinity medium.
Conclusion: Upon an osmotic upshift, the expression of a SigB-dependent antisense RNA is induced, retarding the osmotic induction of the *opuB*, but not the *opuC* operon. The underlying regulatory mechanism and its influence on OpuB- and OpuC-mediated choline transport in wild type and *sigB* mutant cells will be investigated in further experiments.

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GRV06

Denitrification associated transcriptome of the acid-tolerant denitrifier *Rhodanobacter denitrificans*

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Denitrification is the sequential reduction of nitrate via nitrite, nitric oxide, nitrous oxide to molecular dinitrogen and important for nitrogen removal in the environment as well as a source of the greenhouse gas nitrous oxide. *Rhodanobacter denitrificans* is an acid-tolerant, gram negative denitrifier of the Gammaproteobacteria, which is capable of complete denitrification at pH 3 and high nitrate concentrations. Certain acidic, nitrate-rich permafrost affected peat soils are extreme sources of denitrification derived nitrous oxide and host acid-tolerant Gammaproteobacteria. Regulation of denitrification is important to the emission of nitrous oxide. Thus, *R. denitrificans* was chosen as a model organism to study the regulation of denitrification on the transcriptomic level. *R. denitrificans* was incubated with glucose as source of carbon and electrons under oxic and micro-oxic/denitrifying conditions. Exponential growth started without apparent delay and was completed after 14 hours under oxic conditions. Under micro-oxic/denitrifying conditions, biphasic growth was observed. Growth rates decreased after 3 h of incubation concomitant to the production of nitrous oxide, suggesting a transition from aerobic growth to denitrification. Exponential growth was completed after 18 h of

incubation, and nitrate was stochiometrically converted to N-gases. Total RNA was obtained after 3 h (early) of incubation and at the mid- to late exponential phase (late) from all cultures, depleted of rRNA, and subjected to HiSeq analysis. 80 million reads were obtained in total and differentially expressed genes identified by the Rockhopper pipeline. Transcription of 80 and 50 genes were either up or down regulated under micro-oxic/ denitrifying when compared to oxic conditions at the early and late samplings, respectively. Genes that were up regulated included putative oxygen and nitrate sensors only distantly related to those available in public databases, denitrification associated structural genes, and genes of unknown function. The collective data indicates a new transcriptional regulatory network associated with the regulation of denitrification in *R. denitrificans*.

GRV07
RcsB – a versatile transcription factor in *Escherichia coli*
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RcsB is the response regulator of the Rcs phosphorelay and belongs to the family of FixJ/NarL-type transcription factors. RcsB regulates expression of multiple loci related to motility, biofilm formation, and various stress responses. The activity of RcsB is controlled by two mechanisms. First, the Rcs phosphorelay controls RcsB activity by phosphorylating a conserved aspartate residue within its receiver domain. The Rcs phosphorelay is a complex signal transduction system of the two-component type that senses perturbations of the bacterial cell envelope. Second, RcsB in addition to forming a homodimer can be modulated by interaction with auxiliary proteins such as RcsA (regulation of capsule synthesis) [1], BglJ (pleiotropic regulator, activating *bgl* and *leuO*) [2], and GadE (acid stress response) [3]. These auxiliary regulators likewise belong to the FixJ/NarL transcription factor family and their activity depends on RcsB. Here we demonstrate that RcsB interacts with two additional transcriptional regulators, MatA (control of the Mat pili expression) and DctR (encoded in the acid stress island), which also belong to the same FixJ/NarL-family of transcriptional regulators. We further show, that transcriptional activation by RcsB homodimers and RcsA/RcsB heterodimers is dependent on phosphorylation, while the activity of BglJ/RcsB as well as MatA/RcsB heterodimers is phosphorylation independent. In addition, we constructed RcsB mutants with exchanges of highly conserved amino acid residues and analyzed the importance of these residues for transcriptional regulation depending on the dimerization partner. Taken together, our findings underline the versatile and unique role of RcsB being involved in many regulatory networks depending on phosphorylation state and interaction partner.

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GRV08
Staphylococcus lugdunensis; an amplifiable *isd* locus
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Introduction: The *isd* locus of *Staphylococcus aureus* represents an important virulence factor and encodes a heme acquisition system. Isd allows acquiring nutrient iron during infection to overcome iron restriction *in vivo*. The coagulase negative Staphylococcus (CoNS) *S. lugdunensis* is associated with a series of cases of infective endocarditis and is the only CoNS encoding an *isd* locus. The clinical Isolate HKU09-01 harbors a tandem repeat duplication of Isd, indication an important role of the locus. Gene duplications and amplifications (GDAs) are known as a driving force of evolution. They allow high frequency of RecA dependent recombination leading to an accordion-like expansion and contraction of the copy. This results in extensive variation in gene expression levels.
Objectives: GDAs are almost exclusively studied in model organisms. We therefore investigated whether similar processes allow *isd*-copy number variation in *S. lugdunensis*.
Methods: Methods to detect GDAs using PCR and antibiotic resistance levels were developed to trace copy number variation *in vitro*. Site directed mutagenesis was used for constructing stable copy number variants and *in vitro* assays to investigate the physiological effects were established.
Results: The duplication of the *isd* operon was found to be intrinsically unstable and could be stabilized by the introduction of a *recA* mutation. Furthermore, subclones carrying up to seven copies of the *isd* operon could be isolated and the increase in copy number correlated with an increase in Isd protein expression. The effects of the GDA of the *isd*-operon regarding haem acquisition are under current investigation. Apart from heme acquisition, Isd-proteins are known to possess additional functions in immune evasion and the effects of the *isd* copy number variation and its evolutionary importance are therefore investigated
Conclusion: GDAs are important genetic events and frequently neglected. Even in clinical isolates that are difficult to manipulate, such events can be detected and their development can be traced using distinct molecular techniques. This opens novel avenues for studying how pathogens rapidly adapt gene expression levels to the environmental pressures within specific environments such as the hospital setting or during invasive disease.

MAV01
Lactate metabolism in the acetogenic bacterium *Acetobacterium woodii*
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Introduction *Acetobacterium woodii* is an acetogenic bacterium that is able to grow autotrophically on H_2 + CO_2 and heterotrophically on substrates such as sugars, alcohols or acids such as lactate. Lactate is a common substrate for major groups of strictly anaerobic bacteria but the biochemistry and bioenergetics of lactate oxidation is obscure. The low redox potential of the pyruvate/lactate pair of $E_0' = -190$ mV excludes direct NAD^+ reduction ($E_0' = -320$ mV) [1] and indeed, NAD^+ reduction was never demonstrated nor was the electron acceptor identified. The organism must have developed a way to circumvent this energetic obstacle encountered during lactate metabolism [2].
Objective Identification and charactarization of the lactate-oxidizing enzyme and the pathway used for lactate oxidation in *A. woodii*.
Methods The lactate dehydrogenase (LDH) from *A. woodii* was purified by chromatographic steps and analyzed biochmically to identify the mode of lactate oxidation.
Results The LDH forms a stable complex with two subunits of an electron-transferring flavoprotein (Etf) and did not reduce NAD^+ with lactate as electron donor. However, this ability was conveyed by addition of reduced ferredoxin. The LDH/Etf complex of *A. woodii* apparently uses flavin-based electron confurcation to drive endergonic lactate oxidation with NAD^+ as oxidant at the expense of simultaneous exergonic electron flow from reduced ferredoxin ($E^{0'} \approx$-500 mV) to NAD^+ according to: lactate + Fd^{2-} + 2 NAD^+ à pyruvate + Fd + 2 NADH. Inspection of genomes revealed that this metabolic scenario for lactate oxidation may also apply to many other anaerobes.
Conclusion The energetic barrier imposed during lactate metabolism in *A. woodii* is overcome by coupling it to an exergonic redox reaction, the oxidation of reduced ferredoxin with lactate as electron acceptor.

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MAV02
The structure of the NADH- dependent reduced ferredoxin-NADP oxidoreductase: Insights into flavin-based electron bifurcation
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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. Consequently, NADPH binds to b-FAD. On this basis a mechanism of FAD-based electron bifurcation was postulated. Therein, we propose that the second electron transfer to a-FAD, the site of the exergonic reaction, is prevented by a rearrangement of the NfnA and NfnB subunits relative to each other.

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MAV03

The electron transport chain of *Wolinella succinogenes* sulfite respiration

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Introduction: Assimilatory and dissimilatory sulfite reduction are key reactions of the biogeochemical sulfur cycle and several distinct sulfite reducing enzymes have been characterized in the past, for example in sulfate-reducing bacteria and archaea [1]. In addition, the Epsilonproteobacterium *Wolinella succinogenes* has been described to grow by respiratory sulfite reduction (yielding sulfide) using formate as electron donor [2]. Sulfite is reduced in the periplasm by the octahaem cytochrome *c* MccA, a representative of a new class of highly active cytochrome *c* sulfite reductases [1-4].
Objectives: The work aimed to test the proposed model of *W. succinogenes* sulfite respiration and to structurally characterize the cytochrome *c* sulfite reductase MccA.
Methods: Several *W. succinogenes* mutants were characterized as to their sulfite respiration capacity. The sulfite reductase MccA was purified by affinity chromatography and crystallized under anaerobic conditions. The three-dimensional structure was determined by single-wavelength anomalous dispersion.
Results: 8-methylmenaquinone-6 (MMK) was found to be an essential component of sulfite respiration. Reduced MMK is thought to be oxidized by a putative MccCD complex that consists of a quinol dehydrogenase of the NrfD/PsrC family (MccD) and the iron-sulfur protein MccC. Electrons are eventually transferred to sulfite reducing MccA, of which a high-resolution crystal structure was obtained [4]. The MccA structure revealed an unprecedented haem *c*-based active site of sulfite reduction and confirmed the presence of haem *c* group bound by a $CX_{15}CH$ binding motif.
Conclusion: The electron transport chain of *W. succinogenes* sulfite respiration reveals several new features as compared to other types of anaerobic respiration including a cytochrome *c* sulfite reductase with unique enzymatic features.

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MAV04

An enzyme producing rocket fuel – Structure of hydrazine synthase from anammox bacteria

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Anaerobic ammonium oxidation (anammox) plays a major role in the earth's nitrogen cycle and is used in novel waste water treatment procedures. This bacterial process combines nitrite and ammonium to form dinitrogen gas and water, and has been estimated to be responsible for up to 50% of the loss of fixed nitrogen from the oceans. Strikingly, the anammox process relies on the highly unusual, extremely reactive intermediate hydrazine [1, 2], a compound with a very low redox potential (E_0'= -750 mV), which is used as a propellant in spacecraft. So far, the enzymatic mechanism by which hydrazine is synthesized was unknown. We determined the 2.7 Å resolution crystal structure of a hydrazine synthase (HZS) multienzyme complex (kuste2859-61) isolated from the anammox organism *Kuenenia stuttgartiensis*. The structure shows an elongated dimer of heterotrimers, each of which has two distinct c-type heme-containing active sites, as well as interaction points for redox partners. Furthermore, we observed a system of tunnels connecting these active sites. Finally, biophysical analyses using analytical ultracentrifugation (AUC) and small-angle X-ray scattering (SAXS) show the 327 kDa heterodimer to be the oligomeric form of HZS in solution. The crystal structure and biochemical data imply a two-step mechanism for hydrazine synthesis.

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MAV05

Mechanism of dearomatization/dehalogenation by ATP-dependent benzoyl-CoA reductases

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Under anaerobic conditions halogenated aromatic compounds (HAC) were until recently considered to be only degraded by organohalide respiring anaerobes that use HAC as terminal electron acceptors. Here, dehalogenation is catalyzed by corrinoid-containing, membrane-bound dehalogenases (1). However, *Thauera chlorobenzoica* 3CB-1 has the capability to completely degrade 3-Cl- and 3-Br-benzoate to CO_2 and the halide under denitrifying conditions. After activation to the corresponding CoA-thioesters these are reductively dehalogenated to benzoyl-CoA and HCl/HBr by ATP-dependent benzoyl-CoA reductases (BCRs) due to a reductive dearomatization/halide elimination mechanism (2). Here we describe the complete degradation of 4-F-benzoate to CO_2 and HF by *Thauera aromatic* K172. We demonstrate that the reductive C-F-cleavage is accomplished by BCRs in an ATP-dependent manner via a nucleophilic substitution-like reaction at the aromatic ring. Proteomic and transcriptomic analyses revealed that during growth on 4-F-benzoate the expression of genes encoding for BCR is up-regulated, thereby compensating for the low specific defluorination activity of the enzyme. The development of a heterologous expression allowed the functional production of benzoyl-CoA reductases from various organisms. Among these an enzyme recently assigned to a 3-methyl-benzoyl-CoA reductase in *Azoarcus* sp. CIB (3) was shown to catalyze, next to the reductive dearomatization, the ATP-dependent reductive dehalogenation of 2-Cl-, 3-Cl- and 4-Cl-benzoyl-CoA. This observation improves the relevance of reductive dehalogenation by class I BCRs.

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MAV06

Identification of tetrachloroethene respiratory chain components in *Sulfurospirillum multivorans*

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Introduction: *Sulfurospirillum multivorans* is an epsilonproteobacterium able to respire tetrachloroethene (PCE) and trichloroethene. The organism couples reductive dehalogenation of these compounds to energy conservation via electron transport phosphorylation (organohalide respiration). To date little is known about the PCE respiratory chain. The terminal reductase is the PCE reductive dehalogenase (PceA), a corrinoid-containing iron-sulfur protein. The mature form of PceA is located at the periplasmic side of the cytoplasmic membrane [1].

Objectives: Elucidation of the PCE respiratory chain in *S. multivorans*.
Methods: The genome of *S. multivorans* was sequenced [2] and a differential proteome analysis was conducted. The soluble and membrane extracts of cells either grown in the presence or absence of PCE were analyzed. Involvement of quinones and a reverse electron flow were tested by inhibition studies and the quinones were identified.
Results: The experimental approaches allowed for the identification of proteins specifically produced in PCE-grown cells. Among other proteins, a quinol dehydrogenase (similar to NapGH found in nitrate respiration) was identified, which is presumably involved in electron transfer to PceA. With quinone analogues, PCE respiration was inhibited, pointing to the involvement of quinones. In *S. multivorans*, organohalide respiration was blocked by protonophores while there was no effect of uncouplers on PCE-respiration of the gram-positive *Desulfitobacterium hafniense* Y51. The latter organism lacks NapGH-type quinol dehydrogenase genes.
Conclusions: The results of this study lead to development of a more precise model of the PCE respiratory chain in *S. multivorans*. Quinones and a quinol dehydrogenase seem to be involved in PCE respiration. Until now, a NapGH-type quinol dehydrogenase was only identified in respiratory chains of denitrifying organisms. Also, other organohalide-respiring bacteria (OHRB) do not seem to employ such a quinol dehydrogenase, which implies the existence of fundamentally distinct electron transport chains in different OHRB.

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MAV07
Single gene insertion creates a synthetic pathway for bioalcohols in a hyperthermophilic archaeon
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Production of alcohol-based biofuels from renewable feedstocks is currently achieved by only a very limited number of metabolic pathways and only at moderate temperatures. A fundamentally different synthetic pathway for bioalcohol production at 70°C was constructed by insertion of the gene for bacterial alcohol dehydrogenase (AdhA) into the archaeon *Pyrococcus furiosus*. The engineered strain converted glucose to ethanol, thereby being the first evidence for significant alcohol production by any member of the domain Archaea. Ethanol was formed from acetate and without involvement of acetyl-CoA; with the key reductions catalyzed by *P. furiosus*' own aldehyde ferredoxin oxidoreductase (AOR) and the heterologously-expressed AdhA, in an energy-conserving, redox-balanced pathway. Furthermore, the engineered strain converted exogenously-added aliphatic and aromatic carboxylic acids to the corresponding alcohol using as the reductant glucose, pyruvate, or hydrogen gas or, by heterologous co-expression of a dehydrogenase, carbon monoxide. These results call into question the previously suggested physiological function for AOR and suggest that organic acids as electron sinks might have supported forms of ancient life in early earth. The application of the AOR/AdhA pathway in syngas-fermenting microorganisms is potentially a game-changing platform technology for the production of longer bioalcohols.

Basen, M.; Schut, G.J.;*et al.* **(2014)**. ***PNAS*** doi:10.1073/pnas.1413789111

MAV08
Comprehensive view on the thiol proteome of the obligate anaerobe *Clostridium difficile* and its *perR* mutant under infection-relevant conditions
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Introduction: Infections with the spore-forming, Gram-positive human pathogen *C. difficile* have become the most frequent cause of hospital acquired diarrhea that very often results in a serious inflammation of the intestines. An increasing antibiotic resistance of the bacterium and a very high relapse rate call for new antimicrobial strategies. During infection, *C. difficile* faces several conditions (microaerophily, reactive oxygen species (ROS) and bile acids) that could disturb the cellular redox balance.
Objectives: A disturbed redox status could also have an impact on the thiol proteome with dramatic cellular side effects, as cysteine residues play an important role in enzymatic reactions and can serve as regulatory switches. In order to gain a better understanding of the thiol redox proteome of *C. difficile* in general as well as of the specific role of the central redox regulator PerR, we aim to identify and quantify alterations in the thiol proteome of D*perR vs*. its reference wild type strain *C. difficile* 630 D*erm* under control conditions but also after redox stress.
Results: Bioinformatic inspection of the *C. difficile* proteome reveals an extraordinary high content of the amino acid cysteine. Possibly due to its strictly anaerobic lifestyle, *C. difficile* can afford to hold a higher than average proportion of oxidation-susceptible cysteine. It would be interesting to know if these cysteines also have an amplified regulatory function in the bacterium.
We established a workflow based on differential labeling of unmodified and (reversibly) oxidized thiols in *C. difficile* following three approaches, one based on 1D-geLC-MS/MS and two gelfree setups making use of the OxICAT method as well as LC-IM-MS^E. In our first thiol-redox profilings the different experimental setups are compared, and the impact of a *perR* mutation and oxidative stress on the thiol proteome is assessed.
Conclusion: The developed technique allows us to assay the status of the thiol proteome of *C. difficile* which can be used to characterize the response to stress and to unravel the role of redox active regulators and enzymes. An additional comprehensive protein quantification will complement this data aiming at a detailed picture of the adaptation mechanisms of vegetative *C. difficile* cells to the unfavorable conditions in the intestinal tract.

MCV01
X-ray crystallography reveals new surprises in methanogenic enzymes
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Introduction: It is estimated that approximately 1 billion tons of methane (potential greenhouse gas) per year is formed globally by methanogenic archaea in anoxic environments. Enzymes of the methanogenic pathways have been biochemically and some of them structurally characterized [1]. However, crystal structures of the key enzymes involved in energy conservation (such as the integral-membrane Na+-translocating methyltransferase, the Mtr complex, and the electron-bifurcating heterodisulfide reductase) are still unraveled.
Objectives: Our aims are to obtain a three-dimensional structure of these enzymes to understand their catalytic mechanisms. In our presentation, we will mainly focus on crystal structure of the cobalamin-binding subunit (MtrA), which is the potential motor protein of the membrane Na+-translocating pump. We will also report a new post-translational modification in an isoenzyme of the methyl-CoM reductase (MCR II).
Methods: The cytoplasmic domain of MtrA from *Methanocaldococcus jannaschii* and a cytoplasmic MtrA homolog from *Methanothermus fervidus* were purified from recombinant *Escherichia coli* cells. MCR II was purified from *Methanothermobacter marburgensis*, *Methanotorris formicicus* and *Methanothermobacter wolfei*. The purified enzymes were crystallized and their X-ray structures determined.
Results: The structure of the cobalamin-binding protein MtrA showed a unique binding mode of cobalamin. The structure around the axial cobalamin ligand indicated that binding of the methyl group to cobalamin potentially induces a conformational change, which is conveyed to the membrane-spanning pore and finally trigger Na^+ translocation. In MCR II from *M. marburgensis*, a new post- translationally modified amino acid, dehydroaspartate, was found, which was corroborated by the 2.2-Å crystal structure. This modification might be crucial for catalysis as it directly affects substrate binding.
Conclusion: The new cobalamin-binding motif implied a possible conformational change of MtrA triggering Na+-translocation. The dehydroaspartate residue in MCR II imposes a local backbone distortion, which might be required for adjusting the optimal conformation for substrate binding.

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MCV02

DNA-based investigations of plant production and bacterial consumption of chloromethane in the *Arabidopsis thaliana* phyllosphere

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Introduction: Chloromethane (CH_3Cl) is a toxic gas mainly produced naturally and in particular by plants, and significantly contributes to ozone destruction in the stratosphere. Conversely, CH_3Cl can be degraded and used as the sole carbon and energy source by specialized methylotrophic bacteria, which have been isolated from a variety of environments including the phyllosphere, i.e. the aerial parts of vegetation [1].
Objectives: The potential role of phyllospheric CH_3Cl-degrading bacteria as a filter for plant emissions of CH_3Cl was investigated using the *A. thaliana* model.
Materials & Methods: Emissions of CH_3Cl by *A. thaliana* were evaluated by a specific fluorescence-based bioreporter [2]. Bacterial functional and structural diversity in the *A. thaliana* phyllosphere was explored by qPCR, RT-qPCR and 454 pyrosequencing of amplicons of the 16S rRNA gene and of the *cmuA* gene encoding chloromethane dehalogenase.
Results: Three variants of *A. thaliana* with differential (i.e. low, wild-type, high) expression of the *HOL1* methyltransferase gene responsible for most of the CH_3Cl production in *A. thaliana* were investigated. CH_3Cl production paralleled *HOL1* expression, as assessed by the bioreporter [2]. Analysis of phyllosphere nucleic acids by qPCR and RT-qPCR showed that bacterial *cmuA* gene content and expression also increased with plant *HOL1* expression. The genus *Methylobacterium* dominated phyllospheric bacterial diversity in all investigated *A. thaliana HOL1* variants. The *A. thaliana* variant with highest expression of *HOL1* presented the broadest diversity of *cmuA* sequences.
Conclusions: The proportion, activity and diversity of CH_3Cl-degrading bacteria in the *A. thaliana* phyllosphere are modulated by differential expression of a single plant gene involved in the production of CH_3Cl. This suggests that CH_3Cl-degrading bacteria may play a role in controlling plant emissions of CH_3Cl to the atmosphere.

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MCV03

Fungal propionyl-CoA degradation via methylcitrate cycle and citric acid cycle: evidence for a second active site in aconitase AcoA

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Introduction: The filamentous fungus *Aspergillus nidulans* is capable of growing on propionate as sole carbon source. The activated form, propionyl-CoA, is degraded via the methylcitrate cycle (mcc). Propionyl-CoA condenses with oxaloacetate and forms methylcitrate which undergoes a de- and re-hydration to yield methylisocitrate that is finally cleaved by a specific lyase into pyruvate and succinate.
Objectives: The yet uncharacterized enzyme methylisocitrate dehydratase (mid) catalyzes the reaction from methylaconitate to methylisocitrate. This enzyme does not belong to the unique enzymes of the mcc and could not be isolated so far. In former studies, the citric acid cycle (cac) aconitase from mammalian mitochondria was capable of catalyzing this unusual side-reaction *in vitro*. Our previous investigations on the bacterium *Escherichia coli* showed that the cac aconitase AcnB acts as an mid *in vivo*.
Methods: Mid was isolated from propionate-grown fungal cells and analyzed by tryptic digestion and subsequent MALDI-TOF. Isolated protein was tested for activity and ESR and Mössbauer spectroscopies were performed to correlate oxidative state with enzyme activity.
Results: Although mammalian cac aconitases had been described to be only enzymatically active when bearing the intact [4Fe-4S]-Cluster, we clearly saw a segregation between mid and aconitase activity in the same protein in *A. nidulans*! The purified aconitase AcoA with reconstituted cluster showed the main activity with one of the three cac substrates. A significantly smaller activity was measured with the methylated substrates (as in the mcc), the normal side reaction. In contrast, when the aconitase AcoA from *A. nidulans* was slightly oxidatively damaged, the aconitase activity declined as expected but the activity with the methylated substrates increased, together with the occurence of the [3Fe-4S]-cluster.
Conclusion: Thus, our results provide the first example for changes in substrate spectrum of an aconitase by changes in the constitution of the iron-sulfur cluster. We conclude that during loss of the active Fe_a a new active site reconstitutes, either form the residual irons or from an unknown amino acid residue.

MCV04

Dedicated Maturation Factors for the essential Ribosome-associated Iron-Sulfur Protein Rli1

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Introduction: Iron-sulfur (Fe-S) clusters are important cofactors of numerous proteins and are present in all known forms of life. In eukaryotes, Fe-S proteins perform essential functions in cellular processes such as DNA replication, DNA repair and translation. The synthesis of the Fe-S cofactors and their subsequent insertion into apoproteins require three complex proteinaceous machineries. The maturation of cytosolic and nuclear Fe-S proteins is accomplished by the cooperation of the mitochondrial iron-sulfur cluster (ISC) assembly machinery, the ISC export machinery and the cytosolic iron-sulfur protein assembly (CIA) machinery. Currently, eight CIA proteins are known in the model organism *S. cerevisiae*. These proteins are evolutionarily conserved in virtually all eukaryotes, and form three subcomplexes that function in two main stages. First, a [4Fe-4S]-cluster is assembled on a scaffold protein complex. Second, the Fe-S cluster is transferred onto apoproteins by the so-called "CIA-targeting complex".
Objectives: Identification of new CIA factors and target Fe-S proteins.
Methods: Systematic analyses of the CIA-interactome by TAP-MS and Co-Immunoprecipitation. Functional characterization of so far unknown interaction partners and analysis of their role in cytosolic Fe-S cluster biogenesis.
Results: While studying the CIA-interactome, we discovered the essential factors Yae1 and Lto1 as interaction partners of the CIA targeting complex and the Fe-S protein Rli1 (RNAse L inhibitor 1). Rli1 is the first identified essential extra-mitochondrial Fe-S protein and directly interacts with the ribosome executing diverse roles in ribosome biogenesis and function. Depletion of Yae1 or Lto1 resulted in a specific maturation defect of Rli1 but surprisingly of no other cytosolic or nuclear target Fe-S protein. By adapting novel protein depletion technology we showed that the Fe-S cofactors remained stably associated with Rli1 when Yae1 was rapidly degraded suggesting that this protein functions as a Fe-S cluster biogenesis rather than stabilization factor.
Conclusion: Yae1 and Lto1 are dedicated maturation factors for the Fe-S protein Rli1 and represent the first example of specific Fe-S cluster targeting factors in the yeast cytosol.

MCV05

Cell wall recycling in *Staphylococcus aureus* – role of the N-acetylmuramic acid 6 phosphate etherase

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Bacteria can break down their own peptidoglycan (PGN) and reutilize the degradation products for cell wall synthesis or for energy supply. This process is called peptidoglycan recycling and well-studied in Gram-negative bacteria, including *Escherichia coli* (reviewed in[(1)]) and *Pseudomonas aeruginosa* ([2,3]), but still unclear in Gram-positive bacteria. Interestingly, Reith and Mayer (2011) [(4)] identified orthologs of *E. coli* cell wall recycling and N-acetylmuramic acid (MurNAc) reutilization proteins in the pathogenic *S. aureus.*

We generated a markerless deletion mutant of the putative N-acetylmuramic acid 6 phosphate etherase (MurNAc 6P etherase; MurQ), a distinctive recycling enzyme in *E. coli* that cleaves the D-lactyl rest of the substrate MurNAc 6P, yielding N-acetylglucosamine 6 phosphate (GlcNAc 6P). Using HPLC-MS technique we showed that this *S. aureus* mutant but not the wild type accumulates MurNAc 6P, suggesting that peptidoglycan recycling occurs in *S. aureus* and that this requires MurQ.
The accumulation of the MurNAc 6P in the *murQ* mutant already started in the exponential phase (OD≈2), reached a maximum in the early exponential phase (OD≈6.5), and remained at a high level after 24h of cultivation. The amount of MurNAc 6P was reduced after complementation the mutant with *murQ* from *S. aureus* on a plasmid. Moreover, the addition of MurNAc to the nutrient-rich growth medium resulted in up to 10 times higher concentrations of accumulated MurNAc 6P in the *murQ* mutant, but was absent in the wild type. Lastly, differences in the thickness of the cell wall between the *murQ* mutant and the wild type have been measured. We are currently investigating the physiological effect of impaired peptidoglycan recycling in *S. aureus*.
Better understanding the process of cell wall recycling in this pathogen may suggest new therapeutic approaches for treatment of multi-resistant *S. aureus* infections.

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MCV06
Structure-function relationships of the rhodopin 3,4-desaturase (CrtD) of *Rhodospirillum rubrum*
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It is now well established that the carotenoid present in the light-harvesting (LH) 1 complex is in the *all-trans* configuration. It has also been demonstrated that *in situ* modification of the carotenoid "ends" i.e. the 3,4,3´,4´-dehydrogenation, hydroxylation and methoxylation can occur during LH1 maturation. For each of these carotenoid maturation steps mentioned above, only a single enzyme (CrtD, CrtC, and CrtF, respectively) is present, which from hydropathy analysis appear to be localized to the cytoplasmic side of the membrane. This raises the interesting question as to how maturation of an *all-trans* carotenoid can occur *in situ*. In this study we focus on the *crtD* gene to cast light upon this question. The *crtD* gene, encoding the rhodopin 3,4,3´,4´-desaturase of *Rhodospirillum rubrum*, was cloned into a pRK404-derived broad host range vector and then subjected to extensive random mutagenesis. The mutagenized vectors were then used to complement a *crtD* deletion mutant (*R.rubrum* strain ST4 [1]). The resulting complemented mutants yielded colonies showing a variety of different colours (from brown to pink to purple), which were isolated for subsequent carotenoid content analysis. DNA sequencing of the interesting mutants yielded insights into the nature of the active site of CrtD. These results will be presented here.

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MCV07
Acetate assimilation via the methylaspartate cycle in haloarchaea
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The methylaspartate (MA) cycle is an anaplerotic acetate assimilation cycle recently discovered in *Haloarcula marismortui* (***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 MA and its following deamination leads 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 methylmalonyl-CoA and subsequently to succinyl-CoA, thus closing the cycle, whereas the glyoxylate condensation with acetyl-CoA yields the final product of the MA cycle malate. Further studies of the MA cycle in *H. marismortui* were hampered by the lack of the genetic system, and the genes for most of the characteristic enzymes of the cycle could only putatively be identified. Therefore, we decided to switch to *H. hispanica,* for which a gene knockout system is available (***2***). The MA cycle was obviously present in *H. hispanica*, as activities of its enzymes were strongly up-regulated in acetate-grown cells. Knockout mutants of the genes encoding its key enzymes (glutamate mutase, methylaspartate ammonia-lyase, putative mesaconate CoA-transferase and putative mesaconyl-CoA hydratase) were unable to grow in the presence of acetate. The growth of the complementation mutants as well as the growth on pyruvate (or acetate plus pyruvate) was not impaired. The mutants incubated in the presence of acetate accumulated intermediates of the MA cycle, and the enzyme assays confirmed the identification of HAH_1336 and HAH_1340 as mesaconate CoA-transferase and mesaconyl-CoA hydratase, respectively. Analysis of the distribution of the genes of the cycle in 81 sequenced haloarchaeal genomes showed that 53% of the haloarchaea use the MA cycle for acetate assimilation, although 39% are capable to use the glyoxylate cycle. Interestingly, 72% of the species using the MA cycle possess also the genes for polyhydroxyalkanoate (PHA) biosynthesis, whereas only 36% of the species with the glyoxylate cycle are capable to synthesize PHA. This suggests that the MA cycle is shaped for the PHA assimilation during starvation, whereas the glyoxylate cycle is probably adapted for growth on substrates metabolized via acetyl-CoA.

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MCV08
A novel function-based screen suited to seek RubisCOs from metagenomic libraries – improving our understanding of RubisCO regulation and activation mechanisms
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A comparison of the six for now known autotrophic CO_2 fixation pathways reveals that the Calvin Benson (CB) cycle accounts for most of Earth's net primary production (>99.5% of 105 x 10^9 tons/year). Here, the key carboxylation reaction is catalyzed by the Ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO), an enzyme which is present in all plants, but also in most photo- and chemolitoautotrophic prokaryotes. Various studies aiming at the phylogenetic distribution of RubisCO encoding genes substantiate the ubiquity of this enzyme. However, it remains enigmatic if the respective gene products really form functional enzymes. Although the vast majority of functional RubisCOs from uncultured organisms (>99%) remains inaccessible until recently no function-based approach was available that enables the detection of RubisCOs directly from the environment.
We here describe the first solely activity-based approach suited to seek RubisCO active fosmid clones from metagenomic libraries[1]. We identified 12 environmental, recombinant RubisCOs through this screen, which showed homologies to *Thiomicrospira crunogena*. One clone exemplarily was further investigated and comprised a 35.2 kb metagenomic insert, consisting of a RubisCO gene cluster and flanking DNA regions. Knock-out mutants were constructed for each gene encoded on the metagenomic fragment, demonstrating that RubisCO activity was significantly impaired through the deletion of twelve of the investigated genes. Besides having supported the assumption of specific genes and respective products to be involved as transcriptional regulators or posttranslational activators, one gene (*orf06*) was uncovered whose gene product has never been associated with RubisCO activity before, but is involved in positively regulating transcription of the two types of RubisCOs. This screen opens the door to detect up until now unexplored RubisCOs directly from environmental samples.

[1] S. Böhnke and M. Perner, ISMEJ (2014)

MEcV01
Archaea dominate the ammonia oxidizing communities in Savanna soils along a granitic and basaltic toposequence in Kruger National Park (South Africa)
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Ammonia oxidation, the first step of nitrification, is a central process of the nitrogen cycle in terrestrial ecosystems. Numerous studies revealed a numerical dominance of ammonia-oxidizing archaea (AOA) over ammonia-oxidizing bacteria (AOB) especially in non-fertilized and slightly to strongly acidic soils. However, only little is known about the potential role of AOA in nitrification in Savanna soils. We performed comparative investigations of AOA and AOB communities along a

basaltic and a granitic catena in the semi-arid, southern area of Kruger National Park. We hypothesized that (i) soil ammonia oxidizer community composition and abundance vary along and between the two catenas influenced by differences in soil and bedrock geochemistry, and that (ii) ammonia oxidizer communities are dominated by AOA under the given slightly acidic conditions. Soil concentrations of ammonium and nitrate ranged from 0 - 1,8 and 0 - 1,96 µmol/g soil, respectively. Quantitative PCR targeting archaeal and bacterial 16S rRNA genes and *amoA* genes encoding ammonia mono-oxygenase showed that AOA outnumbered AOB by more than two orders of magnitude and accounted for approximately 1% of the total microbial population. Increasing pH along the granitic catena had a positive effect on the abundance of AOA and total bacteria and was linked to changes in AOA community composition. Our results demonstrate a strong influence of local geology on ammonia oxidizer populations and suggest that nitrification in these Savanna soils could be controlled by archaea. Ongoing investigations address the influence of local geology on the total bacterial and archaeal diversity along the two catenas.

MEcV02

Effect of *e*CO_2 on microbial communities involved in N cycling in soils

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Introduction: To predict ecosystem reactions to elevated atmospheric CO_2 (eCO_2) it is essential to understand the interactions between plant carbon input, microbial community composition and activity and associated nutrient dynamics. Long-term observations (> 14 years) within the Giessen Free Air Carbon dioxide Enrichment (Giessen FACE) study on permanent grassland showed next to an enhanced biomass production an unexpected strong positive feedback effect on ecosystem respiration and nitrous oxide (N_2O) production.
Objectives: The overall goal of this study is to understand the long-term effects of eCO_2 and carbon input on microbial community composition and activity as well as the associated nitrogen dynamics, N_2O production and plant N uptake in the Giessen FACE study on permanent grassland.
Methods: Microbial analyses include exploring changes in the composition of microbial communities involved in the turnover of NH_4^+, NO_3^-, N_2O and N_2, i.e. ammonia oxidizing, denitrifying, and microbial communities involved in dissimilatory nitrate reduction to ammonia (DNRA). mRNA based analyses over time of these genes will be employed to comparably evaluate the long-term effects of eCO_2 on the structure and abundance of these communities.
Results: During this study we could observe a stable mRNA abundance and a transcriptionally active community composition for almost all genes for the first analyzed ring pair. Only *nirS* showed a lower abundance under ambient conditions as well as a change in the transcriptionally active community composition under eCO_2. The transcriptional activity of both NO_2^--reducing denitrifier groups (*nirK+nirS*) under eCO_2 lead also to a decrease of the *nosZ*/*nirK+nirS* transcript ratio compared to ambient conditions.
Conclusions: These first results can maybe explain the higher N2O production under the elevation of CO2, since denitrifiers are the major source of N2O emission from grassland soils.

MEcV03

Physiologic and genomic characterization of a novel *Nitrospira* species enriched under anaerobic, nitrate reducing conditions

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Among the genera capable of aerobic nitrite oxidation, members of the genus *Nitrospira* are dominant in many natural habitats and of vital importance for wastewater treatment. They are chemolithoautotrophic organisms capable of growth with nitrite and CO_2 as sole energy and carbon source. It further has been demonstrated that some *Nitrospira* can utilize simple organic carbon compounds and molecular hydrogen as alternative substrates and can switch to nitrate reduction under oxygen limitation. However, this metabolism has been assumed to be a survival strategy and in most systems studied so far *Nitrospira* was outcompeted by denitrifying organisms whenever anoxic conditions prevailed.
In this study a community of nitrogen cycle bacteria was enriched using a sample taken from the anaerobic compartment of a biofilter connected to a recirculating aquaculture system. The reactor was fed with filtered water from the aquaculture system, supplemented with ammonium, nitrite and nitrate. A stable enrichment culture was obtained which anaerobically converted ammonium, nitrite and nitrate into dinitrogen gas. The culture was dominated by an anaerobic ammonium-oxidizing *Brocadia*, a denitrifier belonging to the genus *Denitratisoma*, and a *Nitrospira* species. Fluorescence in situ hybridization analysis showed that anammox bacteria and *Nitrospira* coaggregated, while the denitrifiers formed separate clusters. This enrichment culture showed that anaerobic ammonium oxidizers and nitrite oxidizers, who to date were considered to be mutually exclusive, can be grown as a stable co-culture and, furthermore, that some *Nitrospira* species can be competitive under nitrate-reducing conditions. Phylogenetic and comparative genomic analyses of this *Nitrospira* revealed similarity to *N. moscoviensis*, and transcriptomic analyses allowed insights into the adaptations to anoxic conditions. Thus, after the previous discovery of hydrogenotrophic growth this study demonstrates another unexpected lifestyle for an organism believed to be competitive only under aerobic lithoautotrophic conditions. It further indicates that *Nitrospira* can be of interest for anoxic wastewater treatment systems in combination with anammox with high nitrate and ammonia loads.

MEcV04

Plant polymers modulate the active microbial community in paddy soil

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Rice straw is commonly used as organic fertilizer in rice farming. As a result, paddy soil microbial communities are adapted to plant polymer utilization. Rice straw breakdown has been studied in detail, but its heteropolymeric composition made it difficult to link particular community members to the breakdown of individual rice straw components.
We aimed to identify those paddy soil microbial populations that actively respond to the amendment of particular plant polymers. Using rice straw as a reference substrate, the analysis included individual straw components such as cellulose and xylan and, in addition, the fungal cell wall polymer chitin.
Paddy soil slurries amended with the respective polysaccharide were incubated for four weeks. Short-chain fatty acids and methane were monitored by HPLC and GC. 16S rRNA gene copy and transcript numbers were determined by quantitative PCR and RT-PCR, respectively. Samples for rRNA-based (ribo-tag) analyses were taken after an incubation period of 7 and 28 days. Total RNA was extracted and analyzed by Illumina RNA-Seq
Substrate amendment induced an increase in the 16S rRNA gene transcript numbers by up to three orders of magnitude. Methane formation was strongly enhanced upon substrate amendment. Key metabolic intermediates during rice straw and plant polymer breakdown were acetate, propionate and butyrate, with peak concentrations ranging between 2 and 30 mM dependent on the applied substrate. Acetate was the major discriminant of methanogen composition. *Methanosarcinaceae* (up to 4.5%) and *Methanocellaceae* (up to 1.5%) were the most abundant methanogen families. Pronounced community dynamics were observed for bacteria over time. Microbial communities amended with cellulose or chitin clustered together and distinct from those amended with straw or xylan. The distinct clustering was related to differential abundances of *Fibrobacteres* (up to 15%) and *Ruminococcaceae* (up to 25%).
Amendment of defined polysaccharides stimulated microbial activity and methane formation. Cellulose and chitin triggered a similar community response, although chitin is usually not present in paddy soils.The response to chitin indicated that paddy soil communities have broader polysaccharide-degrading capabilities than previously known.

MEcV05

Chitin Degradation by a Complex Microbial Community in Soil

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Chitin is an abundant biopolymer with high turnover in soils. Aerobic and anaerobic soil microbes degrade chitin by initial hydrolysis through exo- and endochitinases. In an aerated soil, oxygen distribution is highly heterogeneous and dynamic on the micro- to millimeter scale. Thus, different microbial redox guilds are simultaneously active when chitin is degraded. U-[^{13}C]chitin-based 16S rRNA stable isotope probing was used to identify chitinoclastic taxa under oxic and anoxic conditions in an aerated soil. Chitin was degraded during aerobic respiration, ammonification, and nitrification to carbon dioxide and nitrate under oxic conditions. *Chitinophagaceae, Cytophagaceae, Sphingobacteriaceae*, *Comamonadaceae* and *Pseudomonadaceae* were the first labelled genotypes (3-10 days). Subsequently, *Bdellovibrionalles, Planctomycetes and* uncultured *Myxococcales* and *Verrucomicrobia* were labelled suggesting that *Bacteriodetes* and *Beta-* and *Gammaproteobacteria* initially attacked chitin, followed by *Deltaproteobacteria, Planctomycetes* and *Verrucomicrobia,* which were likely secondary utilizers of the chitin-derived ^{13}C, i.e. those that utilized metabolic products. *Bdellovibrionaceae*, *Bacteriovoraceae and Myxococcales* are known to harbor predatory members and were labelled thus indicating the occurrence of potential predator-prey interactions under oxic conditions. When oxygen was absent, butyric and propionic acid fermentation occurred along with ammonification, nitrate and iron respiration and contributed to chitin mineralization. *Ruminococcaceae, Lachnospiraceae, Paenibacillacea,* and uncultured *Bacteroidetes* were predominantly labelled suggesting that they all together contributed to chitin degradation under anoxic conditions. Analyses of 18S rRNA, archaeal 16S rRNA, *chiA* genotypes, and the metaproteome will refine and complement the current findings on the chitinoclastic foodweb. There is first evidence that the detected bacteria have different functions in regard to chitin degradation.

MEcV06

Comparative analysis of the root microbiome of *Verticillium longisporum* resistant and susceptible rapseed line

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Agricultural plants harbor a high diversity of microbes colonizing the rhizosphere and endophytic compartment of roots, among those several plant growth promoting and antagonistic bacteria. Root exudation pattern and other so far unknown plant-derived factors can thereby strongly affect root colonization with specifically adapted microbial communities which can be involved in the protection of plants against root pathogenic fungi. We investigated root-associated rhizosphere and endophytic microbial communities of two contrasting oilseed rape double haploid lines from a cross of a susceptible and a resistant parent exhibiting resistance to *Verticillium longisporum*. Seedlings were germinated and grown for ten days in sand or soil, dip inoculated with a *V. longisporum* spore solution and grown in a sand/soil mix or soil for further four weeks. A clear disease pattern was developed after infection of the susceptible, but not of the resistant cultivar.
DNA was extracted from rhizosphere soil, root and hypocotyl samples (endophytic compartments) of the two cultivars grown in sand or soil. First microbial communities were compared by denaturing gradient gel electrophoreses (DGGE) using universal and actinobacterial-specific 16S rRNA gene-targeting primers. Second Illumina 16S rRNA gene amplicon sequencing was applied for detailed phylogenetic studies of the associated microbiomes. Preliminary data clearly indicated cultivar specific rhizosphere and endophytic microbial communities affected by growth in sand or soil. The comparative analysis of different root compartments furthermore showed clear differences in microbial communities inhabiting different plant compartments with cultivar specific differences. The data indicate that the ability to accumulate specific rhizosphere and endophytic microbial communities by rapeseed can play a role in the resistance to *V. longisporum* infections. Several bacteria were isolated and phylogenetically identified from the resistant cultivars which are potential biologicals for the protection of rape seed for *V. longisporum* infections.

MEcV07

Functional redundancy of electroactive microbial biofilms fed by domestic wastewater

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Introduction: Microbial electrochemical technologies represent a promising platform for sustainable wastewater treatment. They may not only allow energy savings by reducing the need for aeration in activated sludge tanks but even aim at an additional energy gain, e.g. in form of electricity in microbial fuel cells. The key element is the anode community, breaking down complex wastewater constituents and performing extracellular electron transfer.
Objectives: So far, most studies exploiting real domestic wastewater with microbial fuel cells focused on the engineering perspective instead of exploring the ecological niche of an electroactive microbial biofilm. In this study, the role of the microbial community of anodes treating domestic wastewater was investigated and potential functional interactions revealed.
Methods: Wastewater of a primary clarifier was regularly fed to lab scale batch reactors and the systems performance was assessed in terms of COD removal, substrate consumption, current production and coulombic efficiency as well as biomass formation and biomass composition using molecular techniques. In comparison, wastewater treatment in activated sludge basin like aerated reactors was performed.
Results: We have shown that bioelectrochemical reactors fed with identical real wastewater and providing identical habitats differ significantly on their reactor performance as well as in their related microbial planktonic and anodic biofilm communities. This shows that different communities of microorganisms are able to utilize the substrates provided by the wastewater. Different trophic networks were identified serving the same purpose: degrading organic compounds and producing current.
Conclusion: This functional redundancy as well as flexibility bares three main messages: i) wastewater derived electroactive microbial biofilms differ significantly from defined substrate laboratory cultures, ii) studies with real wastewaters need parallelization, especially when aiming on technical aspects like up-scaling, and iii) electroactive microbial biofilms are highly diverse and can occupy broad ecological niches.

MEcV08

Characterization of the active fraction of the chicken microbiota using metaproteomics

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Introduction: Microbiota colonizing animal gastrointestinal tract (GIT) plays an essential role for nutrients availability and animal health. Microbial activities and composition are influenced by feed composition and environmental factors. Phosphorus (P) is an essential mineral for all animals since it is needed for synthesis of P-containing biomolecules and P-lack can affect growth and productivity of livestock. Inorganic P and microbial phytases (MP) are supplied in fowl diets because the feedstuff´s organic P may not be fully absorbed due of the low amount of intrinsic phytases.
Objectives: A metaproteomic approach was used to investigate the effects of supplemental inorganic P and/or MP in the feed and of the formed inositol phosphates on the composition of the active microbial community colonizing the chicken´s GIT.
Materials & Methods: Broilers were grouped and fed with six different diets varying in supplemented P and/or amount of MP units added. For each treatment, two pooled samples (each of 4 animals) of crop and caeca were used for protein extraction. One dimensional-nanoLC-ESI-MS/MS approach was used to analyze the metaproteome in technical triplicates. MS/MS data were analyzed using both Proteome Discoverer and MaxQuant software for a qualitative and quantitative metaproteome

characterization.
Results: Crop samples showed a low bacterial diversity with *Lactobacillaceae* as the dominant specimen. Caecal microbiota composition changed among diets. Proteins belonging to *Ruminococcaceae* and *Erysipelotrichaceae* were identified in higher amount in diets containing MP. Conversely, proteins of *Lactobacillaceae* decrease with the MP addition. Among diets with P addition, proteins of *Erysipelotrichaceae* and *Bacteroidaceae* were more abundant; while proteins of *Eubacteriaceae* were greater in diets without P addition.
Conclusion: We highlighted that dietary P and/or MP addition have a strong effect on the structure and activities of the gut microbiota and that metaproteomic approaches enable deep insights in the active microbial fraction.

MEcV09
Different utilization of alginate among marine *Alteromonas macleodii* ecotypes indicate ecological speciation
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Alginate, a major structural polysaccharide from brown algae, is an important microbial substrate in marine environments. Here, we studied the physiology and genomics of alginate degradation by *Alteromonas macleodii* strain 83-1, which originates from Atlantic upwelling waters off Mauretania. Cell densities when grown on alginate were comparable to those on monosaccharides, but the growth efficiency was lower as determined by HPLC. Whole-genome analyses revealed an "alginolytic operon" containing several putative alginate lyases, and RT-qPCR confirmed their involvement in the degradation by showing a significant induction when grown on alginate. Comparative genomics demonstrated that the alginolytic operon is part of a genomic island only present in strains from the *A. macleodii* "surface clade", while it is absent in strains from the "deep clade". Differing alginolytic capacities between *A. macleodii* clades have been confirmed by comparative physiology including strain 83-1, the type strain (also belonging to the surface clade), and one representative strain from the deep clade. Additional growth experiments revealed that the hydrolytic potential of *A. macleodii* strain 83-1 is not limited to alginate, but includes other algal polysaccharides such as laminarin, xylan, and pullulan. This significantly expands previously documented metabolic capabilities of *A. macleodii* and contributes to the understanding of physiological adaptations among *A. macleodii* ecotypes. Detected biogeographic patterns in the distribution of *A. macleodii* alginate lyases in marine metagenomes furthermore indicated that their involvement in alginate degradation varies between oceanic provinces. Our study overall also demonstrates that polysaccharide utilization in the oceans is performed by more taxa than those commonly considered as prime polymer degraders, such as the *Bacteroidetes*.

MEcV10
Genome and ecophysiology of a gammaproteobacterium of the genus *Reinekea* associated with North Sea spring phytoplankton blooms
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Introduction: In 2009 we investigated the response of bacterioplankton to the release of algal-derived organic compounds during a spring phytoplankton bloom in the North Sea. We observed a swift succession of distinct bacterioplankton clades with different ecological niches, likely due to changes in substrate availability over time [1]. Within the succession, *Reinekea* spp. rapidly increased to 16% relative abundance during the bloom and subsequently vanished again. However, explanations for this short-lived *Reinekea* bloom remained elusive.
Objectives: In 2010 we isolated a *Reinekea* strain (Hel1_31_D35) during the spring bloom at the same location. Metagenome read recruitment showed that this isolate is a representative of *Reinekea* spp. from the 2009 bloom [2]. We currently study this isolate to shed light on the ecophysiological role of *Reinekea* spp. in the environment and their possible recurrence during subsequent blooms.
Methods: We sequenced the *Reinekea* sp. Hel1_31_D35 genome and assessed its physiological potential using (i) *in silico* metabolic reconstruction, (ii) dedicated growth experiments, and (iii) reanalysis of bacterioplankton metatranscriptome data from the 2009 spring bloom. In addition, we investigated the extent of recurrence and the level of clonality of *Reinekea* spp. during subsequent blooms (2009-2012) using metagenome data and 16S rRNA gene sequences.
Results: The *Reinekea* population recurred during the subsequent blooms, albeit with decreasing abundances, and exhibited a high level of clonality. *Reinekea* sp. Hel1_31_D35 has a broad capacity for the utilization of low molecular weight compounds. *In situ* expression of genes showed potential for the acquisition of various phosphorus compounds as well as presence of RTX toxins.
Conclusion: The *Reinekea* population observed in the sampled bloom events likely consisted of a single species. The representative isolate Hel1_31_D35 is a generalist capable of utilizing a broad substrate spectrum with effective phosphorous scavenging. Genome analysis indicated toxin production, but it remains to be seen whether this strain can inhibit growth of other bacteria or algae as a strategy for survival.

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[2] R.L. Hahnke *et. al.*, *Environ Microbiol*,10.1111/1462-2920.12479 (2014).

MEcV11
The sixth element – a 102-kb plasmid of *Dinoroseobacter shibae* modulates chromosomal gene expression
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Dinoroseobacter shibae DFL-12 (DSM 16493^T), a facultative anaerobic photoheterotrophic alphaproteobacterium, is a model organism of the transregio SFB *Roseobacter* (TRR 51). Its genome comprises five extrachromosomal elements (ECRs), two chromids and three plasmids, with sizes between 72-kb and 191-kb. Their relevance has recently been documented in a study based on transposon mutagenesis, which showed that a third of the genes essential for anaerobic denitrifying growth are located on ECRs. Starvation experiments moreover revealed the indispensability of the 72-kb chromid for survival under light-dark cycling. Surprisingly, we detected an additional plasmid with a size of about 102-kb via pulsed field gel electrophoresis (PFGE) in an old glycerol stock of *D. shibae* DFL-12. Sequencing and manual annotation showed that the respective RepABC-9 type replicon harbors a complete type IV secretion system and many genes associated with heavy metal resistance. Comparative genome analyses allowed us to identify a highly conserved syntenous sister plasmid in *Roseobacter litoralis*, thus providing the first example for horizontal plasmid exchange between distantly related Rhodobacterales. Finally, we established transcriptome data from both strains via Illumina sequencing and validated the experimental reproducibility by three biological replicates. Astonishingly, our comparative analyses showed that the 102-kb plasmid is a strong modulator for the expression of chromosomal genes. Its presence correlates with the downregulation of e.g., flagellar genes, whereas a gene cluster for inositol phosphate metabolism and the *nos*-operon including the nitrous-oxide reductase is upregulated. These global effects, which may be triggered by a RNA polymerase sigma-70 factor located on the sixth element, exemplify the close regulatory interplay of chromosome and ECRs in this exciting group of marine Alphaproteobacteria.

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MEcV12

Free-living, particle-associated and benthic *Roseobacter* populations show distinctive distributions along the German/Scandinavian North Sea coast

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The *Roseobacter* clade is regarded to be a major bacterial lineage in marine environments. This clade represents a numerically significant part not only of pelagic, but also of benthic microbial communities. To understand their biogeography and specific metabolic adaptations, we compared the diversity of *Roseobacter*-affiliated bacteria within sediment and water samples from the eastern North Sea by cultivation-independent and cultivation-based methods. North Sea samples were analyzed by DGGE using *Roseobacter*-specific primers to identify regional and spatial differences. Overall, cluster analysis of DGGE patterns revealed specific compositions of free-living and attached populations. Within the attached fraction, communities from surface-near particles (3 meters below sea level; mbsl) clustered separately, while sinking particles (10-40 mbsl) and sediment surfaces (23-181 mbsl) showed similar arrangements. Additionally, all samples grouped according to their geographical origin (German/Danish coast, Norwegian trench). These results were confirmed by high throughput amplicon-based analysis. About 400,000 16S rRNA gene sequences were obtained from all sites and compartments. An average of ~17% per sample were affiliated to the *Roseobacter* clade with ~23% of the free-living fraction and about 18% and 2% at particles and in sediments. Phylogenetic analysis indicated an increasing diversity of *Roseobacter* populations from the sea surface to the seafloor. As roseobacters are known to contribute to sulfur transformations, most probable number (MPN) experiments were set up with media containing either dimethyl sulfide (DMS), dimethyl sulfonium propionate (DMSP) or dimethyl sulfoxide (DMSO). DMSO-containing enrichments showed highest MPN numbers for the sediment surface (up to 2.1 x 10^7 roseobacters/cm^3). A total of 20 roseobacters (12 from sediments) were isolated from DMSP- and DMS-containing dilution series. One *Shimia haliotis*-affiliated isolate showed interesting phenotypic characteristics (bundle forming pili) and was subjected to genome sequencing to identify adaptation mechanisms. As the sum of our isolates represented only 0.03% of all *Bacteria*, pyrosequencing allows to determine their relative abundance in the environment even though they are present in low numbers.

MEcV13

Polysaccharide Hydrolysis and Visualization of Uptake Across Contrasting Oceanic Provinces in the Atlantic Ocean.

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Phytoplankton derived carbohydrates are among the most abundant organic carbon sources in the oceans. The uptake and utilization of polysaccharides requires specialized genetic machinery. Recent genomic analyses have shown that there is a wide distribution of carbohydrate utilization capabilities within marine heterotrophic bacteria. Often they use extracellular enzymes to reduce the polysaccharide to the required size. During the Atlantic Meridional Transect 22 we set out to identify the key organisms, which hydrolyze specific polysaccharides and calculated substrate hydrolysis rates between contrasting oceanic provinces.
To analyse the polysaccharide utilization of marine microorganisms we incubated seawater from five oceanic provinces with fluorescently labeled polysaccharides (Laminarin, Xylan, Chondroitin sulfate) for up to 18 days. During the course of the incubations we analysed changes in the community composition using next generation sequencing. Additionally we analysed the hydrolysis rates within different provinces. Finally we calculated cellular abundance of key groups using fluorescence *in situ* hybridization in combination with fluorescent substrate staining to show cell-substrate interactions.
There were marked differences in the hydrolysis rate between communities from different oceanic provinces, with Laminarin showing the highest and earliest enzymatic activity. Chondroitin sulphate showed the most variable enzymatic activity. It was detected early in high productivity regions, but not until 18 days in the gyral regions. This maybe explained by the variability in the complexity of the polysaccharide substrates. There was a significant change in the bacterial community within each substrate incubation, over time and between different substrates, in all oceanic provinces. The most dominant organisms within the incubations were members of the *Bacteroidetes, Alteromonadales, Verrucomicrobia* and *Planctomycetes*. However the dominant genera between each station within the same substrate incubation varied.
This study combined the rates of polysaccharide utilisation of contrasting communities with the visualisation, identification and enumeration of the key organisms, offering a new in-depth insight into the marine carbon cycle.

MEcV14

Heterotrophic *Proteobacteria* with hydrocarbon degrading potential as major players in intermediate mixing zones at hydrothermal vents

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Introduction: Deep sea hydrothermal vents are habitats characterized by steep gradients in environmental conditions within very small spatial scales, originating from mixing of hydrothermal fluids enriched in reduced compounds with cold oligotrophic sea water. Until now, most of the studies investigating microorganisms at hydrothermal vents addressed chemolithoautotrophic organisms living immediately at the emission point of diffuse or focused fluids, or in hydrothermal plumes in the water column.
Objectives: Our study aims to expand our knowledge of microbial communities at hydrothermal vents by investigating the community structure at different points within mixing gradients, from point of fluid emission, to non-buoyant hydrothermal plume. Additionally, we addressed the role of heterotrophic microorganisms in these habitats.
Methods: At the Menez Gwen hydrothermal field we sampled diffuse fluids at, and in close proximity to points of emission as well as hydrothermal plumes. Microbial community compositions were assessed by 16S rRNA gene tag sequencing and fluorescence *in situ* hybridization (FISH). Metagenome sequencing was conducted and draft genomes of microbial groups of interest were retrieved by binning and targeted re-assembly of metagenomic reads.
Results: We identified three distinct types of microbial communities. At the point of fluid emission the communities were dominated by chemolithoautotrophic *Epsilonproteobacteria*. The distant plume communities were dominated by deep-sea water column bacterial clades. In the intermediate mixing zone surrounding the vents, we detected a third type of community, clearly distinct from the other two. This third type of community was characterized by a dominance of heterotrophic *Gammaproteobacteria* and *Alphaproteobacteria* genera related to alkane degraders. Metagenome analysis confirmed a hydrocarbon degrading metabolic potential in this community. Draft genome analysis allowed a further characterization of these potential hydrocarbon oxidizers.
Conclusion: Free-living microbial communities at hydrothermal vents are clearly structured along a mixing gradient of hydrothermal fluids and sea water. The intermediate mixing zone is dominated by heterotrophic *Proteobacteria*. Our data suggest that hydrocarbon oxidation is an important driver for heterotrophy at hydrothermal fields.

MEcV15

Quantifying Microbial Communities of the Methane Cycle in two Subsea Permafrost Deposits of the Central Laptev Sea

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Introduction: Submarine arctic permafrost was formed when sea level rise flooded terrestrial permafrost and warmed the frozen sediments during the Holocene. This thawing permafrost may play a major role in global warming as it stores huge amounts of organic carbon. Hitherto, the extent and importance of microbial activity on carbon transformations as well as the reactions of microorganisms to the environmental changes

accompanying the inundation of permafrost by sea water are poorly understood.
Objectives: We investigated the impact of sea level rise on methane cycle associated microbial communities in degrading permafrost of the western and central Laptev Sea shelf, Siberian.
Material and methods: Two sediment cores were retrieved (77 m and 52 m deep) from the coastal shelf north of Cape Mamontov Klyk 'C2' (11.5 km offshore) and west of Buor Khaya Peninsula 'BK2' (800 m offshore), respectively. Chemical parameters such as total organic carbon (TOC), methane concentrations and ^{13}C isotope values were measured and correlated with molecular analysis of microbial communities along the cores.
Results: Frozen sediment was encountered at 35.5 (C2) and 28 (BK2) meters below sea level (mbsl), respectively. Methane concentrations varied between 0.21 and 284.31 nmol g^{-1} with highest values in the frozen permafrost and lowest values in the overlaying unfrozen sediments. Low methane concentrations in the unfrozen sediments of BK2 (16.25-28.20 mbsl) correlated with the highest carbon isotope values of methane (-29.8 ‰ VPDB) indicating microbial oxidation of methane under *in situ* conditions in the thawing permafrost. Bacterial cell numbers (16S rRNA) and functional genes (*mcrA*) of methanogenic archaea and sulphate reducing bacteria (*dsrB*) analysed by quantitative PCR often peaked at high methane or TOC concentrations in the frozen permafrost and showed specific $^{13}CH_4$ isotopic values indicating distinct methanogenic populations.
Conclusion: Our data give first insights into how the inundation of permafrost by sea water influences the abundance of active members of the microbial methane cycle both along thawed and still frozen sediments. Further analysis of amplicon sequencing and quantitative analysis by fluorescence *in situ* hybridization will give a better overview of these highly dynamic microbial populations.

MEcV16
Characterization of continental deep-subsurface microbial communities in the Iberian Pyrite Belt (IPB)
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Introduction: From the astrobiological point of view, terrestrial subsurface microbiology is a matter of growing interest. In this context, one of the most fascinating environments is the Iberian Pyrite Belt (IPB), a massive iron-sulfide deposit located in the southwest of Spain. This rather unique environment, that resembles the Mars milieu, sustains a deep subsurface microbial-driven geochemistry that results in oxidation of sulfides in the pyrite to sulfuric acid and solubilization of ferric salts to give rise to the extremely acidic Tinto River.
Objective: To describe the microbial composition in the IPB subsurface and to characterize the metabolic processes that drives this ecosystem.
Methods: A 612 meters depth borehole was drilled in the IPB subsurface where the presence of aquifers could be hosting microbial activity. The rock powder and shards from recovered core samples were analyzed by ICP-MS and ion chromatography in order to elucidate their elemental composition and detect the presence of soluble ions and small organic molecules. DNA was isolated from the samples, amplified by a MDA reaction and used to construct Illumina MiSeq amplicon libraries of the 16S rRNA gene. The presence of metabolically active microbial cells was corroborated by CARD-FISH using specific probes for different microbial taxa.
Results: A rich microbial community was detected in several samples from 103 to 612 meters below ground surface (MBGS) using 16S rRNA gene profiling. This diversity was corroborated in selected samples using CARD-FISH. Interestingly, the bacterial ecosystem was dominated in some depths by organisms belonging to cyanobacteria. Members from proteobacteria were also very abundant along the borehole. The highest bacterial diversity was found at 420 and 496 MBGS, which correlates with small peaks of oxalate, acetate and ammonium. A high microbial diversity was also found at 336.5 MBGS, where high concentrations of acetate, Fe^{2+} and Fe^{3+} are present.
Conclusion: The IPB deep subsurface is inhabited by an unexpectedly rich microbial community, which varies throughout the borehole. A deeper analysis of this unique microbial ecosystem is currently ongoing to further unravel the geomicrobiological cycles in this and other continental deposits.

MEvV01
***Paracoccus denitrificans* and nitrite: 500 generations – problem solved**
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Denitrifying organisms reduce nitrate in four reaction steps to dinitrogen via nitrite, nitric oxide and nitrous oxide. Each step can be performed by one single or by multiple, cross-feeding populations. When growing under carbon limitation, denitrifying bacteria rapidly face toxic concentrations of nitrite. In this experimental evolution study we investigated potential driving forces for adaptive physiological improvements of *Paracoccus denitrificans* Pd1222 under anoxic, denitrifying conditions. We maintained four acetate (carbon and energy source) or nitrate (electron acceptor) limited chemostats for more than 800 generations and analyzed substrate conversions, genome variation and transcriptional activities.
The response of *P. denitrificans* to acetate limitation was more pronounced than to nitrate limitation. Under acetate limitation, nitrite accumulated in the culture, until a transition after 500 generations resulted in the complete conversion of nitrite. The evolved phenotype showed significantly up- and down-regulated transcription of numerous genes encoding the denitrifying respiratory enzymes and various groups of transporters and regulators. The strong physiological responses might be explained by beneficial mutations at increased frequency after 500 generations together with potentially emerged subpopulations. Although similar numbers of mutations were identified under nitrate limitation, this selective pressure had minor effects on the phenotype of *P. denitrificans*. Minor changes of transcriptional profiles were observed over time and conversions of acetate and nitrate remained nearly constant. The results presented here suggest a high potential of denitrifying organisms to adapt to certain environmental conditions and to shape the structure of environmental microbial communities.

MEvV02
Laboratory evolution of a fungal pathogen in macrophages restores virulence of a non-pathogenic mutant
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Laboratory evolution of fungal pathogens has proven highly useful in the investigation of antifungal drug resistance. We used a similar approach to study host-specific adaptations in the fungal pathogens *Candida albicans* and *C. glabrata*. To this end, we established a continuous co-culture model with macrophages, which play a central role in host defense against fungi (1,2).
Wild-type *C. albicans* can readily escape macrophages *in vitro* by forming hyphae. These are induced via a complex, well-investigated signalling pathway in response to environmental stimuli. We used a *C. albicans* mutant defective in hyphal formation as a case study to determine the adaptation potential of fungi to their host. This mutant lacks two central transcription factor genes of the hyphae formation program (*EFG1* and *CPH1*) and thus cannot respond to triggers of hyphae formation. Hence, the mutant was stuck within the phagosome, where it survived and replicated under severe stress.
During *in vitro* evolution, the *efg1*Δ/*cph1*Δ strain nearly completely regained its ability to form hyphae, allowing it to escape from macrophages, and the transcription program of hyphae formation. Importantly, the connection between triggers of hyphae formation and response was re-established. Consequently, virulence in a mouse model of systemic infection, essentially absent in the parental mutant, was mostly restored.

Genome sequencing in combination with RNA-Seq revealed a single nucleotide exchange to be responsible for this phenotypic reversal. We detected this mutation in the Ssn3 kinase, a part of the Mediator complex. Introduction of a single mutant allele into the *efg1*Δ/*cph1*Δ mutant was sufficient to bypass the two transcription factors, hinting toward a hitherto unknown layer in morphogenesis regulation (1).
In summary, we show that experimental evolution can force pathogens to bypass even central factors of a signalling pathway. This suggests that laboratory evolution can be used as a powerful, unbiased tool to uncover novel and unexpected pathways.

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MEvV03
Sociomicrobiology of *Bacillus subtilis* pellicle biofilms
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Biofilms are structurally complex bacterial communities that are considered to be the most common lifestyle of bacteria in nature. The cells in a biofilm are enclosed in an extracellular matrix that mediates the attachment of the cells to each other or to surfaces, holding the biofilm together. Essential components of the extracellular matrix in *Bacillus subtilis* biofilms are exopolysaccharides (EPS) and the protein TasA that forms extracellular amyloid-like fibers, which are considered to be public goods. When colonizing a surface, spatial segregation provides a solution for public good producers to outcompete non-cooperators [1,2]. Interestingly, different mechanisms seem to benefit matrix producer wild type strains at the air-liquid interface biofilms, called pellicles.
Direct co-cultivation tests and experimental evolution provide a powerful approach to examine the evolutionary dynamics of bacterial populations and interactions among its members. Using a fluorescent based method to quantify the fitness of bacterial strains in cocultures, various traits were examined during pellicle development that provided additional insights as compared to experiments with single strains. Using direct competition tests with various mutants against the wild type, we demonstrate how motility and aerotaxis contribute to fitness during *B. subtilis* pellicle development.
Strains lacking either of the matrix components reap the benefit from the matrix produced by the wild type population and increase their relative numbers in pellicle. However, the double mutant strain that lacks both EPS and TasA are excluded from the pellicle built by the wild type cells. The double mutant non-producer strain was co-cultivated with the matrix producer wild type strain and reinoculated every 2-3 days including an artificial dispersal and a sporulation bottleneck. In this experimental evolution, the non-producer strain regained its fitness after several rounds of pellicle growth. Using competition assays, we determined that the non-producer strain evolved in the co-culture and increased its ability to incorporate into the pellicle.

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MEvV04
On the evolution of wrinkly spreaders, wrinkleality and fitness
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Introduction: Bacterial adaptive radiation has been extensively studied using *Pseudomonas fluorescens* SBW25 which gives rise to the Wrinkly Spreader (WS), a class of adaptive mutants recognized by a wrinkled colony morphology and capable of producing a robust biofilm at the air-liquid interface of static microcosms (reviewed by [1]). The WS phenotype is believed to be the result of mutations targeting different diguanylate cyclases (DGCs) to up-regulate intracellular levels of c-di-GMP and induce the same final pathway. Although variations in colony morphology within the WS class have been noted, variations in the WS phenotype (wrinkleality) determined in static microcosms has not been quantified, nor related to the fitness advantage WS isolates have over the ancestral strain or other non-biofilm-forming competitors.
Objectives: To investigate the relationship between wrinkleality and fitness using WS isolates evolved in microcosms containing different growth media.
Methods: Wrinkleality was quantified using the combined biofilm assay (measuring growth, biofilm strength and attachment levels) in microcosms containing five different media including King's B (KB). Competitive fitness was determined in KB microcosms compared to the non-biofilm-forming reference strain SM-13. Data were analyzed by ANOVA and fitness modeled using a GLM approach with environment as the main factor and wrinklealities as covariates.
Results: Significant variation in wrinkleality and fitness was observed between and within WS isolates evolved in different environments. Environment, growth, strength and attachment were all found to have significant effects on fitness.
Conclusion: Environments were found to select for subtly different classes of WS that could be quantified and differentiated on the basis of wrinkleality. The underlying differences in phenotype could also help to explain the variation seen in fitnesses. These findings also provide substantial evidence to suggest that WS mutations might have pleiotropic effects, or that WS isolates accumulate secondary mutations that effect the final phenotype.

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MEvV05
Evolutionary dynamics of *Salmonella* Typhimurium cooperative virulence
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The expression of virulence by *Salmonella* Typhimurium correlates with substantial fitness cost for the pathogen (1). On the other hand, virulence factors trigger inflammation in the intestinal tract of *S.* Tm hosts which provides the pathogen with a competitive advantage over the protective microbiota (2). Using experimental evolution, we found that defector mutants unable to express virulence and therefore not paying the associated fitness cost are selected during within-host growth (3). Defectors profit from the inflammation triggered by the virulent wild-type population. This makes the virulence of *S.* Tm an unstable cooperative trait and raises the question of its evolution. We discovered that several factors could favor cooperative virulence. First, the fine-tuned bistable virulence expression restrains the rise of avirulent defectors during within-host competition (3). Second, antibiotic treatments select for virulent bacteria able to form persisters inside the host tissues (4). Third, adaptive immunity limits the growth of defectors in vaccinated hosts. Fourth, defectors alone cannot reach maximum population size in hosts protected by their microbiota which should impair the transmission of defectors to the next host. Overall, our observations demonstrate that evolution of cooperative virulence is a complex process depending on genetic and ecological factors. Understanding this dynamics provides fundamental insights for the design of innovative strategies to fight pathogenic bacteria.

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MEvV06
Microevolution of *Pseudomonas aeruginosa* in cystic fibrosis lungs
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Introduction: Chronic airway infections with the opportunistic pathogen *Pseudomonas aeruginosa* determine the clinical course of most patients with cystic fibrosis (CF). During the course of the chronic infection the *P. aeruginosa* bacteria undergo microevolution which is supposedly linked to better adaptation to the lung habitat.
Objectives: At our local CF clinic, serial *P. aeruginosa* isolates were collected in half year intervals from 35 CF patients who became colonized with *P. aeruginosa* in the 1980s. The microevolution events occurring in CF lungs were investigated for isolates from the six patients with the mildest and the six patients with the most severe clinical courses in order to investigate associations between microevolution during chronic infection and the course of the disease.
Methods: Serial isolates were genotyped with a customized microarray to determine the clonal lineages. Sequential isolates of the initially colonizing clone were then subjected to whole genome sequencing by SOLiD5500 technology. Nucleotide variations compared to the PA14 reference genome were extracted, filtered, annotated and used for the reconstruction of clades. The 250 sequenced bacterial isolates were

characterized in mutation rates and some phenotypic traits such as morphology, motility and secretion of virulence effectors.
Results: Exopolysaccharide biosynthesis, antimicrobial resistance and global regulators of lifestyle and metabolism are the most common functional categories of *P. aeruginosa* genes hit by mutations in the CF lungs. The observed microevolution was not uniform. *P. aeruginosa* clones in lungs of severely affected patients repetitively generated descendants with stop mutations or drastic amino acid changes in key genes of lifestyle, but these loss-of-function mutants were not recovered from the lungs at later time points. In contrast, *P. aeruginosa* clones predominantly acquired mutations causing neutral or benign amino acid substitutions in patients who maintained a normal function of their chronically colonized lungs for up to 30 years.
Conclusion: Results obtained so far indicate an association of *P. aeruginosa* microevolution modes in CF lungs with the severity of the chronic lung infection.
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MEvV07
The mtDNA sequence of the *Mucor*-related fusion parasite *Parasitella parasitica* – evidence for an unusually high number of intron-situated homing endonuclease genes
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Introduction: Infection of the mucoralean fungus *Absidia glauca* by the mycoparasite *Parasitella parasitica* is accompanied by mycelial fusion between the partners. This process involves the formation of an inter-specific cytoplasmic continuum, leading to transfer of nuclei and presumably of mitochondria. Whereas transfer of nuclear DNA has been studied at the molecular level [1, 2], the behaviour of mitochondria has never been investigated.
Objective: We intend to provide the necessary experimental prerequisites for analyzing migration, establishment, and possibly recombination of *Parasitella* mtDNA following infection.
Methods: The mtDNA sequence of *P. parasitica* was recovered from total genome data of of *P. parasitica*. Sequencing was performed by 454 and Illumina sequencing.
Results: The mitochondrial DNA of *P. parasitica* (ENAKM38227)is represented by a single circular molecule with the length of 83,361 bp, which places it among those fungi, containing very large mtDNA. It harbours all the genes for proteins involved in making up the respiratory chain that are normally found in mitochondria. On the whole, we identified and annotated 41 protein-coding genes and 26 genes for tRNAs. The most prominent property is the presence of 27 genes for homing endonucleases, most of which are found to reside in introns [3].
Conclusions: The availability of the mtDNA sequence of *Parasitella parasitica* enables us to follow the fate of mitochondria after mycelial fusion between *Parasitella* and its hosts. Experiments along this line have been started. We like to forward the hypothesis that the acquisition of the unusually high number of homing endonuclease genes is the consequence of the evolutionary trend of *Parasitella* introns towards higher mobility and selfishness. Such behaviour may be considered as a reasonable evolutionary strategy for selfish elements of a parasite, undergoing fusion with its hosts.

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MEvV08
Proteome and carbon flux analysis of *P. aeruginosa* clinical isolates from different infection sites
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Introduction: *P. aeruginosa* is an opportunistic pathogen, which can be frequently isolated from different infection sites such as burn, lung and urinary tract.
Objectives: To shed light on molecular niche-specific adaptation mechanisms of *P. aeruginosa* clinical isolates, absolute quantities of soluble proteins expressed by the different strains were determined. Moreover, the metabolic diversity of the different isolates has been investigated by ^{13}C-metabolic flux analyses.
Methods: Twelve different *P. aeruginosa* isolates from lung, burn and urinary tract infections, including the type strains PAO1 and PA14, were cultivated in minimal medium containing ^{13}C-labeled glucose. Extracted amino acids and metabolites were analyzed by GC-MS or HPLC. Proteins were identified and quantified by a gel- and label-free proteomics approach (LC/MS^{E}).
Results: In total around 500 proteins belong to the "core-proteome" of the tested clinical isolates. The vast majority of these proteins is rather constantly expressed in all the strains and exhibits a relatively low coefficient of variance. In contrast, 60 proteins, e.g. the outer membrane protein H1 (OprH) and the corresponding response regulator PhoP, were found to be expressed in different amounts depending on isolate. Notably, OprH and PhoP expression rates seem to be elevated when isolates from burn and the urinary tract were compared to lung isolates. Moreover, ferritin was found to be more abundant in burn isolates compared to lung or urinary tract isolates. Analysis of carbon fluxes indicates that all isolates metabolize glucose via the Entner-Doudoroff pathway. However, only urinary tract isolates were utilizing the labeled substrate to synthesize alginate.
Conclusion: Our combined label-free quantitative proteomics and metabolic flux analyses of *P. aeruginosa* isolates indicates that processes such as membrane stabilization (OprH, PhoP), iron storage (ferritin) and exopolysaccharide synthesis (alginate) can be involved in niche-specific adaptation processes.

MIV01
Hiding in plain sight, root endophytic community is the 'true' producer of maytansine in *Putterlickia* plants
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Introduction: Endophytes, a diverse group of microorganisms ubiquitous in plants colonize living internal tissues of plants in a mutualistic fashion [1]. Given the central role of chemical crosstalk in plants and endophytes, it is compelling that certain compounds or their precursors formerly believed to be synthesized only by plants or exclusively considered plant metabolites can be produced by endophytes or other plant-associated microorganisms [2]. For the important anticancer and cytotoxic compound maytansine, the actual producer(s) responsible for its biosynthesis in plants has been an open question since its discovery in the 1970s.
Objectives: The main objective of our work was to experimentally prove whether maytansine is produced by *Putterlickia verrucosa* and *Putterlickia retrospinosa* plants, or by the endophytic microbial community harbored in them.
Methods: After isolating the endophytic community from different tissues of *Putterlickia* plants [3], we used the 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), a maytansine-specific selective microbiological assay, and gene discovery methods to elucidate the source and site of maytansine biosynthesis.
Results: Evaluation of the root endophytic community by chemical characterization of their fermentation products using HPLC-HRMS^{n}, along with a selective bioautography, revealed the endophytic production of maytansine. This was further confirmed by the discovery of specific AHBA synthase genes only in the root endophytic communities and not in the host plants. Finally, MALDI-imaging-HRMS demonstrated that maytansine produced by the endophytes is accumulated mainly in the root cortex of both plants.
Conclusion: Our study demonstrated that maytansine is actually a biosynthetic product of root-associated endophytic microorganisms. The knowledge gained from this study provides fundamental insights on the biosynthesis of so-called plant metabolites by endophytes residing in distinct ecological niches.

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MIV02

Influence of different phosphate sources on active bacterial microbiome in the rhizosphere and endorhiza of barley (*Hordeum vulgare* L.)

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Introduction: Phosphate is a macronutrient and often the limiting growing factor of many ecosystems due to scarce mobility, related to the different forms of organic and inorganic phosphate occurring in the various soils. Despite its primary role in ecosystem productivity, the effect of different phosphate sources on soil- and rhizosphere bacteria were not investigated yet.
Objectives: To assess the effects of different phosphate sources on both the rhizosphere and the endorhiza bacterial microbiome of barley, under greenhouse conditions.
Methods: Barley was grown in greenhouse on nutrient depleted soil amended with each 100 mg P kg^{-1} soil of either Ca_3PO_4 (CaP), Gafsa rock phosphate (GAFSA), sodium hexaphytate (NaHex), or without amendment. Total RNA was extracted from both the rhizosphere and the endorhiza of 4 pooled samples per phosphate treatment/control. cDNA was obtained by RT-PCR of the bacterial 16S rRNAs, and sequenced by IonTorrent. The sequences were analyzed with the QIIME software pipeline. Relationships between bacteria and phosphate sources were assessed by correlation network analysis of phosphate-specific OTUs.
Results: Phosphate amendment significantly affected the structure of the active microbiome, but only CaP determined higher diversity indices. Sixty-two OTUs were significantly different between phosphate sources, accounting for 50.6% and 11.3% of the total reads in the rhizosphere and in the endorhizal microbiome, respectively. The co-occurrence correlation network showed that all Nocardioaceae and Acidobacteriaceae OTUs were enriched by CaP, while some Oxalobacteriaceae OTUs were depleted by all phosphate sources and other Oxalobacteriaceae OTUs were enriched by NaHex or GAFSA. Similarly some *Rhodanobacter* OTUs were depleted by all phosphate sources and others slightly enriched by CaP, and *Lysobacter* OTUs were enriched by GAFSA. Negative correlations were also identified, indicating complementary OTUs.
Conclusions: This is the first study investigating the effect of different phosphate sources on the active fraction of the rhizosphere and endorhiza microbiome. Phosphate source affected more the rhizosphere than the endorhizal microbiome. The most affected taxa were identified and their relationships within and between the two microbial habitats were unraveled.

MIV03

Metabolic cross-feeding via inter-cellular nanotubes among bacteria

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Bacteria frequently exchange metabolites by diffusion through the extracellular environment, yet it remains generally unclear whether bacteria can also use cell-cell connections to directly exchange nutrients. Here we address this question by engineering cross-feeding interactions within and between *Acinetobacter baylyi* and *Escherichia coli*, in which two distant bacterial species reciprocally exchange essential amino acids. We establish that in a well-mixed environment *E. coli* but not *A. baylyi* can connect to other bacterial cells via membrane-derived nanotubes and use these to exchange cytoplasmic constituents. Inter-cellular connections were induced by auxotrophy-causing mutations and ceased to establish when amino acids were externally supplied. Electron and fluorescence microscopy revealed a network of nanotubular structures that connected bacterial cells and enabled an intercellular transfer of cytoplasmic materials. Together, our results demonstrate that bacteria can use nanotubes to exchange nutrients among connected cells and thus help to distribute metabolic functions within microbial communities.

MIV04

Iron adaptation mechanisms by the pathogenic yeast *Candida albicans* are responsive to a bacterial quorum sensing molecule

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Introduction: Little is known on interkingdom signalling between pathogenic fungi and co-infecting bacteria.
Objectives: Here, we examined whether the human pathogenic yeast *Candida albicans* can specifically respond to a low physiological concentration of 3-oxo-C12-homoserine lactone (3-oxo-C12-HSL), a quorum sensing molecule from the Gram-negative bacterium *Pseudomonas aeruginosa*.
Materials & Methods: Focussing on selected *C. albicans* genes involved in iron adaptation mechanisms, we measured their transcriptional response to 3-oxo-C12-HSL. Subsequently, *C. albicans* yeast growth was examined under iron-limited conditions after pre-exposure to 3-oxo-C12-HSL.
Results: Monitoring the transcriptional response of *C. albicans* genes involved in iron adaptation, a specific activation of key factors from high-affinity iron acquisition systems was detected. At the same time, factors involved in iron consuming processes were found to be repressed. In accordance to these observations, growth experiments evidenced that *C. albicans* cells, which were pre-exposed to 3-oxo-C12-HSL, showed an increased potential to adapt to iron starvation conditions.
Conclusion: These findings provide novel insights into the molecular basis of host adaptation mechanisms in *C. albicans*, and moreover exemplify the interspecies crosstalk between fungi and bacteria.

MIV05

FIB/SEM and (serial) Electron Tomography of the enigmatic *Ignicoccus hospitalis*/ *Nanoarchaeum equitans* Co-Culture

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Introduction: The marine hyperthermophilic crenarchaeon *Ignicoccus hospitalis* exhibits an outer cellular membrane (OCM) in addition to a cytoplasmic membrane (CM). Furthermore it supports the propagation of *Nanoarchaeum equitans* on its surface [1]. Remarkably, proteomic and transcriptomic analysis revealed only little impact of *Nanoarchaeum* on *Ignicoccus* [2, 3].
Objectives: We aimed for a detailed look into the unusual cell architecture of *Ignicoccus hospitalis* and its relationship to *Nanoarchaeum equitans*.
Methods: For this we used cryopreparation in combination with 3D methods: serial sectioning, FIB/SEM and (serial) electron tomography.
Results: Between the OCM and the CM of *Ignicoccus* there is an intermembrane compartment (IMC) whose volume makes up ~40% of the whole cell volume in average. In few cells it can reach an extent much larger than the volume of the cytoplasm. In the IMC, elongated protrusions of the cytoplasm are present. Apparently, these structures can constrict from or fuse with the CM or themselves. We also observed interactions of these protrusions with the OCM via pore complexes. All interacting structures are connected via thin filaments (~3-6 nm in diameter), that span through the whole IMC. The same structures also seem to be involved in building up the contact to *Nanoarchaeum*. The S-Layer of *Nanoarchaeum* appears to be disintegrated at the contact site, but might play a role in the initial adherence process. Intriguingly, at the contact site a fusion of cytoplasms of both cells was observed.
Conclusion: The 3D-models obtained reveal a highly complex and dynamic endogenous membrane system in *Ignicoccus* that is unrivaled among prokaryotes. *Nanoarchaeum* gets in direct contact with this endogenous membrane system.

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MIV06

Tin3 of *Ustilago maydis* – A dual function effector?

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Introduction: The corn smut fungus *Ustilago maydis* establishes a compatible interaction with its host plant maize leading to massive fungal proliferation in the host tissue and the induction of tumors. This biotrophic interaction is governed by the secretion of fungal effector proteins that exert their function either in the apoplast or are taken up by plant cells to suppress or reprogram host responses. The effector gene cluster 19A comprising of 24 effector genes is essential for full virulence and tumor formation.
Objectives: In this study we functionally analyze the cluster 19A effector gene *tin3*.
Materials and Methods: To determine the relevance of *tin3* for virulence we deleted the gene, followed by virulence assays. To identify Tin3 interacting proteins we performed yeast two hybrid screening against a maize library. To further characterize interactions we performed co-immunoprecipitation analysis and bimolecular fluorescence complementation. Enzyme assays were performed to functionally analyze Tin3 in vitro.
Results: We demonstrate that Tin3 inhibits C1A-type cysteine proteases that are secreted by the plant in response to the infection. This inhibitory function of Tin3 most likely contributes to the virulence function of Tin3 since expression of other C1A inhibitors partially complements the virulence phenotype of *tin3* deletion mutants. Besides the interaction of Tin3 with C1A proteases, Tin3 also interacts with maize Atg6/Beclin1, an autophagy-related protein.
Conclusion: We propose that Tin3 is a dual function effector with an apoplastic, cystein protease inhibiting function and an additional function after uptake by plant cells, where Tin3 may interfere with the autophagy pathway.

MIV07

Role of PilY1 in adhesion to host cells and intracellular infection of *Legionella pneumophila*

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Legionella pneumophila is a Gram-negative bacterium which causes a severe form of pneumonia - Legionnaires' disease. A key feature of *L. pneumophila* pathogenicity is the ability to reprogram the degradative intracellular trafficking. In both host systems, human macrophages and *D. discoideum*, *L. pneumophila*-containing vacuoles (LCVs) exclude endocytic and lysosomal markers and associate with the rough endoplasmic reticulum at the early stage of infection. In previous work we have selected *L. pneumophila* transposon mutants that are attenuated in escaping the lysosomal degradation. One of the mutants that shows significantly higher co-localization with the lysosomal compartment exhibits several insertions in the *pilY1* gene. PilY1 represents a unique structure as it possesses a von Willebrand factor A (vWFA) domain at the N-terminus and a PilY1 domain at the C-terminus. The vWF domain is often involved in cell adhesion and is commonly found in extracellular eukaryotic proteins. In contrast the PilY1 domain shares homology with the virulence factor PilY of *P. aeruginosa* and PilC of *Neisseria* spp. and is proposed to influence type IV pilus biogenesis and stability. The *pilY1* gene is present predominantly in *L. pneumophila* strains and cellular fractionation experiments revealed that *pilY1* is located at the outer membrane. *PilY1* knock out mutants show attenuated replication as well a reduced adhesion and uptake in THP-1 macrophage-like cells and *D. discoideum*.Moreover these mutants have defects in twitching and sliding motility. Further complementation analysis demonstrated that this multiple surface behaviors are mediated by the PilY1 domain. Taken together, these results indicate a role of PilY1 as a potential adhesion factor participating in the initial step of the infection.

MIV08

Inflammasome activation in *Salmonella*-infected primary human macrophages is dependent on flagellin

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Introduction: During *Salmonella* infections, flagellin is translocated into the host cell cytosol by the *Salmonella* Pathogenicity Island 1 Type III secretion system (SPI-1 T3SS). In mice, the cytosolic presence of *Salmonella* flagellin triggers formation of the NLRC4 (NLR family CARD domain-containing protein) inflammasome. NLRC4 acts as a platform for activation of Caspase1, which promotes two major events: release of the pro-inflammatory cytokines IL-1b and IL-18, and the induction of a pro-inflammatory form of cell death termed pyroptosis. Mice utilize the Naip receptors (NLR family, apoptosis inhibitory proteins) Naip1, Naip2 and Naip5/6 to detect the T3SS PrgI needle, the PrgJ rod subunit and flagellin, respectively. In contrast to mice, a single Naip protein isoform has been described in humans, which is believed to only sense the T3SS needle protein PrgI and not flagellin. This seems unlikely, as flagellin is a very potent trigger of acute inflammatory processes and humans are routinely challenged with flagellated pathogens.
Objectives: To our knowledge, all past studies that have attempted to investigate the role of hNaip in human macrophages and its response to flagellin were performed with macrophage-like cancer cell lines U937 and THP-1. We hypothesize that primary human macrophages from healthy donors would be responsive to bacterial flagellin.
Methods: We differentiated primary macrophages from the blood of human volunteers, infected them with wild type and non-flagellated mutant strains of *Salmonella* and measured inflammasome performance.
Results: Infection of primary human macrophages with *Salmonella* enterica Serovars Typhimurium or Typhi activates the inflammasome in response to flagellin and this detection requires a full-length isoform of the human Naip sensor. Our data reveal, that this full-length Naip isoform is expressed in primary monocyte-derived macrophages from healthy human donors but absent in monocytic tumor cells, U937 cells, rendering them insensitive to bacterial flagellin. However, ectopic expression of full-length Naip rescues the ability of U937 cells to trigger a robust response towards *Salmonella* flagellin, resulting in enhanced cell death. In conclusion, human Naip functions to activate the inflammasome in response to flagellin, similar to murine Naip5/6.

MSV01

Long and short range heterogeneity of production of amyloid curli fibres and cellulose is essential for morphogenesis of *Escherichia coli* macrocolony biofilms

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Introduction. In *E. coli* macrocolonies the emergence of elaborated structures, such as ridges and wrinkles, relies on a precise spatial distribution of flagella, amyloid curli fibers and cellulose. Over a long range, this spatial matrix distribution is based on *E. coli* differentiating into different physiological layers in response to nutrient gradients building up in the biofilm: vegetatively growing cells entangled by flagella in the bottom layer (i.e., close to the nutrient-providing agar) and starving stationary-phase cells producing cellulose and curli fibers in the top layer.
Objectives. This study aims at investigating how heterogeneity of matrix synthesis occurs over very short distances inside macrocolonies and how does it impact macrocolony microarchitecture.
Methods. Heterogeneity of matrix synthesis inside biofilms of *E. coli* strains W3110 and AR3110 and derivative mutants was examined by combining macrocolony cryo-sectioning, specific labelling of matrix components and fluorescence and SEM microscopy.
Results. We found that the lower zone of the stationary-phase macrocolony layer represents a highly heterogeneous transition area with cells that have switched to stationary phase and the production of mainly cellulose being located immediately adjacent to 'naked', elongated and flagellated cells. Importantly, we found that YciR, a 'trigger enzyme' with c-di-GMP degrading phosphodiesterase activity which acts as a central switching device in the control of *csgD* expression, is a key player in generating this heterogeneity. A knockout mutation in *yciR* resulted in confluent and homogeneous distribution of cellulose and curli in the *entire* stationary phase zone. At the macroscopic level this translated into large, rigid and extremely flat macrocolonies with scared breaks at the surface,

thus reflecting extreme cohesiveness but reduced elasticity of the macrocolony.
Conclusion. Overall, these results highlight that heterogeneity in matrix synthesis is essential for the development of complex morphological patterns in macrocolony biofilms.

MSV02
Genes Involved in the Formation of Multicellular Aggregates in *Staphylococcus aureus*
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Many bacterial species are able to grow in multicellular aggregates or biofilms and efficiently colonize diverse surfaces, such as implants, catheters and tissues, which serve as a reservoir for the generation of hard-to-treat infections. Among those bacterial species, the opportunistic pathogen *Staphylococcus aureus* is an efficient biofilm former on natural and synthetic surfaces and is responsible for the generation of biofilm-associated chronic infections.The extracellular matrix of proteins, exopolysaccharides and eDNA provides a diffusion barrier for many antimicrobials and a means for immune evasion [1,2]. Biofilm formation in *S. aureus* is traditionally studied in a model where cells adhere to a submerged polystyrene surface and yet, it is known that this pathogen exerts many very different strategies to colonize a large number of surfaces. In this work, we have developed a new approach to study biofilm formation in *S. aureus*. We have used an *in-vitro* model for biofilm formation that mimics the conditions of chronic infections when associated with bones, joints or soft tissues [3]. We will show how our approach promotes biofilm formation on a solid surface in a large variety of genetically different *S. aureus* strains. Moreover, we have used a genome-wide mapped transposon mutant library [4] to screen for genes that play a relevant role in biofilm formation using our new biofilm formation model. Using this approach, we have identified a new battery of genes that are involved in staphylococcal biofilm formation and we have studied their role in detail, which will be discussed in this presentation. Furthermore, we could confirm the role in biofilm formation of previously described genes. Overall, our new biofilm formation assay allows us to discover genes that play a role in *S. aureus* biofilm formation and facilitates the understanding of biofilm formation in this important pathogen.

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MSV03
Regulation of thermotolerance development in *Bacillus subtilis*
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Cells of bacterial and eukaryotic organisms such as *B. subtilis* or *S. cerevisiae* survive an otherwise lethal heat shock once they are primed by a mild pre-heat shock. The molecular mechanism and regulation of this important cell protective mechanism is not well understood or characterized.
Using *B. subtilis* as a model organism, we investigated the thermotolerance development by analyzing cell survival and in vivo protein aggregate formation in severely heat shocked cells of wild type and mutant strains primed by a mild heat shock. In the course of these experiments we could also identify the thiol stress regulator Spx as a heat shock regulator important for thermotolerance development, which suggest that protection of misfolded proteins from thiol oxidation during heat shock plays a general role in prevention of cellular protein aggregation (Runde et al (2014) MolMi 91:1036).
We continued our investigation by analyzing mutant strains where stress response regulators such as HrcA, CtsR or SigB are deleted. In addition, we investigated changes in the transcriptome and reporter fusion proteins during thermotolerance development. Our results suggest that the stringent response of *B. subtilis* is involved in heat shock response.
Finally we propose a preliminary model, where the regulation of thermotolerance development can be separated in a fast reversible and a second more long-term adaptation.

MSV04
Structural basis for (p)ppGpp catalysis by an oligomeric alarmone synthetase and its allosteric regulation by pppGpp but not ppGpp
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Introduction: When facing nutrient limiting conditions, bacteria adapt by rearranging their metabolome to increase cell viability. This stringent response is mediated by the second messengers ppGpp and pppGpp [1]. In *B. subtilis*, three (p)ppGpp-synthetases have been identified so far, RelA and two small alarmone synthetases (SAS): SAS1 and SAS2. These enzymes utilize ATP and GDP (or GTP) to synthesize ppGpp and pppGpp, respectively [2].
Objectives: We aimed at investigating the structural and biochemical properties of SAS1 from *B. subtilis* and its relevance *in vivo*.
Methods: X-ray crystallography, hydrogen-deuterium mass-spectrometry (HDX-MS), biochemical assays, *in vivo* analysis, light microscopy
Results: SAS1 forms a stable homotetrameric complex, connecting the active sites in a highly cooperative manner. ATP and GDP/GTP bind to the enzymes active sites in a sequentially-ordered binding mode. Interestingly, K_m and v_{max} for GDP and GTP differ and ppGpp is the predominant product. However, pppGpp but not ppGpp, acts as a positive allosteric regulator of its own synthesis. In minimal medium, *B. subtilis* wildtype grows better than a strain carrying an inactive or non-allosterically regulated variant of SAS1. Furthermore, these variations within SAS1 lead to an increased amount of unchained cells compared to the nearly exclusively chained wildtype strain.
Conclusion: We present an in depth structural and biochemical analysis of a (p)ppGpp-synthetase, including the structure of SAS1 in different nucleotide-bound states, determination of the catalytic mode of (p)ppGpp-synthesis and kinetic analysis. Moreover, the relevance of SAS1 *in vivo* is shown by the observed growth defect of strains carrying variants of SAS1 compared to wildtype strains. SAS1 affecting cell heterogeneity displays a remarkable feature which needs to be further investigated.

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MSV05
Iron-, heme- and cobalamin-binding properties of the *Streptomyces* sensory protein HbpS involved in anti-oxidative stress response
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The extracellular protein HbpS acts as an accessory protein of the two-component system SenS-SenR from the cellulose degrader *Streptomyces reticuli*. HbpS-SenS-SenR is involved in the protection of this soil bacterium against the toxic effects of oxidative stress and is conserved in many other actinobacteria [1]. Analysis of the 3D crystal structure and biochemical studies revealed that HbpS assembles as an octamer [2]. Additional spectroscopic studies showed that HbpS specifically interacts ferrous ions as well as the tetrapyrroles heme and aquo-cobalamin (H_2OCbl^+) [3,4]. Based on 3D crystal structures, structural and sequence comparisons, mutagenesis, and comparative biochemical investigations, we identified the coordination sites for iron ions, heme and aquo-cobalamin, and the corresponding binding kinetics were calculated [3,4]. While the physiological relevance of iron- and heme-binding by HbpS has been analysed in detail, the *in vivo* role of the HbpS-H_2OCbl^+ complex remains to be elucidated. Given that HbpS is conserved in many other Gram-positive and Gram-negative bacteria, the presented data are applicable to related species.

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MSV06

Fungoglobin – a novel member of the sensor globin family supports hypoxic growth of the pathogenic fungus Aspergillus fumigatus.

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Infection with conidia of the pathogenic fungus *Aspergillus fumigatus* is a frequent health threat for immunocompromised human individuals. Low oxygen partial pressures is a property which transiently occurs in most natural growth environments, but also defines deep layers of infected human tissue. *A. fumigatus* survives and prospers in such hypoxic areas but metabolic and energetic consequences of low O_2 availability are less understood. We hypothesized that the fungus could also sense and react to O_2 directly. In a first approach, we used Next Generation Sequencing to study the dynamic and short term response to a transient exposure to low O_2. Deprivation of O_2 triggered a more than threefold induction of 680 genes after only 15 min while 420 genes were down regulated at the same time point. Among the highest upregulated genes we identified a gene encoding a hypothetical protein which appears to be conserved in filamentous fungi. In sharp contrast, reoxygenation of the growth medium resulted in the complete repression of its mRNA. Besides O_2 limitation, low iron also induced its expression, but transcription was largely independant of the two major transcription factors. The encoded protein comprised a globin-like N-terminal domain and was identified as a member of the large protein family of sensor globins. The function of these proteins is largely unknown but heterologous expression and purification gave evidence for a functional heme binding site. Furthermore, the deletion of the gene led to an impaired growth of *A. fumigatus* in low oxygen atmospheres. The biochemical function of these "fungoglobins" will be discussed.

MSV07

The garlic sulfur compound Allicin causes protein Sulfoallylation and disulfide stress in *Bacillus subtilis* and *Staphylococcus aureus*

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Introduction: Allicin is a diallylthiosulfinate that is produced upon wounding in garlic. Allicin is active against a broad spectrum of Gram-positive and Gram-negative bacteria, including also antibiotic-resistant strains like methicillin-resistant *Staplylococcus aureus* (MRSA). Allicin was shown to react with thiols groups of proteins and depletes the thiol-redox buffer glutathione in eukaryotes [1]. However, the antimicrobial mode of action and post-translational thiol-modifications caused by Allicin have been not yet studied in Gram-positive bacteria.
Objectives: Here, we analyzed the changes in the redox proteome and transcriptome caused by Allicin in *Bacillus subtilis* and *Staphylococcus aureus* to reveal the antimicrobial mode of action of Allicin.
Material & Methods: We applied MS-based thiol-redox proteomics for identification of abundant protein Sulfoallylations in *B. subtilis* and *S. aureus* under Allicin treatment. Transcriptomics and Northern blot analyses were used to study the mode of action and stress response caused by Allicin. Voronoi Treemaps were used for visualization of the functions of Sulfoallylated proteins.
Results: In *B. subtilis* and *S. aureus* USA300 we found strong sensitivity of the bacillithiol (BSH)-deficient mutants towards Allicin indicating that BSH is important for Allicin detoxification and resistance. Treatment of *B. subtilis* and *S. aureus* cells with Allicin resulted in strongly increased protein Sulfoallylation of redox-sensitive Cys residues in more than 100 proteins including redox-sensing transcriptional regulators. Allicin further caused a disulfide stress response in the transcriptome of *B. subtilis* as revealed by the induction of the PerR, Spx, OhrR and HypR regulons. In addition, mutants with defects in several redox-sensing regulators displayed sensitivities towards Allicin in growth phenotype assays.
Conclusions: We show here that the garlic sulfur species Allicin causes protein sulfoallylation of many redox-sensitive proteins in *Firmicutes* bacteria which acts as thiol-protection and redox-regulatory mechanism and induces a thiol-specific oxidative stress response in the transcriptome.

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MSV08

Bacterial stress response to daptomycin

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Daptomycin is a last resort antibiotic against multi-resistant superbugs. Despite more than 10 years of clinical use, its antibacterial action is still not well understood. Early studies suggested a block of cell wall synthesis[1] but none of the enzymatic steps in this pathway was inhibited.[2] More recent results indicated that daptomycin selectively interacts with phospholipids[3] and affects membrane organization.[4]
In order to get deeper insight into the effect of daptomycin on the bacterial cell, we employed global profiling and microscopy techniques. Comparison of the *Bacillus subtilis* proteome response with a reference library showed a dual impact of daptomycin on cell wall synthesis and the membrane. Light microscopy confirmed effects on cell wall synthesis and electron microscopy showed aberrant cell wall morphology. Likewise, aberrant membrane organization was observed by both fluorescence and electron microscopy.
While membrane integrity was not affected, impact on membrane function was corroborated by reduced cellular ATP levels. A global ionomics approach revealed a 3-fold increase of cellular potassium, while ion homeostasis was otherwise unaffected. Further experiments pointed to a cellular mechanism being involved in this potassium translocation. Antimicrobial peptides have been shown to activate mechanosensitive channels.[5] We showed correlation between channel activation and membrane deformation. However, there was no effect for daptomycin. Poglino *et al*. proposed that daptomycin induces membrane areas with altered curvature and correlated changes in cell morphology to relocation of the cell division protein DivIVA.[4]
In order to relate our observations to daptomycin's impact on protein localization, we investigated the localization of the cell wall synthesis enzyme MurG, the lipid synthases PlsX and PgsA, the cell division protein MinD, and the ATPase subunit AtpA. Our results indicate that the observed effects of daptomycin are caused by delocalization of membrane proteins due to changes in membrane architecture.

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SAV01

IN and OUT, ON and OFF - An efficient concept of stimulus perception by a histidine kinase

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Two-component signal transduction (TCS) is a common strategy in bacteria to adapt to changing environments. The KdpD/KdpE system is the most widespread TCS among bacteria and important for survival under certain stress conditions. Under K^+-limitation KdpD/KdpE activates *kdpFABC* expression, encoding a high affinity K^+-importer. As potassium is crucial for different cellular functions in prokaryotic and eukaryotic cells, the intracellular K^+-concentration has to be maintained at a constant level. The histidine kinase KdpD is responsible for sensing the cellular "need" for K^+ and exhibits not only kinase and but also phosphatase activities [1]. Until now it was unknown, how KdpD senses K^+ and regulates the ratio between kinase and phosphatase activities. In this study we identified a periplasmic K^+-recognition site, which is responsible for

the regulation of the kinase activity. Furthermore, we found that the phosphatase activity is adjusted by K^+-recognition of the C-terminal cytoplasmic domain. This is the first example of a histidine kinase that senses the same stimulus from the extra- and intracellular side and accordingly adapts its kinase and phosphatase activities. Modelling combined with biochemical and genetic analyses provide first insights, how KdpD efficiently regulates the switch between kinase and phosphatase activities dependent on the extra- and intracellular K^+-concentration. This dual sensing strategy might be a widespread mechanism in other signal transduction systems.

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SAV02

LuxR solos as central players in communication of *Photorhabdus* species

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Bacteria constantly need to sense their environment and current host to accordingly adapt their bacterial behavior. Bacterial communication via small diffusible molecules to mediate group-coordinated behavior is referred to as quorum sensing. The typical quorum sensing system of Gram-negative bacteria consists of a LuxI-type autoinducer synthase that produces acyl-homoserine lactones (AHLs) as signals, and a LuxR-type receptor, which detects AHLs to control expression of specific target genes [1]. However, many bacteria possess LuxR homologs but lack a cognate LuxI-type synthase and those LuxR-type receptors are designated as LuxR solos.
Using a bioinformatics approach, we could identify extraordinarily high numbers of LuxR solos, ranging from 22 to 40, in the three *Photorhabdus* species, *P. luminescens*, *P. temperata* and *P. asymbiotica*, however all three lack LuxI homologs [2]. The overall 100 LuxR solos could be grouped into three types dependent on their N-terminal signal-binding domain (SBD). Moreover, the motif of six conserved amino acids in the SBD of AHL-sensing LuxR-type receptors is altered in the majority of the *Photorhabdus*-specific LuxR solos, suggesting the use of other signaling molecules than AHLs, like eukaryotic hormones. Recently, we identified two LuxR solos, which are responding each to non-AHL endogenous signaling molecules. The LuxR solo PluR from *P. luminescens* detects a-pyrones, named photopyrones (PPYs), whereas the LuxR solo PauR from *P. asymbiotica* recognizes 2,5-dialkylresorinols (DARs) [3]. Furthermore, both regulators contain an altered amino acid motif in the SBD, import for the sensing and specificity towards its cognate signaling molecule [2].
In summary, the presence of the several diverse LuxR solos gain first insight into the complexity of the communication network between bacteria among each other as well as with their hosts.

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SAV03

Need-based activation of antibiotic resistance by a flux-sensing mechanism

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Question: To control the expression of antibiotic resistance genes, bacteria often utilize two-component systems that directly sense the extracellular antibiotic concentration. However, a class of resistance modules directed against peptide antibiotics was recently found to be controlled by two-component systems that lack dedicated ligand-binding domains in their histidine kinases (HKs). Instead, as shown for the prototypic BceRS-BceAB system in *Bacillus subtilis*, stimulus perception via the HK BceS is contingent on complex formation with the cognate transporter BceAB. At the same time, the transporter mediates resistance to the peptide antibiotic bacitracin, which inhibits the lipid II cycle of cell wall biosynthesis by binding to undecaprenyl pyrophosphate (UPP). To date, the nature of the stimulus perceived by the BceS-BceAB sensory complex remains poorly understood.
Methods & Results: Here, we discriminate between alternative sensing scenarios using a combination of mathematical modeling and experimentation. We provide evidence that the BceRS-BceAB sensory complexes respond to changes in the transport activity of BceAB rather than to changes in the extracellular or intracellular bacitracin concentration. We thereby show that BceRS-BceAB implements a novel type of "flux-sensor", which adjusts the rate of *de novo* transporter synthesis to precisely the level needed to keep a significant fraction of UPP bacitracin-free.
Conclusion: Based on these results, we propose that flux-sensing has evolved as an efficient "produce-to-demand" strategy in Bce-like antibiotic resistance modules, which are also found in many clinically relevant bacterial species.

SAV04

A phosphorylation-dependent network regulates motility of *Sulfolobus acidocaldarius*

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Introduction: In the archaeon *S. acidocaldarius* expression of the motility structure, the archaellum, is induced by starvation employing a network of positive and negative regulators[1,2]. Two important motility repressors are ArnA and ArnB which interact in a phosphorylation-dependent manner. Reversible phosphorylation of these regulators is mediated by at least two kinases (ArnC and ArnD) and one phosphatase (Saci_PP2A)[2].
Objective: The mechanism underlying motility regulation in Crenarchaea is poorly understood. We aim to elucidate the role of phosphorylation and to characterize the proteins that are involved in establishing the network necessary for archaellum expression using *S. acidocaldarius* as a model organism.
Methods: Structural details about ArnA/ArnB interaction were gained applying X-ray crystallography. Interaction assays, mass spec. and *in vitro* phosphorylation assays were used to study phosphorylation of ArnB and the interaction of the repressors. Motility assays of ArnB mutants and the kinases ArnC and ArnD were performed to assess their function in motility by testing their ability to produce archaella subunits and to swim. Western Blot analysis and qRT PCR of *ΔarnC* and *ΔarnD* were applied to characterize the kinases and to reveal their influence on archaellum expression and the hierarchy of the network.
Results: Structural analysis revealed the localization of phosphopeptide-binding sites in ArnA and phosphorylated residues in the C-terminus of ArnB. Truncation of ArnB reduced interaction to ArnA and abolished its phosphorylation. Complementation of *ΔarnB* cells showed that the C-terminus of ArnB is required for its function. Both kinases ArnC and ArnD were able to phosphorylate the repressors but had different effects on motility, only *ΔarnD* cells were hypermotile and produced high amounts of the archaellin FlaB.
Conclusion: Taken together our results deepen the knowledge about the phosphorylation-dependent network that regulates motility of *S. acidocaldarius* even though we are just beginning to understand the complexity of this network.

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SAV05

Comparative proteomic analysis reveals that the Tor pathway in *Aspergillus fumigatus* is involved in iron regulation

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Introduction: The opportunistic fungal pathogen *Aspergillus fumigatus* is a major cause of death among immunocompromised patients. The ability of *A. fumigatus* to sense and to adapt to specific environmental cues is crucial in making this microorganism such an effective pathogen. To drive all those changes, which lead to responsive strategies, several conserved pathways mediate intracellular signalling. The Tor (Target Of Rapamycin)

kinase is one of the major regulatory nodes in eukaryotes.
Objectives: According to our analysis, *A. fumigatus* possesses a unique gene that showed significant similarity with the Tor kinases previously identified. Here, we present data about the function of the Tor pathway in *A. fumigatus*.
Methods: Because deletion of the single *tor* gene of *A. fumigatus* was apparently lethal, we generated a conditional *tor* mutant. This was achieved by fusing the *tor* gene with the inducible xylose promoter and replacing the endogenous *tor* gene by the inducible *xyl*p-*tor* gene cassette. The generated transgenic strain opened up the possibility to activate or silence the *tor* gene under controlled conditions. By using both a 2D-gel electrophoresis approach and a gel-free LC-MS/MS workflow for the analysis of mycelial proteins, we dissected the regulatory network of Tor.
Results: In accordance with the reported role of Tor in other organisms, most of the proteins with altered abundance were assigned to nutrient sensing and metabolism. Interestingly, a large number of identified proteins were related to processes occurring in mitochondria, including also proteins involved in iron sensing and storage belonging to the ornithine metabolic pathway. The incapability of the inducible *tor* mutant strain to sense the iron stress was also proven by quantifying the production of extracellular siderophores during iron depletion.
Conclusion: We showed that Tor controls a variety of genes and proteins involved in nutrient sensing, cell cycle progression, protein biosynthesis and degradation. Additionally, Tor regulated also important processes occurring into the mitochondria, e.g. respiration and ornithine metabolism. Deregulation of ornithine metabolism had consequences in iron acquisition and siderophore biosyntheses. The effects of Tor on iron stress response revealed a new function of Tor in iron regulation.

SAV06
Structure and function of a phycobiliprotein lyase from a marine cyanophage
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Cyanobacteria are among the most abundant organisms is the ocean and greatly contribute to the world's primary production. However, the number of cyanobacteria is by far outnumbered by cyanophages, viruses that infect cyanobacteria. Those cyanophages play an important ecological role as they control cyanobacterial population size and contribute to lateral gene transfer. This is most obvious from sequenced cyanophage genomes which were shown to contain gene clusters of cyanobacterial origin. Interestingly, many photosynthesis-related genes like the D1 protein of photosystem II were identified. Several reports suggest that these cyanophage genes support the host in keeping photosynthesis active during infection. In addition to the reaction center proteins, cyanophages encode pigment-biosynthesis enzymes and so-called phycobiliprotein-lyases, proteins involved in the coupling of pigments to the light-harvesting phycobiliproteins.
Here we present the first results on the structure and function of a CpeT-like phycobiliprotein lyase from the cyanophage P-HM1. PBP-lyases are molecular chaperones that facilitate the correct region- and stereospecific attachment of chromophores to conserved cysteine residues of the apo-phycobiliprotein. Recombinant, affinity purified P-HM1CpeT binds the chromophores phycoerythrobilin (PEB) and 15,16 dihydrobiliverdin (DHBV) with high affinity. Interestingly, the very stable complex between P-HM1CpeT and (*3Z*) PEB is highly fluorescent suggesting that the chromophore is rigidly bound. The X-ray structure of P-HM1CpeT was solved at 2.8 Å revealing a β-barrel with a modified lipocalin-fold. Based on the crystal structure, a comparison between host and phage phycobiliprotein lyases will be discussed in the light of the putative function of such genes in the genomes of cyanophages.

SAV07
The bacteriochlorophyll biosynthesis of the marine bacterium *Dinoroseobacter shibae* and its light-dependent regulation
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Dinoroseobacter shibae is a member of the *Roseobacter* clade of marine bacteria which represents one of the major lineage isolates of seawater and marine sediments in the global oceans and is known for its variety of metabolic processes. The majority of this clade belongs to the aerobic anoxygenic photosynthetic bacteria (AAP), which indicates that in the presence of oxygen light is used as supplementary energy source to metabolize organic matter [1]. Spheroidenone and bacteriochlorophyll *a* act as the main light harvesting pigments. Moreover, *D. shibae* is able to generate energy by anaerobic respiration including denitrification. We use the model organism *D. shibae* to study the bacteriochlorophyll biosynthesis. Changing light or 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, light dependent regulation is assumed.
We established a fluorescence microscopy setup to measure bacteriochlorophyll *a*-dependent infrared fluorescence on single cell level. This enables us to monitor the bacteriochlorophyll *a* biosynthesis and degradation during the growth of living cells at defined temperatures and various light conditions using time-lapse microscopy. Therefore, the software TLM-Tracker was developed within our group to analyze time-lapse movies [4]. In parallel, we extracted bacteriochlorophyll pigments from cells grown in the dark and under light conditions and measured the bacteriochlorophyll *a* content using UV/Vis spectroscopy. This experimental setup will be used to study mutant strains of regulators potentially involved in the regulation of photosynthesis genes. One putative candidate is Dshi_2006 which shows a high degree of similarity to known LOV domains from blue light receptor proteins. These results will lead to new insights into the biosynthesis of bacteriochlorophyll and the influence of photosynthesis as an adaption strategy for aerobic anoxygenic photoheterotrophs.

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SAV08
Distinct features of closely related *Natrialba* species suggest an adaptation towards different ecological habitats.
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Members of the genus *Natrialba* in the family *Halobacteriaceae* (**halophilic Archaea**), have been isolated from habitats with NaCl concentrations ranging from 15% NaCl to saturation (~32%). These highly **saline habitats** may differ in their pH, with either pH > 8 (**alkaline**) or pH ~ 7 (**neutral**). We investigated the **phenotypic and genomic features** of the currently available type strains of species in the genus *Natrialba* with the aim of extending our insights into their **adaptation strategies**.
The neutrophilic strains contained a significantly greater number of enzymes showing activity towards carbohydrates (**glycoside hydrolases, polysaccharide lyases, carbohydrate esterases, carbohydrate binding modules**) 64 - 77 vs. 28 - 40 in alkaliphilic strains. In contrast a substantially lower number of **peptidases** were detected (mostly metallo- and serine-peptidases) 19 vs. 25 - 149 in alkaliphilic strains. Furthermore, neutrophilic strains utilized a greater diversity of **saccharides** faster and were able to decompose **polysaccharides** (e.g. cellulose). In contrast, alkaliphilic strains quickly decomposed peptides (proteins) such as casein and gelatin. We identified events of **loss/gain of genes and gene cluster in the genomes** that might explain the differences in substrate utilization (e.g. starch and laminarin). Additionally, we identified one of the main **metabolic features** that explains **why neutrophilic strains in contrast to alkaliphilic strains** utilize such a great diversity of saccharides.
These results suggest, that the currently known *Natrialba* species have undergone **contrasting phenotypic adaptations** to cope with either neutral or alkaline habitats. Furthermore, a number of these phenotypic changes could be linked to events in the genome of the strains. We hypothesise that neutrophilic *Natrialba* species may be **adapted** to marine salterns in which **decomposition of algal polysaccharides** might be an advantage.

SMeV01
The antibiotic roseoflavin from Streptomyces davawensis: mechanism of action and resistance
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Question: Roseoflavin (RoF) produced by *Streptomyces davawensis* and *Streptomyces cinnabarinus* is the only known natural riboflavin/vitamin B_2 (RF) analog with antibiotic function and is studied as a model compound in our laboratory (1) in order to pave the way for the structured analyis of other vitamin analogs yet to be discovered.
Methods: Standard procedures of molecular biology, microbiology and biochemistry were used.
Results: RoF is taken up by RF transporters and is converted to the flavin mononucleotide (FMN)/flavin adenine dinucleotide (FAD) analogs RoFMN/RoFAD by flavokinases/FAD-synthetases (1,2). The addition of RoF leads to a decrease of intracellular RF/FMN/FAD levels. This is the consequence of RoFMN mediated blocking of bacterial FMN riboswitches, which leads to a reduced expression of genes involved in RF transport and/or biosynthesis (3). A specialized FMN riboswitch thereby confers roseoflavin resistance to *S. davawensis* and *S. cinnabarinus* (4). Additional cellular targets for RoF are flavoproteins, proteins, which depend on the cofactors FMN and FAD. We could show that 37 out of 38 *Escherichia coli* flavoproteins contained either RoFMN or RoFAD when cells were treated with just toxic doses of RoF (5). FMN-dependent AzoR (EC 1.7.1.6) from *E. coli* exemplarily was analyzed in greater detail with regard to the molecular effect of RoF. RoFMN binds to AzoR apoenzyme with a higher affinity compared to that of FMN, however, AzoR-RoFMN is less acitve (30% of AzoR-FMN activity). Structural analysis (1.07 Å) revealed that RoFMN binding did not affect the overall topology of the enzyme and also did not interfere with dimerization of AzoR (6).
Conclusion: Although, until now, only very few natural vitamin analogs with antibiotic function have been identified, we expect that a multitude of yet unknown vitamin analogs can be isolated from microorganisms/plants/etc. Since most vitamins are active at more than one site, vitamin analogs in principle have multiple cellular targets (as shown for RoF). Moreover, many microorganisms (target organisms) have efficient vitamin transporters which ensure the delivery of the antibiotic to the target molecules in a cell.

SMeV02
Molecular probing as novel tool to dissect and control the stereochemistry of enoylthioester reductases – lessons from fatty acid biosynthesis for polyketide engineering
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Introduction: Enoylthioester reductases (ETRs) are essential to fatty acid biosynthesis and serve as an important antibiotic target. ETRs are also essential to polyketide biosynthesis and control the stereochemistry of natural products. Despite their essential role in biology, the general mechanism of these reduction reactions is not really understood. Here we demonstrate a molecular probing approach that allows uncoupling single steps of ETR catalysis to dissect, manipulate and control the ETR of the yeast *Candida tropicalis* at unprecedented detail.
Objectives: To study the detailed mechanism of the ETR from *Candida tropicalis* using a covalent NADPH-ene-compound[1] as molecular probe. To understand and manipulate the molecular basis for stereochemical control in ETRs.
Methods: Putative proton donors of yeast ETR were identified from the crystal structure and changed by site-directed mutagenesis. ETR wild type (wt) and mutants were characterized using a novel approach of molecular probing in combination with UV-Vis and NMR spectroscopy. The stereochemistry of the protonation reaction for ETR wt and mutants was tested using D_2O labeling in combination with mass spectrometry.
Results: Tyrosine 79 serves as proton donor in yeast ETR. Mutagenesis of this residue leads to a protonation-deficient mutant that is surprisingly still active and forms a dead end adduct. This new adduct explains the unexpected findings of many previous studies. At the same time our study reveals that standard spectrophotometric assays used in biology are heavily prone to data misinterpretation. With the new understanding of the ETR reaction, the stereochemistry of the enzyme was successfully changed from *re-* to *si-face*.[2]
Conclusion: We could for the first time dissect and manipulate single steps of the ETR reaction. Our results provide the biochemical basis for engineering ETRs and lay the foundation for the rational design of novel polyketides with altered stereochemistry. At the same time our results also call for a careful use of standard biochemical assays to avoid data misinterpretation.

SMeV03
Recombinant production of the lipopeptide biosurfactant Serrawettin W1
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Introduction: Surfactants are important for several industrial, pharmaceutical and everyday household applications. Industry increasingly looks at biosurfactants of microbial origin which can be produced from renewable raw materials feature good biological degradability and exhibit often antimicrobial side benefits thus leading to various potential applications.
Objective: The non-ionic lipopeptide biosurfactant serrawettin W1 produced by several strains of *Serratia marcescens* shows antimicrobial, antitumor and plant protecting properties, but is nonetheless nearly unexplored regarding its production for biotechnological purposes, maybe due to the pathogenicity of the natural production strain and the complex regulation of biosurfactant production. Here, we aimed for a simple method for heterologous production of this biosurfactant.
Methods: We introduced a single gene from the newly identified producer *S. marcescens* DSM12481 into different host organisms under control of different inducible promoters. Serrawettin W1 was extracted from culture supernatants and analyzed via TLC and HPLC-MS.
Results: For the first time, the production of serrawettin W1 was successfully established in the heterologous host organisms *E. coli*[1], *Erwinia billingiae*, and *Pseudomonas putida*. Further studies indicated transcriptional control by a rather weak promoter as well as a cultivation temperature of 30°C as prerequisites for the functional expression of the non-ribosomal peptide synthetase SwrW in all expression systems.
Within this work, previously unpublished congeners of this lipopeptide with longer fatty acids were identified. The congener compositions of serrawettin W1 isolated from different production hosts were surprisingly slightly dissimilar[1]. Initial characterization of the recombinantly produced lipopeptide revealed antimicrobial effects towards different bacteria.
Conclusion: The expression strategy described here paves the way for the large scale production of Serrawettin and enables further characterization of this lipopeptide.

[1] Thies *et al*., 2014. *Heterologous production of the lipopeptide biosurfactant serrawettin W1 in Escherichia coli.* J. Biotechnol. 181, 27-30.

SMeV04
Insights into the structural diversity of bacterial lasso peptides
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Introduction: Lasso peptides are a class of ribosomally assembled and posttranslationally modified peptides (RiPPs) that are characterized by a unique topology, which is reminiscent of a lariat knot. This natural product family of small peptides (13 to 26 residues)[1,2] can be subdivided into three different classes, distinguished by the number of disulfide bridges present, ranging from two in class I to none in class II and a single disulfide bridge in class III lasso peptides. The first lasso peptide was reported in 1991 and until 2008 most of them were uncovered only by bioactivity driven research. These lasso peptides show a range of activities including enzyme inhibitory, receptor antagonistic, anti-HIV and antimicrobial properties.
Objectives: The utilization of the vast amount of available DNA sequence data by directed genome mining approaches ought to allow the prediction and isolation of new lasso peptides. Their thorough characterization should enhance the understanding of their physical and biochemical properties as well as their biosynthetic machineries and probe the correctness of existing assumptions.
Methods: Cloning, mutagenesis, MS/MS², natural product isolation, HPLC, NMR, thermal stability assays, protease assays.

Results: Directed genome mining approaches facilitated the discovery of more than a dozen new representatives of this interesting RiPP family, shedding more light on their fundamental biosynthetic machineries, heat stability and ring structure.[1-5] Several conserved sequence aspects, particularly, the restriction of the size of the threaded macrolactam rings to either eight or nine residues were revised[2] as well as the necessity for the N terminal amino acid of a lasso core peptide to be either a cysteine (class I lasso peptides) or glycine (class II and III lasso peptides) residue.[3]
Conclusion: These outcomes not only broaden the structural and chemical diversity of this class of RiPPs, but furthermore allow a deeper understanding of the restrictions for a precursor peptide to be transformed into this unique, native lasso fold by a machinery comprising of only two biosynthetic enzymes.

[1] Hegemann et al. *Biopolymers* **2013**, 527-542. [2] Hegemann et al. *Angew. Chem.* **2014**, 2230-2234. [5] Zimmermann et al. *Chemical Science* **2014**, 4032-4043. [4] Hegemann et. al. *J. Am. Chem. Soc.* **2013**, 210-222. [5] Zimmermann et al. *Chem Biol* **2013**, 558-569.

SMeV05
Glycolipid biosynthesis in *Ustilago maydis*
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Under nitrogen starvation the smut fungus *Ustilago maydis* produces a bunch of secondary metabolites. Among these are the glycolipids Ustilagic acid (UA) and Mannosylerythritol lipid (MEL), which consist of a sugar moiety esterified with fatty acid side chains of variable length (from C2 - C16). The biosynthesis of UA is encoded by the UA gene cluster (11 genes). MEL production depends on the MEL gene cluster composed of the genes *mat1*, *mmf1*, *mac1*, *emt1* and *mac2*. Deletion of *mac1, mac2* or *emt1* in *U. maydis* resulted in the complete loss of MELs (1).
Recently, we have characterized the role of peroxisomes in MEL biosynthesis. The two mannosylerythritol lipid acyltransferases Mac1 and Mac2 contain bona fide peroxisomal targeting sequences 1 (PTS1) at the C-termini. Cytosolic targeting of both Mac1 and Mac2, altered MEL production and interfered with UA biosynthesis (2).
Here, we further characterise the functions of Mmf1, a protein of the major facilitator superfamily, and Emt1, the Erythritol-mannosyl-transferase. The analysis of glycolipid production by thin layer chromatography and mass spectrometry from wild type strain MB215 revealed a mixture of MELs with different grade (non-, mono- and di-acetylated) of acetylation. Strains without Mmf1 show no mono-acetylated variants whereas overexpression of Mmf1 resulted in increased amounts of mono-acetylated MELs. This indicates a role of this cell membrane located transporter in the secretion of MELs. Further results will be discussed.

(1) Hewald et al., *Appl. Environ. Microbiol.* (2006)
(2) Freitag et al., *Mol. Microbiol.* (2014)

SMeV06
Comparison of the exometabolome composition of two members of the Roseobacter clade
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Marine dissolved organic matter (DOM), the marine geometabolome, is an extremely complex mixture composed of a wide variety of compounds. The molecular chemodiversity affects the function and turnover rate of DOM in the ocean. We hypothesize that the active microbial community essentially contributes to the complexity of the DOM pool through uptake and excretion of compounds, and that these processes are regulated by the composition of the respective microbial community. We investigated the exometabolome of two pelagic representatives of the *Roseobacter* clade; *Phaeobacter inhibens* DSM 17395 and *Dinoroseobacter shibae*. The organisms were grown separately in cultures on defined substrates (fatty acids, amino acids, monosaccharides). The exometabolome was characterized on molecular level using Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) as well as High Performance Liquid Chromatography (HPLC) for amino acids, mono and polyssacharides. The exometabolome composition varied between the tested strains, i.e. while for *P. inhibens*, >1000 formulae could be detected, the exometabolome of *D. shibae* only comprised ~ 400 formulae. Furthermore, both strains exhibit differential excretion of metabolites depending on the growth phase as well as growth conditions.

SMeV07
Identification of activated secondary metabolite clusters on protein and metabolic level of *Aspergillus nidulans* after inducing low temperature stress
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Introduction: Fungi produce an arsenal of secondary metabolites for various purposes, e.g. intercellular communication or chemical defense [1]. Many of these molecules are only produced under certain conditions. By a specific trigger, e. g. a stress condition, silence gene clusters are activated. So far, little is known about how fungi adapt to cold stress and whether these conditions induce the biosynthesis of secondary metabolites. In our study, we investigated the stress response of the industrially important filamentous fungus *Aspergillus nidulans* during cold stress.
Objectives: To identify key processes induced at low temperature stress, the proteome such as the secondary metabolite production of the filamentous fungus *Aspergillus nidulans* was analyzed under defined cold stress conditions.
Methods: In order to identify activated secondary metabolite gene clusters, the proteins that were differentially expressed at cold-stress were analyzed in a comparative gel-based (DIGE) and gel-free (LC-MS) proteomic approach. Further on, the secondary metabolites were identified directly via LC-MS.
Results: The comparative protein analysis led to the identification of proteins involved in cellular transport, transcription, cell cycle and protein modification. Furthermore, we identified indirectly on proteomic and directly on metabolic level factors that influence the sexual development in *Aspergillus nidulans* or compounds that exhibit toxic activity.
Conclusion: This work provides first insights into the regulation of sexual development in *Aspergillus nidulans* after inducing cold stress. Moreover, it shows the successful combination of different experimental strategies to improve the identification of activated secondary metabolite clusters. The combination of proteomic and metabolomic approach leads to an increased number of identified activated secondary metabolite clusters in comparison to the direct analysis alone.

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SMeV08
Unraveling the biosynthesis of secondary metabolite 'Sodorifen' of *S. plymuthica 4Rx13*
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The existence of microorganisms has been a blessing and a curse for mankind ever since. Nowadays, microorganisms are a popular research object due to their many beneficial characteristics, not only for being pathogenic. Especially bacteria produce many solid compounds like antibiotics but also diverse volatile compounds that are compiled in the database mVOC (1).
Thus, bacteria are investigated more detailed for their action potential. But the HOW and WHY are also exciting questions which inevitable occur. This study focuses on the elucidation of the biosynthesis of volatile compounds and on the uncovering of their function. The rhizobacteria *S. plymuthica* is of interest because of its rich VOC-profile. The main compound 'Sodorifen', was elucidated in 2010 (2) and has a unique and remarkable structure ($C_{16}H_{26}$). The analysis of Sodorifen with NMR revealed its bicylic ring structure that is utterly methylated and has no heteroatoms. Therefore, the classification of this compound remains unclear and so does the biosynthesis. The approach for investigating the biosynthesis was threefold: Firtsly, the comparison of the genome of Sodorifen producer to non-producer. Secondly, the proteome comparison and thirdly, the transcriptome analysis between producer and non-producer strains. Potential candidate genes were extracted throgh the approaches and knock-out mutants generated. After checking their VOC-profile, one gene proved to be of interest because of its Sodorifen negative phenotype. Further investigations on genome level revealed a cluster of four genes.

The other three genes within the cluster were investigated and pointed to a Sodorifen negative phenotype, with one exception that is possibly partially involved. A number of new compounds were emitted which could represent intermediates or a byproducts of the biosynthesis. Structure examination of the genes within the cluster with labeled compounds and NMR analysis were done and gave hints for both hypotheses.
First genes with a potential involvement in the biosynthesis are found but not sufficient for building the compound. Thus, additional candidate genes have to be discovered and mutants generated.

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SMV01

Construction and analysis of synthetic RNA modules derived from prokaryotic regulatory RNAs

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Synthetic biology often combines natural occurring cellular elements to generate new mechanisms of gene regulation. Here, we make use of regulatory RNA molecules from prokaryotes to design regulatory devices with novel functionality. The constructed RNA modules consist of a riboswitch and an RNA thermometer (RNAT). Riboswitches regulate gene expression by binding to cellular metabolites that cause a switch in their structural conformation. As a result, the expression of the downstream gene is changed (1). In contrast, RNATs modulate translation efficiency by temperature-responsive RNA structures. In a zipper-like manner they sequester the Shine-Dalgarno (SD) sequence at low temperatures, hence inhibiting translation initiation. With rising temperatures, the secondary structure unfolds and liberates the SD sequence, allowing translation of the mRNA (2, 3).
By connecting a transcriptional riboswitch with an RNAT, a consecutive fusion was designed, which allows regulation on transcriptional and translational level. Reporter gene assays with a heat-stable β-galactosidase and the chemotaxis protein CheZ showed dual regulation by a metabolite and temperature. The functionality of the RNAT in the RNA module was confirmed by site-directed mutagenesis.
Moreover we were able to integrate a temperature-responsive RNA hairpin of a fourU thermometer (4) into a ligand-sensing riboswitch, and thus generated a temperature-controlled riboswitch. These examples show how modular regulatory RNA elements are and how they can be exploited in synthetic biology.

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SMV02

Towards synthetic CO_2 fixation in *Methylobacterium extorquens*: establishing genetic tools for Alphaproteobacteria and realizing artificial CO_2-fixation *in vitro*

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Introduction: The problem of steadily rising atmospheric CO_2 concentrations requires society to find novel creative ideas to control this greenhouse gas. Instead of optimizing existing natural pathways (e.g., the Calvin cycle in plants), the emerging field of synthetic biology provides an alternative approach to address this question. By combining different enzymatic reactions, artificial pathways for CO_2-fixation are intended to be created that are of improved efficiency compared to natural existing CO_2-fixation routes.
Objectives: The alphaproteobacterial model organism *Methylobacterium extorquens* is to be established as a host for the implementation of artificial CO_2-fixation pathways. Such synthetic CO_2-fixation pathways are realized and tested on a dedicated *in vitro* platform prior to their *in vivo* implementation.
Methods: To assemble genetic elements in a standardized and convenient fashion, a toolset for synthetic biology in Alphaproteobacteria has been developed that includes a suite of brick-like vectors and various promoters. In parallel, a mass-spectrometry based platform for the assembly and characterization of synthetic CO_2-fixation pathways has been established.
Results: A genetic toolset for metabolic engineering of *M. extorquens* and other Alphaproteobacteria was established[1]. Using these so-called MethyloBricks, a three-enzyme module of a synthetic pathway was successfully implemented in *M. extorquens*, allowing for assimilation of the otherwise toxic compound glyoxylate. In addition, a synthetic CO_2 fixation pathway comprised of twelve enzymes was successfully reconstituted *in vitro* from purified enzymes.
Conclusion: The genetic engineering of *M. extorquens* towards a CO_2-fixing organism, by using new tools and innovative approaches, revealed new details about the physiology and (artificial) evolution of the fundamental biological process of autotrophy. A first non-natural CO_2-fixation pathway was successfully realized that provides the basis for *in vivo* implementation.

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SMV03

A LOV2 DOMAIN-BASED OPTOGENETIC TOOL FOR SYNTHETIC REGULATION OF PROTEIN STABILITY IN EUKARYOTIC CELLS

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Regulation of cellular behavior by light requires a photoreceptor fused to an output domain that influences a cellular response. The photo-sensitive degron (psd) module consists of the photoreceptor domain LOV2 of *Arabidopsis thaliana* phototropin 1 fused to the murine ornithine decarboxylase-like degradation sequence cODC1. The degradation sequence induces proteolysis of the fusion protein by the proteasome. Thus, the psd module provides synthetic light-control of protein stability. Variants of the psd modules with altered light-sensitivity were generated by site-specific and random mutagenesis as well as using different LOV2 domains. Characterization of the variants in the model organism *Saccharomyces cerevisiae* showed that psd modules with increased and decreased light sensitivity were obtained. In total, the mutational analysis resulted in psd module variants, which provide tuning of protein stability over a broad range by blue light. Several variants showed characteristics that are profoundly improved compared to the original construct. The modular usage of the LOV2 domain in optogenetic tools allows the usage of the mutants in the context of other applications in synthetic and systems biology as well.

SMV04

MreB paralogs of *Bacillus subtilis* associate with a lipid bilayer and co-polymerize to a single curved filament *in vitro*

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Many non-spherical bacteria express one or more actin-like MreB proteins. Among other cellular tasks, these cytoskeletal elements are suggested to function in cell shape maintenance by spatially organizing cell wall synthesis. Super resolution microscopy revealed that in the model bacterium *B. subtilis*, MreB and its paralogs Mbl and MreBH assemble into dynamic, discontinuous filaments of variable length underneath the lateral membrane. It is not clear how average length and localization to the curved membrane surface are achieved.
In this study, we performed an *in vitro* assay that allowed inspection of the polymerization properties of purified *B. subtilis* MreB proteins by fluorescence microscopy and electron microscopy. Taking advantage of the intrinsic membrane affinity of MreB proteins, we immobilized the paralogs on flat lipid bilayers attached to glass slides.
In the presence of divalent cations, each MreB paralog polymerized to helical filaments of concentration-dependent length. MreB visualized by a maleimide dye formed filaments indistinguishable to those of YFP-MreB showing that neither the formation of filaments nor their architecture are artifacts of the fluorescence marker but an intrinsic property of MreB. The helical turns exhibited diameters in a range of the cell diameter of *B. subtilis*, indicating that the curved architecture is not determined by the cylindrical cell envelop but that it may be an intrinsic feature of the

filaments. Monovalent cations inhibited the polymerization in a dose-dependent manner. Efficient inhibition occurred at about 20 fold higher concentration of monovalent cations compared with stabilization through divalent ions, suggesting that cations take an active part in the remodeling of the filaments *in vivo,* and that their ratios in cells determine the average length of filaments. When applied as a mixture, the three paralogs polymerized to a single helical filament consistent with their co-localization in filaments in *B. subtilis*. Electron microscopy could resolve MreB filaments as sheets of protofilaments, with dual protofilaments being the minimal structure that was observed as individual filament.
Our data reveal that many properties of the localization of MreB can be explained by intrinsic polymerization properties of the actin-like protein.

SMV05
Synthetic secondary chromosomes in *Escherichia coli* based on the replication origin of chromosome II in *Vibrio cholerae*
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Engineering of bacteria for biotechnological applications is typically reached either by modifying primary chromosomes or construction of plasmids. An alternative approach is the addition of synthetic secondary chromosomes to the cell. We constructed the replicon synVicII based on the replication origin of *Vibrio cholerae* chromosome II and successfully transferred it to *Escherichia coli*. To measure replicon stability, a new flow-cytometry-based assay was established. Respective measurements showed that synVicII is maintained more stable than a synthetic chromosome based on the *E. coli* replication origin *oriC*. Cell-cycle analysis and determination of cellular copy numbers of synVicII indicate that replication timing in *E. coli* is comparable to the natural chromosome II in *V. cholerae*. Additional features of synVicII allow the assembly of larger replicons for synthetic-biology studies on chromosome biology and biotechnology applications.

SMV06
Global Signal Integration of Bacterial Two-component Systems
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Background: Bacteria are endowed with a unique family of protein pair known as the two-component system (TCS) to sense various environmental signals encompassing pH, temperature, light, chemo-attractants and osmolarity. A phosphorelay of the TCS allow bacteria to modulate the expression of the downstream genes which controls abroad variety of phenotypes spanning central metabolism, cell differentiation, motility through bio-film formation and even virulence of pathogenic bacteria.
Objectives: Considering the functional importance of the TCS in the physiological processes and virulence *per se* of pathogenic bacteria, the non-conventional cross-talk between non-cognate pairs has been shown *in vitro*. However, such studies were focused to elucidate their cross-interaction in parts and little had been focused on integrating the total TCS of bacteria with an aim to bring out the mechanism of signal integration as a whole.
Methods: We have adopted a theoretical approach to build an interactome comprising the proteins from the two component signal transduction systems of different classes of bacteria. These interactomes were then analyzed by using different graph theoretical network parameters like centrality and k-core measures.
Results: We have delineated a signature of these different networks and figured out the most indispensable proteins from them by parametric analyses, of which PhoP and CheY have significant scores. Finally, we have built a model to correctly predict the order of the dynamic behavior of such signal transduction systems.
Conclusion: We have attempted to delineate the global picture of all the interacting two-component systems in an organism using sets of graph theoretical parameters. These might serve as models to give a bird's eye view of the gross behaviour of the TCS interactions. Understanding these classes of proteins would contribute in identifying novel targets for antimicrobial agents.

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SMV07
Site-directed mutagenesis switching a dimethylallyl tryptophan synthase to a specific tyrosine *C3*-prenylating enzyme
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Introduction: Tryptophan prenyltransferases FgaPT2 and 7-DMATS catalyze *C4*- and *C7*-prenylation of the indole ring [1,2], respectively. 7-DMATS was found to accept l-tyrosine as substrate as well and converted it to an *O*-prenylated derivative. SirD and TyrPT, which catalyze the *O*-prenylation of the phenolic hydroxyl group in tyrosine, accept l-tryptophan as substrate as well and catalyze the same *C7*-prenylation as 7-DMATS [3, 4].
Objectives: Encouraged by the results obtained for l-tryptophan with SirD and TyrPT and l-tyrosine with 7-DMATS, we investigated the behavior of FgaPT2 towards l-tyrosine and its derivatives.
Methods: pIU18 was used for FgaPT2 overproduction [5] and as DNA template for site-directed mutagenesis experiments. One step site-directed mutagenesis protocol was used to generate the mutated derivatives of FgaPT2.
Results: FgaPT2 catalyzes *in vitro* regular *C*3-prenylation of l-tyrosine and 4-amino-l-phenylalanine. Molecular modeling and site-directed mutagenesis led to creation of a mutant FgaPT2_K174F, whose catalytic efficiency towards l-tyrosine was 4.8-fold compared to that of non-mutated FgaPT2, while the activity towards l-tryptophan was less than 0.4 % of that of the wild-type.
Conclusion: The first l-tyrosine *C*3-prenylating enzyme was created by molecular modeling-guided mutagenesis. Single mutation on the key amino acid switches the tryptophan *C*4-prenyltransferase to a tyrosine *C*3-prenylating enzyme.

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SMV08
Corynebacterium glutamicum as a host for the production of GABA
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Introduction: *Gamma*-Aminobutyric acid (GABA) is a non-protein amino acid widespread in nature from microorganisms to plants and animals. In microorganisms, GABA is involved in the spore germination, is an important component for acidic pH resistance and is required for the normal oxidative stress tolerance [1]. GABA also represents a building block of bio-plastics. For example, the bio-plastic polyamide 4 (PA4) is the linear polymer of GABA. PA4 has the advantage of be biodegradable either in the soil as well as in activated sludges [2].
GABA is usually produced by glutamate decarboxylase (GAD), a pyridoxal 5'-phosphate-dependent enzyme, which catalyzes the α-decarboxylation of glutamate to GABA. *Corynebacterium glutamicum*, a natural L-glutamate producer, has been successfully engineered for the conversion of L-glutamate to GABA without addition of L-glutamate [2].
Objectives: Create a new pathway for the production of GABA in *C. glutamicum* and optimize the production of GABA.
Methods: Metabolic engineering of a *C. glutamicum* putrescine production strain (PUT21) involving the overexpression of putrescine transaminase (*patA*) and γ-aminobutyraldehyde dehydrogenase (*patD*) from *Escherichia coli* and deletion of *C. glutamicum* endogenous genes.
Results: The heterologous expression of *patA* and *patD* in PUT21 led to the production of GABA in *C. glutamicum*. The GABA production was optimized by modification of CGXII medium and by deletion of GABA catabolic genes and the GABA uptake gene of *C. glutamicum*.
Conclusion: For the first time the production of GABA from putrescine is reported. The production achieved by the new pathway is very competitive comparing with the traditional pathway.

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SYV01

Evolution from pathogen to commensalist – Pseudomonas aeruginosa obtains selfish fitness gains through virulence attenuation during in vivo adaptation to immunocompetent and immunocompromised metazoan hosts

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Symbiotic interactions are indispensable for metazoan function, but their origin and evolution remains elusive. Host expansion, the initial infection of hosts that microorganisms have not met before, may create particular opportunities to study how novel interactions evolve. Host immunity likely plays an important role in the establishment and evolution of new symbionts. To understand the origin of novel biological interactions, we performed an evolution experiment in which initially pathogenic Pseudomonas aeruginosa adapted to populations of immunocompetent and immunocompromised C. elegans host strains. Phenotypic analyses revealed rapid, to a large extent parallel evolution across all treatments. Intriguingly, all strains strongly increased fitness by loss of virulence. Genomic analyses suggest the evolving commensalist interaction is driven by few crucial bacterial mutations in central regulator genes that arose early in evolution. These results provide important insights for the understanding of bacterial adaptation to host populations, and suggest bacteria may drive metazoan function and evolution.

SYV02

***Endomicrobium proavitum*, a free-living relative of flagellate endosymbionts in termite guts – a real-time model of reductive genome evolution**

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Introduction: Obligately intracellular symbionts in insect tissues have experienced the progressive loss of genes that are no longer required in the symbiosis, whereas essential pathways are retained. While the original uptake of these endosymbionts occurred more than 150 Mya ago [1], the intracellular symbiosis between "Endomicrobia" and termite gut flagellates is considerably younger [2]. A comparison of the genome of the obligately endosymbiotic *Candidatus* Endomicrobium trichonymphae [3] with that of a free-living relative would allow an examination of the earlier stages of reductive genome evolution from a free-living bacterium to an intracellular symbiont.
Objectives: Isolation of a free-living "Endomicrobium" and comparative analysis of the genome reduction process in the endosymbionts.
Methods: Strain Rsa215 was isolated from the 0.2-µm filtrate of *Reticulitermes santonensis* gut homogenate and physiologcally and ultrastructurally characterized. The genome was sequenced using PacBio RS II and assembled with HGAP3.
Results: We isolated *Endomicrobium proavitum*, the first cultivated representative of the Endomicrobia clade (phylum *Elusimicrobia*). It is a free-living, obligately anaerobic and nitrogen-fixing ultramicrobacterium. However, its set of *nif* genes (Group IV) had previously been considered not to encode a functional nitrogenase. Its circular genome (1.59 Mbp) is larger than that of its endosymbiotic relative. As is typical of endosymbionts, many of the pathways that are pseudogenized in the endosymbiont are intact in *E. proavitum*, but we detected some pathways present in the endosymbiont are absent in the free-living strain.
Conclusions: Comparative analysis of the genomes of the free-living *E. proavitum* and its endosymbiotic relative indicates that differences in the respective habitats are responsible for the retention and elimination of specific genes and pathways. Further studies on Endomicrobia promise a better understanding of the evolutionary processes that occur during the earlier stages of symbiogenesis.

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SYV03

An arsenal of toxin-related genes in the genomes of beneficial symbionts from deep-sea hydrothermal vent mussels

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Introduction: *Bathymodiolus* mussels are prominent members of deep-sea hydrothermal vent and cold seep communities worldwide. The key to their success is their symbiotic association with intracellular sulfur-oxidizing bacteria that provide them with nutrition. The mussel symbionts are horizontally transmitted, and each new host generation must be infected from the environment. Nothing is known about the molecular mechanisms that underpin establishment and maintenance of the *Bathymodiolus* symbiosis.
Objective: Gain a better understanding of the genomic basis of the *Bathymodiolus* symbiosis
Methods: We sequenced symbiont genomes from two mussel species and compared these with the genomes of their closest symbiotic and free-living relatives. These are the sulfur-oxidizing vertically transmitted symbionts of vesicomyid clams, and the widespread free-living SUP05 bacteria.
Results: The *Bathymodiolus* SOX symbionts have undergone massive genome rearrangements, and up to 33% of its genes may be of foreign origin. Many of the potentially foreign genes were homologs of virulence genes. We discovered a diverse array of toxins similar to insecticidal toxins of nematode and aphid symbionts, and toxins of pathogens such as *Yersinia* and *Vibrio*. Transcriptomics and proteomics revealed that the SOX symbionts express these toxins in their hosts.
Conclusion: We hypothesize that the symbionts 'tamed' some ofuse their arsenal of toxins to use inin beneficial interactions with their host. Intriguingly, some toxins may be used to protect the host against parasites. Defensive symbioses based on protein toxins are common in insects, but are so far unknown in marine habitats.

SYV04

Interaction of non-seed plants with arbuscular mycorrhizal fungi

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Introduction: The arbuscular mycorrhizal (AM) symbiosis is one of the most ancient and widespread plant-fungi associations, leading to a great ecological and agronomical importance.
Material-Methods: *P. patens, M. marginata*, *M. paleacea*, *M.polymorpha. Pseudomonas*, *Caulobacter*, *Agrobacterium and Streptomyces*. Glomeromycota and Mucoromycota fungi.
Co-cultivation, Microscopy, Molecular cloning, Bioinformatics
Results: *Although Physcomitrella patens* is not known to establish AM association in nature, it could occasionally be observed under *in vitro* conditions[1]. Phylogenomic analyses of the essential genes of the symbiotic pathway reveal at least one ortholog of each gene in the *P. patens* genome[2]. The focus of this study is the optimization of *P. patens* and fungi *in vitro* association through: 1) Use of *P.patens* mutants overexpressing the Calcium-calmodulin kinase (CCaMK)[3]. These lines are now been genotyped and phenotyped. 2) Screening of AM fungi candidates. 3) Use of helper bacteria as a potential third factor of the AM association. For the establishment of an accurate AM symbiosis detector we are generating *P. patens* mutants with a cameleon sensor that facilitates the detection of the Calcium spiking occurring during AM establishment.
Conclusions: *The* reason of *P.patens* for retaining essential AM genes remains elusive. We expect to optimize *P. patens* through dominant expression of CCaMK [4] and to overcome a possible AM fungi-host plant specificity by screening of fungi. In this way and considering the broad palette of molecular tools (unique capability of homologous recombination among plants) and together with its position in the land plant phylogenetic tree, *P. patens* could be established as a tractable model for AM studies.

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SYV05

Bacterial symbionts as major players in the biology of the whitefly *Bemisia tabaci*

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Introduction: The whitefly *Bemisia tabaci* is an extremely devastating insect pest that inflicts damage by direct feeding and by transmitting more than 100 plant viruses. *B. tabaci* harbors several symbiotic bacteria, including *Portiera aleyrodidarum*, the primary obligatory symbiont, and several secondary symbionts the function of which is unknown.
Objectives: The main objective of our studies is to unravel functions that secondary symbionts play in the biology of *B. tabaci*, that contribute to the damage it inflicts in agriculture such as virus transmission, and the molecular mechanisms that determine the insect-symbiont interactions. Specifically, the role of *Hamiltonella* and *Rickettsia* in begomovirus transmission by *B. tabaci,* and the role of insect proteins in maintaining proper symbiosis with *Rickettsia* will be presented.
Materials & Methods: *B. tabaci* populations that harbour different symbiont combinations and iso-female strains that differ in the content of one bacterium were used. Next generation sequencing, bioassays and molecular verifications, as well as functional validation of gene functions such as RNA interference, were used to investigate insect and symbiont proteins involved in the insect-symbiont interactions.
Results: *Hamiltonella* [1] and *Rickettdia* [2] play important roles in begomovirus transmission by *B. tabaci,* involving one known GroEL protein encoded by *Hamiltonella*. Other insect and symbiont proteins are under investigation. Deep sequencing analysis revealed proteins with essential roles in maintaining proper symbiosis with *Rickettsia,* including the vitellogenin protein. Other proteins such as *vATPase*, *cathepsin D* and *hsp70* have roles in maintaining proper structure and function of bacteriosomes that harbour symbiotic bacteria.
Conclusion: Symbiosis is a wide term that represents complex interactions between bacterial symbionts and their host. In the case of *B. tabaci* and its secondary symbionts, we show that these interactions involve proteins that have essential roles in the damage that *B. tabaci* causes, and in maintaining proper symbiosis between the symbionts and their insect host.

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SYV06

Towards exploring a novel symbiosis between Planctomycetes and macroalgae

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Planctomycetes are ubiquitous environmentally important bacteria. Only attached living cells can divide while daughter cells are flagellated planktonic swimmers. The unusual planctomycetal FtsZ-independent cell division through budding comes at the price of slow growth (doubling times: 6 hours - 1 month). However others and we found Planctomycetes to dominate biofilms on certain macroalgae, a surprising finding since many fast growing heterotrophic bacteria compete for this habitat.
Hypothesis: Planctomycetes live in symbiosis with certain macroalgae. While algae feed Planctomycetes with complex polysaccharides, the bacteria in return produce small molecules that prevent other organisms from biofilm formation.
Our hypothesis is supported by the thin biofilm that covers e.g. *Laminaria* spec. while surfaces in marine habitats get otherwise immediately colonized by various organisms and are covered with thick biofilms within days. Thus an unknown antifouling strategy protect kelp from colonization. Since others and we demonstrated that Planctomycetes could exclusively degrade certain complex (sulfated) polysaccharides, such algae might employ a planctomycetal specific 'food' to provide them a growth advantage.
We used phenotypic microarrays and an ecomimetic approach to simulate the interaction of Planctomycetes and macroalgae *in vitro*. HPLC measurements indicate changes in the secondary metabolite profile of planctomycetal extracts when fed with certain compounds. Simulation of the 'attack' of other heterotrophs with antibiotic threads led to changes in HPLC profiles and such extracts were found to comprise antibiotic activity, further supporting our hypothesis. Besides *in vitro* experiments, we analyzed the colonization of macroalgae from Helgoland (North Sea) and kelp forests of Monterey Bay (Pacific Ocean) employing cultivation dependent and independent methods. We were able to cultivate and describe four novel planctomycetal genera and two novel species that were characterized through various (bio) chemical-, microbiological and microscopic techniques. Our results point towards a putative symbiosis of certain macroalgae and Planctomycetes that might represent a useful source for novel secondary metabolites with various biological activities.

SYV07

Mutualistic root endophytism is not associated with the reduction of saprotrophic characters and requires a non-compromised plant innate immunity

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Introduction: During a compatible interaction, the sebacinoid root endophytes *Piriformospora indica* and *Sebacina vermifera* induce modification of root morphology and enhance shoot growth *in Arabidopsis thaliana.*
Objectives: We investigated the genomic traits common in these two fungi and compared them with those of other root associated fungi and saprotrophs.
Materials & Methods: The transcriptional responses of the two sebacinoid fungi and of the plant host Arabidopsis to colonization at three different symbiotic stages (3, 7 and 14 days post infection) were analyzed via microarrays.
Results: We identified key genomic features specific to orchid mycorrhizal fungi, such as expansion for lectin-like proteins and proteins involved in protein-protein interaction [1 and unpublished]. Additionally, we demonstrate that colonization of Arabidopsis by the sebacinoid fungi leads to a biotrophic interaction which correlates to a dramatic shift in the transcriptome profile of the roots [2 and unpublished]. Induction of salicylic acid at early time point was suppressed at 14 dpi where jasmonate, ethylene and glucosinolate were among the most induced pathways in both symbioses. Genes involved in developmental processes were specifically induced in *S. vermifera* at 14 dpi.
Conclusion: These data provides a starting point for understanding which pathways are involved in the establishment and maintenance of an Arabidopsis root association with endophytic fungi.

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SYV08

Volatiles of symbiotic bacteria of the human skin microbiome

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The human skin is the organ which covers the body and serves as barrier to protect the body against excessive loss of water, harmful substances and pathogens. It is the part of the body which is in contact with the external environment and therefore is colonized by diverse species of microorganisms called the skin microbiome. This microbiome is dominated by bacteria; they are mainly composed of actinobacteria (51.8%, largely *Corynebacterium* sp.) followed by firmicute (24.4%, largely *Staphylococcus* sp.) (Grice et al., 2009). Mostly harmless, they are symbiotic bacteria since they perform useful task for the host. It is well known that some of them synthesize compounds with interesting antimicrobial properties albeit most of those compounds are antimicrobial peptides (Bastos et al., 2009). In contrast knowledge on the nature and the function of volatile compounds which are emitted during their metabolism is scarce. Thus, the volatile profile of 7 *Corynebacterium* strains and 10 *Staphylococcus* strains were analysed using headspace collecting system

and GC/MS. The results revealed that they can emit a wide number of volatile organic compounds (VOCs). Some of these VOCs are species-specific and others can be emitted by several strains. Interestingly, the volatile profile of *Staphylococcus schleiferi* strains revealed new amino/imino ketones and alcohol. They are new in the nature and have never been reported to be emitted by any organism and therefore, the investigation of their biosynthetic pathway was initiated.
The role of VOCs emitted by the skin microbiata in communication between skin microorganism communities and their effect on pathogens remains to be investigated for the better understanding of the balance between host and microorganisms.

TAV01
How does *Staphylococcus aureus* generate membrane potential?
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Introduction: In many aerobic and facultative aerobic microorganisms, the NADH:quinone oxidoreductase represents the entry point of the respiratory electron transfer. The protein oxidizes NADH generated during glucose degradation, and reduces quinone. The complex I-type enzyme (Ndh1) links this reaction to the translocation of cations. In *Escherichia coli*, the complex I-subunit NuoL accomplishes cation translocation and thus generation of membrane potential ($\Delta\psi$). NADH oxidation may also be achieved through the alternative, non-electrogenic type-2 NADH:quinone oxidoreductase (Ndh2), that is not translocating cations.
Objective: As these enzymes were unknown in *Staphylococcus aureus* so far, we aimed to identify the entry point of electrons in the *S. aureus* respiratory chain.
Methods and Results: NADH oxidation measurements revealed Ndh2 as the major NADH:quinone oxidoreductase in *S. aureus*. Additionally, we found a hypothetical protein in *S. aureus* with sequence similarity to the proton-translocating subunit NuoL in *E. coli*'s complex I: the NuoL-like protein Sao412. Deletion mutants of *sao412* and its corresponding operon *sao412-413-414* showed a small colony variant-like phenotype and were strongly impaired in $\Delta\psi$ and oxygen consumption rates. No NADH oxidation activity could be assigned to the Sao412-414 proteins. The cation-translocating function of the Sao412-414 system was shown using a Na^+/H^+ antiporter-deficient *E. coli*.
Conclusion: Sao412-414 represent an important system of the *S. aureus* respiratory chain acting as an electrogenic unit and accomplishing the generation of $\Delta\psi$.

TAV02
The crystal structure of the Na^+-translocating NADH ubiquinone oxidoreductase from *Vibrio cholerae*
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Introduction: The human pathogen *Vibrio cholerae* maintains a Na^+ gradient across the cytoplasmic membrane. The generated sodium motive force is essential for substrate uptake, motility, pathogenicity, or efflux of antibiotics. This gradient is generated by an integral membrane protein complex, the NADH:ubiquinone oxidoreductase (NQR). It catalyzes the same reaction like mitochondrial complex I but both respiratory enzymes exhibit a completely different architecture. NQR is closely related to the so-called RNF complex that is very common in bacteria and occurs as well as in archaea. The NQR complex consists of six different subunits, NqrA-NqrF.
Objectives and Methods: In order to get insights into the mechanism of redox driven Na^+-transport we have isolated and crystallized the NQR of *Vibrio cholerae* [1,2].
Results: The crystals of the entire membrane complex diffract to 3.5 Å. Moreover, we determined independently the structures of the major soluble domains of subunits NqrA, C and F at 1.9 Å, 1.6 Å and 1.7 Å, respectively, completing large parts of the structure of the respiratory complex at high resolution [3,4]. Altogether, the structural information gives a detailed picture of the NQR and allows also a close view on the core subunits of homologous RNF complex.
Conclusion: The structural information available now allows for the first time the detailed analysis of the ion translocation pathway across the membrane and of the coupling between redox and translocation reactions.

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TAV03
ATP-dependent conformational changes trigger substrate capture and release in an ECF-type biotin transporter
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Introduction: ABC-type ECF transporters mediate vitamin and transition metal ion uptake in prokaryotes. They comprise two ABC ATPases, a transmembrane coupling (T) component and a membrane-embedded substrate-specific (S) unit [1]. Based on crystal structures in which the S unit lies nearly horizontally in the membrane, a unique transport mechanism was proposed [2, 3]. Experimental evidence for this toppling hypothesis was not reported.
Objectives: Getting insights into the transport cycle by biochemical and biophysical methods using a biotin transporter as the model.
Methods: Purified BioMNY was reconstituted into nanodiscs, phospholipid bilayers in which the protein/lipid complex is encircled by an amphipathic helical protein belt. Distance changes within BioMNY were recorded by site-specific crosslinking and by EPR upon nitroxide-spin labeling. Reorientation of the MIANS-labeled BioY S unit within the lipid phase was inferred from hypsochromic/bathochromic shifts of fluorescence emissions. Substrate capture/release was analyzed with radioactive biotin.
Results and Conclusion: ATP-induced distance changes between the two BioM ATPases suggested a closure of the dimer. In consequence, the positioning of a coupling helix in the BioN T unit relative to the first hydrophobic helix in BioY was moderately altered but the interaction was not broken. Fluorescence emission shifts argue for repositioning of the substrate-binding site rather than an overall tilting of BioY. Biotin capture and release by BioMNY was dependent on binding of ATP and hydrolysis of Mg-ATP, respectively.
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TAV04
Transport meets Systems Biology: Characterization of Fructose Efflux in *Corynebacterium glutamicum*
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The Gram-positive, non-pathogenic *Corynebacterium glutamicum* is well known for its application in industrial amino acid production. Sugar blends derived from molasses, containing high concentrations of sucrose, are used for industrial fermentations as feedstock. The PEP:carbohydrate phosphotransferase system (PTS) substrate-specific EII_{Suc} permease (encoded by *ptsS*) takes up sucrose and concomitantly phosphorylates it into sucrose-6-phosphate, which is then hydrolyzed via sucrose-6-phosphate hydrolase ScrB to glucose-6-phosphate and fructose. Although phosphorylation to fructosephosphate is a prerequisite for fructose metabolization, *C. glutamicum* does not possess enzymes with fructokinase activity[1]. Therefore, intracellular fructose has to be exported by unknown transporter(s) and re-imported by the fructose specific EII_{Fru} (encoded by *ptsF*)[2].
We here present a Systems Biology approach for characterization of sugar export in bacteria. We developed a minimal *C. glutamicum* model strain, which is capable of efficient sucrose uptake, unable of fructose re-uptake as well as in any fructose converting reaction. This strain allows easy modelling of fructose fluxes as it is a highly specific and selective tool to determine kinetic parameters of fructose efflux, namely intracellular fructose accumulation, steady-state-levels and export velocity. By using ^{14}C-labelled sucrose, fructose-efflux-experiments were performed for short-term observation of intracellular fructose fate. Based on these data, we developed a kinetic model which allows prediction of internal fructose

concentrations and export kinetics. This model was verified in long-term observations of fructose steady-state levels in both, *C. glutamicum wildtype* and model-strains. By this means, we here show the contribution of mechanosensitive channels to fructose efflux, resulting in fructose steady state levels dependent on the external osmolality. Moreover we demonstrate and characterize for the first time the specific export of fructose by transport proteins in *C. glutamicum*.

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TAV05

The Influence of the *N*-terminal Helix on Formate Translocation by the Pentameric Formate Channel FocA

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During fermentation *E. coli* is able to convert up to one third of the carbon derived from glucose to formate, which results from the cleavage of pyruvate by PflB (pyruvate formate-lyase). Formate reduces the internal pH, therefore during growth it is translocated across the cytoplasmic membrane into the periplasm. In the stationary phase formate is quantitatively re-imported for internal consumption by the formate hydrogenlyase complex. One means by which formate is translocated across the membrane is via the channel FocA, which belongs to the formate-nitrite transporter (FNT) superfamily of homopentameric membrane proteins. FocA translocates formate bi-directionally. Recently, we have shown that PflB regulates formate passage through FocA by direct protein-protein interaction via the flexible, approximately 35-amino acid, *N*-terminal helix of FocA[1]. Furthermore, during a recent amino acid exchange programme we noted that amino acid K26 of FocA influenced formate transport[2]. Therefore, here we analysed the effects of *N*- terminal amino acid truncations of FocA on formate translocation. These variants were analysed by monitoring changes in intracellular formate levels using a chromosomal formate-inducible *fdhF'-'lacZ* reporter fusion. We also analysed the influence of these exchanges on the growth-inhibitory effects caused by the toxic formate analogue hypophosphite, which is also translocated by FocA. *N*- terminally truncated FocA variants were membrane-associated, but some were synthesised at a low level and showed no FocA-dependent transport of formate. These results indicate that the *N*-terminus of FocA is involved in the control of formate translocation through the FocA pore. These findings suggest that formate translocation by FocA is not only dependent on the constriction sites within the pore of each protomer[3], but also on the flexible cytoplasmic *N*-terminus, which controls formate passage through the channel. The potential mechanisms underlying this control will be discussed.

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TAV06

Extracellular loop 4 acts as periplasmic gate of the proline transporter PutP

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Introduction: The sodium/L-proline symporter PutP of *Escherichia coli* is a member of the sodium/solute symporter family containing proteins of prokaryotic and eukaryotic origin [1]. PutP is of significance for various bacteria-host interactions including the virulence of *Helicobacter pylori* and *Staphylococcus aureus* [2,3]. The transporter belongs to the LeuT-fold structural family [4].
Objectives: Based on structural similarities to LeuT, extracellular loop (eL) 4 of PutP is proposed to function as a periplasmic gate regulating access to the ligand binding sites. Here, we set out to test the functional significance and the dynamics of eL4.
Methods: A complete spin-labeling scan of eL4 was performed [5]. Amino acid replacements and L-proline uptake assays were performed to analyze the functional importance of eL4. Cysteine accessibility studies were used to show potential opening and closing of cavities.
Results: The spin-labeling site scan of eL4 revealed the presence of two α-helical segments [5]. Amino acid residues which are crucial for transport activity were identified. Phe314 in eL4 was suggested to anchor the loop by hydrophobic contacts to transmembrane domain (TM) 1 close to the ligand binding sites [5]. Furthermore, our homology model of PutP and experimental data suggest interactions between Glu311 (close the tip of eL4) and Ala404 (periplasmic end of TM10) which might be crucial for closure of the periplasmic gate.
Conclusion: The results support the idea that eL4 controls ligand access to their binding sites by acting as periplasmic gate. Residues of particular functional importance of this loop might be crucial for closure of the extracellular cavity.

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TAV07

Phosphate specific porins of *Pseudomonas aeruginosa* outer membrane: Structure, dynamics and ion-selectivity

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Introduction: *Pseudomonas aeruginosa* is an opportunistic pathogen that can adopt many ecological niches. It is also the major cause of chronic lung infections in individuals who suffer from the genetic disease cystic fibrosis. It causes nosocomial infections and the reasons for it, is its high intrinsic resistance to antimicrobials, including antibiotics and disinfectants. This resistance makes *P. aeruginosa* one of the most difficult organisms to treat. Basis of for its high resistance against antibiotic treatment is the low permeability of its outer membrane for antibiotics. Its outer membrane contains only one protein, OprF, which is only partially active as general diffusion porin. All the other outer membrane porins are from the OprD family of substrate-specific porins, which highly contribute to the limited outer membrane permeability. Members of the OprD family are also OprP and OprO. OprP is specific for phosphate and is induced under phosphate limitations. OprO is highly homologous to OprP and is specific for polyphosphate uptake.
Objectives: We focused on OprP and OprO as these are important porins for the uptake of phosphate and polyphosphate. These porins exhibit 74% of sequence identity and 86% sequence homology indicating high structural similarities. For further detailed analysis of their structure and function we focused on the central narrow regions of the two pores where phosphate and polyphosphate binds.
Methods and materials: In particular, we performed site-specific mutagenesis, where two tyrosines, Y62 and Y114 in OprP were replaced by phenylalanine F62 and aspartic acid D114 of OprO and in case of OprO it was opposite.
Result: By mutating these tyrosine groups in OprP, we observed that the single channel conductance of mutant Y62FY114D protein increased and also its specificity towards diphosphate also increased rather than to phosphate when compared to OprP. However the diphosphate binding constant were found to be smaller than the diphosphate constant of OprO.
Conclusion: We expect substantial changes in binding because it has been found previously that phosphate ion binds about 4-times higher to OprP than OprO, whereas diphosphate binds about 16 times more effectively to OprO as compared to OprP.

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TAV08

Unlocking the secret of MOMP the major porin from Campylobacter jejuni – Revealing binding from translocation

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Introduction: For the survivability, bacterial cells need to exchange small molecules, nutrients, nucleic acids, peptides with the surrounding environment. To date, two porins identified in *C. jejuni* namely major outer membrane protein (MOMP) and Omp50. The interaction of polypeptides with protein pores is an underlying fundamental question in biology. Here, in this work we re-constituted MOMP in artificial lipid bilayer. The single-channel properties such as ion-conductance and selectivity were investigated. We further analyzed the interaction of short

range cationic peptides and measured the binding kinetics which help us in differentiating binding from translocation
Objectives: To observe, the pore-forming activity of MOMP, through single-molecule electrophysiology measurements. To elucidate, the possible interactions and hence to differentiate binding from translocation
Materials and methods: Wild type MOMP 85H from *C. jejuni* was purified to homogeneity with the final concentration of 1.0 mg/ml in 0.3% octyl-POE. The short range positively charged peptides used in the present study were tri-arginine (H-RRR-OH acetate salt), penta-arginine (H-RRRRR-OH trifluoroacetate salt) and hepta-arginine (H-RRRRRRR-OH trifluoro acetate salt).
Results: MOMP protein extracted directly from the outer membrane of *C. jejuni,* forms channels in the artificial planar lipid bilayers. Interestingly, the MOMP channel exists as monomers and trimers, not depending on detergent concentration. High-resolution ion conductance measurements in the presence of peptides of different length and charge revealed the kinetics of peptide binding. In the case of cationic peptides, the external voltage acts as a driving force that promotes the interaction of the peptides with the channel surface [1].
Conclusion: In the present study, we investigated an experimental strategy to illuminate various kinetics parameters for polypeptide translocation through the MOMP channel. The applied trans membrane voltage acts as a main driving force for pulling the peptides across the channel. Hence, we hypothesize, that binding is favoured at low voltage and with further increase in the applied voltage results in successful translocation of the peptide.

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BTV-FG01
Proteomic analyses of bacteria used in industrial fermentation processes
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Bacteria are important organisms used in industrial fermentation processes. Especially bacilli are well established expression hosts, for example for the production of technical grade enzymes. During large scale fermentation processes cells are subjected to physiological and metabolic stress partly due to high cell density. Nutrient and oxygen gradients are formed in large bioreactors leading to further starvation and stress condition for the host bacteria. Identifying and understanding such physiological and metabolic bottlenecks in industrial fermentations could improve their productivity.
Using gel-based methods such as 2D gel electrophoresis as well as gel-free mass spectrometry based methods, we studied the proteomes of growing cells and of cells subjected to different stress and starvation conditions relevant for production processes. Since bacilli are known to secrete proteins directly into the extracellular medium, we analysed the extracellular proteome to reveal further information about the response of the cells to different growth conditions.
The data obtained from our proteome studies allow the description of proteomic signatures for growing and non-growing cells. Furthermore, general and specific marker proteins for stress and starvation conditions could be derived. A complex stress/starvation signature library is a useful tool for understanding the cellular physiology of cells grown in the laboratory or in large bioreactors.

BTV-FG02
Leveraging the perfect interplay of diverse expression tools for high level protein production with *Pichia pastoris*
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Along the path from transcription to translation/translocation and subsequent post-translational processing to potential final product secretion, numerous parameters play a more or less pronounced role in recombinant protein production. Their importance may vary for different target proteins. VTU´s library of genetic variants of the *Pichia pastoris* AOX1 promoter allows for efficient fine-tuning of gene expression under methanol induction or even under methanol-free conditions. In addition, this feature, together with the properly adjusted co-expression of helper proteins, will also foster more ideally suited conditions for posttranslational processing thereby enabling the generation of high performance *Pichia pastoris* protein production strains.
The application of VTU´s yield enhancing *Pichia pastoris* expression platform and examples will be presented.

BTV-FG03
Fungal strain development for improved protein production: protease mutant approaches and genome mining for novel enzymes discovery
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Optimized protein production in filamentous fungi requires the availability of fungal strains with low levels of secreted protease activity in order to improve secreted protein levels, including shelf life. Already for several decades research has been carried out to obtain these type of mutants, leading to the isolation of mutants with very favorable characteristics, one being a mutation in a transcriptional regulatory gene, *prtT* (e.g. Punt et al;., 2008). Based on these results further improved strains have been developed using different selection approaches. Controlled fermentation experiments with selected mutant strains revealed different improved characteristics, whereas full genome sequencing was carried out to identify the genetic basis of the mutant phenotypes. Identification of relevant protease-regulatory genes has also been carried out using collections of regulatory gene knock-out strains in *N. crassa* and *A. niger.* Several examples of the use of selected mutant strains in our research to discover and produce novel hydrolytic enzymes will be presented.

EMV-FG01
Correlative imaging of microbial activity in Nature
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Correlative imaging is widely applied to obtain comprehensive information on the identity, activity and physiology of cells in eukaryotic cell biology. In the field of environmental microbiology this method is still little used despite its obvious potential. During the past years substantial progress was made at the MPI for Marine Microbiology (MPI-MM) to establish this method for our field. A particularly powerful approach used in our institute is the use of nano-scale Secondary Ion Mass Spectrometry (nanoSIMS) coupled to stable and radio-isotope labelling experiments to determine single-cell activity in the environment. By combining this approach with Fluorescence in situ Hybridization (FISH) we are able to link the identity of microbial cells in a complex microbial community to their in situ nutrient incorporation, which allows us to calculate cellular uptake rates and directly determine nutrient fluxes. The recent acquisitions of an Environmental Scanning Electron Microscope combined with an EDX system (ESEM/EDX) and a confocal laser Raman microscope coupled with an atomic force microscope provides further elemental, chemical-bond and structural information at a single cell level. I will present results of our correlative imaging studies of complex microbial communities in various marine environments.

EMV-FG02
Application of Raman activated cell sorting to single cell genomics
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Single cell Raman spectra (SCRS) provide intrinsic chemical 'fingerprint' of individual bacterial cells, containing rich information on nucleic acids, protein, carbohydrates and lipids. We employ Raman micro-spectroscopy to develop Raman single cell technology to characterise cell types, physiological states and phenotypic changes. A recent development is to develop Raman activated cell sorting (RACS), which combines SCRS for cell identification and sorting device for isolating cell of interest. RACS technology has been applied to identify and sort uncultured bacteria for single cell genomics. RACS was also combined with stable isotope probing (SIP) to address the fundamental questions of life science '*who (bacteria) is doing what (biological roles), where and when*?' RACS will revolutionise our ability to study the roles of single cells and uncultured bacteria in microbial population. It will be a powerful research tool to open a new frontier for single cell '-omics'.

EMV-FG03
Tracking heavy water (D_2O) incorporation for identifying and sorting active microbial cells
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Microbial communities are essential to the function of virtually all ecosystems and eukaryotes including humans. However, it is still a major challenge to identify microbial cells active under natural conditions in complex systems. In this study, we developed a new method to identify and sort active microbes on the single cell level in complex samples after addition of heavy water (D_2O) using Raman microspectroscopy. Incorporation of D_2O-derived D into the biomass of auto- and heterotrophic bacteria and archaea could be unambiguously detected via C-D signature peaks in single cell Raman spectra and the obtained labeling pattern was confirmed by nano-scale resolution secondary ion mass spectrometry. In fast-growing E. coli cells, label detection was already possible after 20 min. Detection of D incorporation from D_2O in individual microbial cells via Raman microspectroscopy can be directly combined with fluorescence in situ hybridization for the identification of active microbes. Labeled cells can subsequently be sorted using optical tweezers, allowing for targeted single cell genomics. The utility of this approach for activity profiling and targeted (meta-)genomics will be demonstrated by examples from the study of mucosal sugar utilization by the gut microbiota as well as nitrifier micro-diversity in wastewater treatment.

EMV-FG04
Combined Epifluorescence- and Kelvin Probe Force Microscopy (EFM-KPFM) for the study of bacterial attachment to ferrous surfaces
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Initial bacterial attachment to ferrous surfaces (steel, pyrite) was studied using EFM-KPFM for opaque substrata. By KPFM, relative surface potentials can be visualized with the high spatial resolution of an atomic force microscope. Different species and surfaces were examined: attachment of the sulfate-reducer *Desulfovibrio vulgaris* to steel, attachment and biofilm formation on steel surfaces accompanied by biomineralization of manganese-oxides by *Leptothrix discophora* and attachment of the acidophilic iron oxidizer *Leptospirillum ferriphilum* to pyrite. The former two ones are important for microbially influenced corrosion, while the latter one is used for bioleaching of sulfidic ores. Electrochemically active sites (where dissolution occurs) play a crucial role in the initial attachment to both surfaces. Furthermore, attached cells influence electrochemical processes and, thus, surface potentials. Aim of the study is to correlate sites for attachment with microbial attachment and to investigate the bacterial influence on surface potentials.
A BioMAT Workstation equipped with a Kelvin force module (JPK Instruments) was used. Potential and topography were visualized in intermittent contact mode (trace: profile acquisition; retrace: potential mapping) using ElectriTap300 cantilevers (Budgetsensors). Fluorescence staining (DAPI) was used for identification of cells and fluorescently labeled Concanavalin A, which binds to some glycoconjugates present in the extracellular polymeric matrix.
First results indicate an increasing surface potential in close proximity to cells of *D. vulgaris* on non-alloyed steel (+50 mV after 2h incubation). On stainless steel, the increase of the surface potential is significantly reduced (+5 to +10 mV). Manganese oxides show the potential of stainless steel ennoblement and the potential of pitting corrosion by increasing the free corrosion potential up to 200 mV (measured with a electrochemical 3 electrode setup). On pyrite, a significant decrease of the surface potential was noted in close proximity to colonies of *L. ferriphilum* (approx. -150 mV).

EMV-FG05
A novel technology for single-cell manipulation and adhesion force quantification – fluidic force microscopy
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There is an increasing interest to manipulate and analyze single cells, e.g. to investigate cell heterogeneity, to identify single cells in complex consortia, or to uncover cell-cell interactions. Novel single-cell technologies are thus in demand. The fluidic force microscope (FluidFM) is a recently invented technology which consists of a modified atomic force microscope (AFM) equipped with micro-channeled probes and is mounted on top of an inverted optical microscope. The AFM system provides a high precision force-controlled z-positioning of probes which are directly connected to an external fluidic circuit for over- and under-pressure application. Cells are reversibly attached to the cantilever by the application of underpressure, which can be exploited for pick-and-place experiments. Spatial manipulation was shown for bacteria and yeast cells, such as *Escherichia coli* and *Saccharomyces cerevisiae*. Furthermore, FluidFM was used to isolate targeted bacteria from environmental samples for further investigations. In addition, the combination of the pressure-based cell manipulation with the ability of the FluidFM to perform single-cell force spectroscopy, allows for a better understanding of microbial adhesion. Force measurements showed distinct adhesion behavior of yeasts and bacterial cells on engineered substrates. For both types of microorganisms, adhesion forces increased with longer contact times. *Candida albicans* interaction forces on hydrophobic substrates increased from 12 to 37 nN at 23 °C, while for bacteria, such as *E. coli* and *Streptococcus pyogenes* forces in the range of 500 pN up to only a few nN were measured. Results obtained so far highlight the advantages of FluidFM as a versatile platform to manipulate and analyze single cells.

1) Potthoff *et al.* (2012) PLOS ONE 7:e52712.
2) Stiefel *et al.* (2013) Appl Environ Microbiol. 79:4895-905.
3) Guillaume-Gentil *et al.* (2014) Trends Biotechnol. 32:381-8.

EMV-FG06
Nitrite- and Nitrate-Dependent Methanotrophs – Environmental Detection and Relevance in Freshwater Ecosystems
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Humans continue to have an enormous impact on global C and N cycles. While a clear stimulation of methane emissions through human activities is evident, the role of also increasingly released nitrogenous compounds as electron acceptors for microbial methane oxidation is not well constrained. We have developed diverse methods for environmental detection of nitrate- and nitrite-dependent methanotrophs, which have been applied to several freshwater environments.
In contrast to most metabolically flexible heterotrophic denitrifiers, the microorganisms responsible for methane-dependent nitrate/nitrite reduction seem to be specialized to use methane only, are sensitive to oxygen, grow slowly and employ pathways different from each other and from model organisms. This necessitates new approaches for the assessment of their environmental relevance.
Nitrite-dependent methane oxidation is carried out by bacteria related to *Methylomirabilis oxyfera* (NC10 phylum), whereas nitrate-dependent methane oxidizers are close relatives of methanogenic archaea and sulfate-dependent anaerobic methanotrophs (ANME-2). Both can be targeted and quantified (qPCR) by specific primers for their 16S rRNA genes and key functional genes (pmoA and mcrA).
For *M. oxyfera*, laboratory enrichment cultures have formed the basis for its genetic and physiological characterization and the development of several independent methods for its sensitive detection. Whereas their isotope fractionation during methane oxidation is similar to that of other methanotrophs, *M. oxyfera* has a completely different pathway of carbon assimilation than proteobacterial methanotrophs: C is not assimilated from methane, but from CO_2 via the Calvin cycle, with important consequences for the interpretation of environmental isotope labelling studies. In addition, *M. oxyfera* is characterized by a distinct PLFA profile, including methylated lipids so far not found in any other organism.
Case studies using specific primers in combination with lipid profiles and ^{13}C-labelling in peatlands, ricefields and other freshwater environments illustrate the role of these previously overlooked anaerobic methanotrophs in the environment.

ISV-FG01
The impact of the Genomic Encyclopaedia of Bacteria and Archaea project on microbial taxonomy
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The Genomic Encyclopedia of *Bacteria* and *Archaea* (GEBA) project started in 2007 with a pilot project for 165 type strain genomes to be sequenced and analyzed in a collaboration between the DOE Joint Genome Institute (JGI, Walnut Creek, CA) and the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig). Analysis of the first set of genome sequences confirmed the key idea of a significantly accelerated access to genomic diversity by phylogeny-driven selection of the sequencing targets, as well as rapid progress in the discovery of novel protein families. Furthermore, the genome sequences were successfully used to support taxonomic revisions in problematic regions of the bacterial diversity, not least because of statistically well supported whole genome sequence-based phylogenies in combination with classical polyphasic taxonomic data. Inspired by the success of the pilot project, the GEBA project was meanwhile several times extended to a total of over 4000 type strain genomes, representing about one third of all species with validly published names. The steadily increasing coverage of microbial diversity with complete and draft genome sequences in combination with improved genome sequence-based methods for species delimitation and inference of reliable phylogenies will contribute to qualitative progress in the description of novel species within the yet unexplored microbial majority. Genome sequence-based technology will increasingly be complemented by high throughput phenotyping technology to provide a wealth of information about the type strains representing novel species, complementing the classical chemotaxonomic and biochemical data. The new dimension of data will inevitably lead to a discussion about an updated format for the description of novel species.

ISV-FG02
Bacterial species in the light of genomics and metagenomics
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We have recently shown using a metagenomics approach that natural microbial communities comprise predominantly sequence-discrete populations, with intra-population genomic sequence divergence ranging from ~1% to ~6%. The identification of discrete clusters represents a major discovery given that, as several scientists believe, there is no intrinsic reason why the processes driving diversification and adaptation of bacteria must produce sufficiently coherent groups of individuals (species). These populations may constitute the important units of microbial diversity (species?) and their identification the first step towards linking genetic to functional and ecological diversity. I will also summarize how the traditional standards for species demarcation based on 16S rRNA gene identity and DNA-DNA hybridization values translate to the identified discrete populations as well as our efforts to develop new genomic approaches to identify discrete populations.
Recommended Reading:
Rodriguez-R and Konstantinidis. Microbe Magazine, 2014.

ISV-FG03
Genome-Based Taxonomy of the Genus *Corynebacterium*
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Question: The actinobacterial genus *Corynebacterium* today comprises of 97 validly published species and therefore represents one of the largest bacterial genera. It includes species of medical relevance for humans or animals as well as environmental isolates of biotechnological value (1). Several decades of taxonomic classification based on physiological parameters and on ribosomal DNA sequences have shed much light on the taxonomy of this genus, but open issues remained.
Methods: Since the sequencing of genomes nowadays has reached maturity, we took the challenge to decipher the complete genome sequences of all type strains in this genus and to apply bioinformatic tools to derive a genome-based phylogeny.
Results: In total, more than 70 genomes were newly sequenced within this project and the rest was already present in databases. The corynebacterial genomes range between 1.8 Mbp (*C. caspium*) and 3.5 Mbp (*C. glyciniphilum*) in size and have around 1,600 to 3,300 protein-coding regions, respectively. Remarkably, their genomic G+C content varies greatly and lies between 46.4 mol% (*C. kutscheri*) and 74.8 mol% (*C. sphenisci*).
Subsequent analysis by bioinformatics tools such as EDGAR (2) allows to derive comprehensive protein sequence-based phylogenetic trees. This data can than be compared to the already used taxonomic markers such as 16S rDNA or ribosomal protein sequences.
Conclusions: The presentation will discuss findings with taxonomic relevance, derived from the analysis of whole genomes. The project is still ongoing, since novel isolates or even deposited patent strains might comprise novel species worth to formally proposing them (*C. glyciniphilum*, 3).

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3. Al-Dilaimi A, et al. (2014) Revisiting *Corynebacterium glyciniphilum* (ex Kubota *et al*. 1972) sp. nov., nom. rev., isolated from putrefied banana. Int J Syst Evol Microbiol. 2014 Oct 16. doi: 10.1099/ijs.0.065102-0. [Epub ahead of print]

MCV-FG01
Actin homolog MreB has a conserved function in organizing bacterial cytoplasmic membranes
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The eukaryotic cortical actin cytoskeleton creates specific lipid domains, including lipid rafts, which determine the distribution of many membrane proteins. Here we show that the bacterial actin homologue MreB displays a comparable activity. MreB forms membrane-associated filaments that coordinate bacterial cell wall synthesis. We noticed that the MreB cytoskeleton influences fluorescent staining of the cytoplasmic membrane. Detailed analyses combining an array of mutants, using specific lipid staining techniques and spectroscopic methods, revealed that MreB filaments create specific membrane regions with increased fluidity (RIFs). Interference with these fluid lipid domains (RIFs) perturbs overall lipid homeostasis and affects membrane protein localization. The influence of MreB on membrane organization and fluidity may explain why the active movement of MreB stimulates membrane protein diffusion. These novel MreB activities add additional complexity to bacterial cell membrane organization and have implications for many membrane-associated processes.

MCV-FG02
Spatial organization of the bacterial plasma membrane
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The bacterial plasma-membrane is a highly organized cellular compartment. Accurate spatio-temporal sorting of proteins and lipids allows for functioning of membrane-associated processes such as signaling, transport, cell growth and division. Membrane heterogeneity with respect to protein and lipid distribution is therefore an intrinsic and essential feature of bio-membranes. It is well established that lipid distribution affects protein localization. Physico-chemical characteristics of different lipid species result in their lateral segregation into membrane microdomains. This leads to a heterogeneous organization of the membrane, which appears essential for membrane protein functionality. However, how lipid assemblies are organized and whether proteins are actively involved in these processes remains poorly understood. In recent years, flotillins were found to be responsible for lateral segregation of defined lipid domains in bacteria. Flotillins and related proteins form scaffolds for spatial organization of the membrane and recruit proteins (and maybe lipids) into microdomains. Absence of flotillins *in vivo* leads to coalescence of distinct domains of high membrane order and, hence, loss of flotillins in the bacterial plasma-membrane reduces membrane heterogeneity. This loss of heterogeneity leads to impairment of vital cellular processes such as protein secretion. Therefore, it can be concluded that bacteria actively organize their membrane using specialized protein scaffolds. Consequently, influencing membrane organization is an emerging target for anti-bacterial compounds.

MCV-FG03
Domain organization of membrane proteins in *Bacillus subtilis*
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Very little is known about how membrane proteins are organized in 3D. Some membrane proteins and protein complexes are specifically located at the cell poles, including chemotaxis receptors or proteins involved in DNA uptake from the environment. Many other proteins have been observed in a patch-like pattern, versus uniform localization, and it has been proposed that the membrane contains microdomains, in which certain proteins are clustered, possibly in a dynamic and exchangeable manner.
We have started a systematic study of the distribution and dynamics of *Bacillus subtilis* membrane proteins. We have localized an initial set of 250 proteins, and are measuring their mobility. We are using TIRF microscopy for screening, and STED super resolution microscopy for maximum resolution of localization patterns. Further, we are employing stream acquisition to follow the movement of protein assemblies or of individual molecules in real time. Automated tracking is used to collect many trajectories, whose dynamics are mathematically analysed.
Proteins were grouped according to their localization pattern: diffuse, polar, patchy and punctate fluorescent profiles could be observed. Interestingly, almost 20% of all proteins localized to the cell poles, highlighting this subcellular localization as a preferred position for many membrane proteins. The specific localization patterns of some uncharacterized membrane proteins suggests a possible function, e.g. in cell division.
Flotillins have been used as markers for so called "detergent resistant microdomains", which comprise a variety of proteins. We show that flotillin assemblies have a size of 75 nm in *B. subtilis*, and their soluble part forms large assemblies, up to several hundred kilodaltons, *in vitro*. Assemblies move in a random manner within the membrane, at a speed corresponding to their large size. On average, flotillin assemblies (between 10 and 15 per cell for FloT or FloA, respectively) require about one minute to scan the entire surface of the cell dependent on their diffusive movement. At least one protein suggested to be part of a flotillin microdomain does not frequently colocalize with flotillins, indicting that in spite of common extraction properties, proteins may not form large microdomains, but possibly rather small defined assemblies. The implications of our live cell analysis will be discussed.

MCV-FG04
The evolution of molecular order in the cell membrane
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Sterols, such as cholesterol, are ubiquitous eukaryotic membrane lipids with a unique planar geometry that endows them with a crucial role - modulating the thermodynamic order of other membrane lipids and thereby directing molecular dynamics and packing. This unique capacity allows eukaryotes to construct fluid, yet mechanically robust membranes resistant to harsh external environmental conditions, to tune enzymatic activities, and laterally compartmentalize membrane biochemistry. However, many organisms such as bacteria cannot synthesize sterols**.** Furthermore, sterol biosynthesis requires molecular oxygen, but it is known that life was present on Earth at least a billion years before cyanobacteria first started enriching the atmosphere with oxygen. **So, how do organisms that lack sterols modulate membrane order and what preceded sterols in the evolution of membrane ordering?**
We recently demonstrated that hopanoids, putative bacterial sterol surrogates, are biophysically similar to sterols in their ability to order membrane lipids and to form liquid ordered domains in model membranes. This observation alters our understanding of membrane evolution, suggesting that the emergence of ordered biochemically active liquid membranes, and thus the ability to compartmentalize membranes, is independent of oxygen and could have preceded the evolution of sterols. We have now begun to examine how the physicochemical properties imparted to membranes by hopanoids are employed in the physiology of bacteria using the Gram-negative model organism *Methylobacterium extorquens*. Our observations show that hopanoids are located in the outer membrane and interact with the major outer membrane lipid, lipopolysaccharide, in a similar manner as cholesterol interacts with sphingomyelin in the eukaryotic plasma membrane to achieved a highly ordered liquid membrane. This suggests a functional and compositional convergence of the bacterial outer membrane with the eukaryotic plasma membrane and raises the possibility that lipid-dependent lateral compartmentalization could be a feature of the bacterial outer membrane.

MPV-FG01
Unraveling the function and structure of a lipocalin lipoprotein affecting virulence in *Streptococcus pneumoniae*
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Introduction: *Streptococcus pneumoniae* (pneumococci) is a serious pathogen expressing a plethora of lipoproteins involved in various pathophysiological processes which makes them indispensable for bacterial fitness and virulence. The family of lipocalin proteins consists mainly of small extracellular proteins binding hydrophobic ligands and fulfilling numerous biological functions. Here we introduce the **P**neumococcal **c**alycin fold **c**ontaining **L**ipoprotein (PccL) as the first member of the lipocalin-family in pneumococci.
Objectives: The main goals of this work were the structural characterization of a novel surface-exposed lipoprotein and the assessment of its impact on the pathophysiology of pneumococci.
Methods: The impact of PccL on uptake by macrophages and colonization or survival under *in vitro* and *in vivo* conditions, respectively, was tested in cell culture infection assays and by employing an intranasal or sepsis mouse infection model. The structure of PccL was deciphered by X-ray crystallography of the rPccL protein produced in *E. coli*.
Results: The exposure of PccL on the pneumococcal cell surface was indicated by flow cytometry. Depletion of PccL had only a moderate impact on *S. pneumoniae* growth while cell culture-based phagocytosis and adherence experiments showed dramatic differences between wild-type and *pccL*-mutants. *In vivo* experiments revealed a significant attenuation of the *pccL*-mutant and hence, striking differences in colonization and dissemination were visualized in real-time bioimaging when comparing the *S. pneumoniae* D39 wild-type and isogenic *pccL*-mutant. Protein crystals were obtained with a resolution of 2.6 Å and the PccL structure showed main characteristic features of a member of the calycin-fold protein super family. Importantly, members of the calycin protein super family share overall structure similarity, which is not necessarily based on sequence similarity. PccL contains eight β-sheets in antiparallel arrangement, which form a β-barrel, and a short C-terminally located short α-helix. Currently, a model is generated how PccL is assembled on the pneumococcal cell surface.
Conclusion: The results revealed that the PccL lipoprotein is a member of the lipocalin protein family and contributes significantly to the virulence of *S. pneumoniae*.

MPV-FG02
On the role of autocleavage of the switch protein of bacterial type III secretion systems
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Type III secretion systems are employed by Gram negative bacteria to inject effector proteins that can alter host cellular functions in order to promote bacterial survival and colonization. The core unit of type III secretion systems is a so-called needle complex consisting of a base that anchors the structure to the bacterial membranes, a cytoplasmic and inner membrane export apparatus serving in substrate targeting and export, and a hollow, substrate-channeling needle protruding from the bacterial cell surface.
Protein export through type III secretion systems is required to be a strictly hierarchical process. The switch from assembly of the needle filament to secretion of later substrates occurs after the needle has reached a sufficient length. Needle length control and substrate specificity switching are thought to be closely linked events facilitated by the secreted needle length regulator and the switch protein. For switching to occur, the switch protein needs to undergo autocleavage at a conserved N|PTH motif, but how needle length control, autocleavage, and substrate specificity switching are connected is only poorly understood.
To further elucidate the timing of *Salmonella* SpaS autocleavage and its role in needle length control and substrate specificity switching, we analyzed secretion profiles and needle lengths of autocleavage mutants,

studied kinetics and timing of autocleavage in respect to needle complex assembly, looked at the complex-association of the C-terminal fragment after cleavage, and challenged the principle necessity of autocleavage by cleaving SpaS with an exogenous site-specific protease.
We could show that needle length control and substrate specificity switching are disconnected in SpaS autocleavage mutants. Autocleavage turns out to be an unregulated process occuring just after protein folding and before incorporation of SpaS into the needle complex. Functional needle complexes could be obtained from provided, already autocleaved SpaS, suggesting that the autocleavage event does not play a role in substrate specificity switching. The principle cleavage of $SpaS_C$ was sufficient to achieve an enhanced flexibility of the C-terminal domain that is critical for productive substrate specificity switching.

MPV-FG03
A protein quality control mechanism might contribute to survival of world-wide distributed *Pseudomonas aeruginosa* clone C strains in environmental and clinical niches
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The environmental species *Pseudomonas aeruginosa* is the prototype of a highly successful nosocomial pathogen capable to cause a wide variety of infections. World-wide most prevalent is clone C, the variants of which are found in patients, the clinical and environmental aquatic habitats. The factors that determine the success of clone C strains in host infection and environmental transmission and survival have not been defined, but could be alternatively assigned to clone-specific features or unique survival mechanisms in individual strains. In this study, we initially characterize a molecular mechanism of survival unique to clone C strains. We identified a *P. aeruginosa* clone C-specific genomic island (PACGI-1) of 86 kbp, which contains the highly expressed small heat shock protein sHsp20c, the founding member of a novel subclass of class B bacterial small heat shock proteins. sHsp20c is involved in resistance against heat shock and oxidative stress. Deletion of *shsp20c* adjacent genes, *dna* and *clpBc* encoding DNA-binding protein and ClpB-like ATPase, respectively, has an additive effect on these phenotypes. Heat stable sHsp20c is unconventionally expressed in stationary phase in a wide temperature range from 20-42°C. Purified sHsp20c has characteristic features of small heat shock proteins as it forms sphere-like 24-meric oligomers as detected by electron microscopy and exhibits significant chaperone activity. As the *P. aeruginosa* clone C population is significantly more heat shock resistant than genetically unrelated *P. aeruginosa* strains without sHsp20c, the horizontally acquired *shsp20c* operon contributes to survival under stress of world-wide distributed clone C strains.

MPV-FG04
***Legionella pneumophila* outer membrane vesicles are potent pro-inflammatory stimulators**
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The formation and release of outer membrane vesicles (OMVs) is a common phenomenon of gram-negative bacteria, including *Legionella pneumophila* (*L. pneumophila*), which is a causative agent of severe community- and hospital-acquired pneumonia. Upon its transmission into the human lung, *L. pneumophila* primarily replicates in macrophages.
In this study, we analyzed the influence of *L. pneumophila* OMVs on macrophages. For this, THP-1 cells were PMA-differentiated and incubated with rising doses of OMVs for 24 and 48 h, respectively. The supernatant was used to measure the amount of different pro-inflammatory cytokines including IL-8, TNF-alpha, IL-1beta and others. By use of murine bone marrow derived macrophages of different toll like receptor (TLR) knockouts, we determined the impact of TLR-signaling on the recognition of *Legionella* OMVs. To prove the effect of *L. pneumophila* OMVs on bacterial replication, THP-1 cells were pre-incubated with different doses of OMVs and were subsequently infected with *L. pneumophila*. Bacterial replication was determined by colony forming unit (CFU) assay.
Our study shows that OMVs from *L. pneumophila* are potent pro-inflammatory stimulators for macrophages by activating TLR-signaling. Furthermore, they can influence the replication of *Legionella* in macrophages. This provides a new means of communication for *L. pneumophila*, which could explain the spreading of inflammation.

MPV-FG05
The role of Fur in iron homeostasis in *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. Diseases caused by *C. difficile* are referred to as *Clostridium difficile* associated disease (CDAD). Since the late 1970s *C. difficile* has become the most common cause of hospital-acquired infectious diarrhea and has been recognized as a significant cause of morbidity and mortality. *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. We were able to demonstrate that *C. difficile* uses different iron sources in order to grow. BLAST analysis displayed a gene homolog of *Bacillus subtilis fur* gene in *C. difficile*. In *B. subtilis* Fur acts as the global regulator of ferric iron. 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 elucidate 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 regulon, a transcriptomic and proteomic approach using iron-limited conditions was applied. This systems biology approach will contribute to a better understanding of the role of iron in the infection cycle of *C. difficile.*

MPV-FG06
A glycosphingolipid receptor activates Abl signaling for bacterial invasion
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Introduction: *Pseudomonas aeruginosa (P. a.)* is a Gram-negative opportunistic human pathogen which causes severe infections of the respiratory tract, urinary tract, skin and eyes. Internalization of *P. a.* by host cells significantly contributes to its pathogenicity. The entry mechanism(s) and the host cell factors involved in this process are incompletely understood. Recently, Abelson kinase (Abl), a non-receptor tyrosine kinase, has been identified to promote uptake of *P. a.* [1]. However, bacterial factors and host cell receptors activating Abl during bacterial invasion are unknown. One of the virulence factors produced by *P.a.*, the galactophilic lectin LecA, binds to globotriaosylceramide (Gb3) a host cell glycosphingolipid (GSL). Since LecA represents a multifunctional pathogenicity factor involved in biofilm formation, adhesion, and invasion of *P.a.* [2], the question arises if LecA is also capable to induce Abl kinase via a GSL receptor.
Objectives: In our study we address the hypothesis that LecA binds to Gb3 to activate Abl signaling for bacterial uptake.
Methods or Materials & Methods: We used human lung epithelial cells (H1299) as a model cell line for *P.a.* invasion, measured by Amikacin protection assay and Abl-dependent Crk phosphorylation detected by Western blotting. Abl kinase and host GSL synthesis were inhibited using Imatinib and PPMP, respectively. A soluble sugar (PNPG) was used to block LecA binding to Gb3.
Results: Abl kinase activity is required for uptake into H1299 cells. Preliminary data suggest that LecA-secreting but not LecA-deficient *P.a.* strains activate Abl. Soluble LecA induces significant, Abl-dependent Crk phosphorylation in a dose- and time-dependent manner. PPMP- or PNPG-treatment prevents LecA-triggered Crk phosphorylation.

Conclusion: We identified a previously unrecognized function of LecA, which activates Abl kinase to promote bacterial invasion. Of note, our study suggests a novel role for Gb3 in Abl activation.

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QDV-FG01
Bioaerosols from agricultural and waste management facilities: Measurement and assessment
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Microorganisms are ubiquitous on our planet; they are widespread and abundant in hydro-, pedo-, and cryosphere as well on higher organisms' inner and outer surfaces. Natural outer motive forces (e.g. wind) result in the emission of microorganisms from their habitats. Consequently they are detectable as viable or non-viable units in the atmosphere, too. The natural background concentration e.g. of airborne bacteria and fungi varies between 10^3 - 10^5 cells m^{-3}. Therefore, inhalation of bioaerosols naturally occurs during breathing.
In agricultural and technical enterprises like waste treatment plants and animal husbandry, microbial habitats or colonized materials are mechanically processed and the amount of airborne microorganisms can exceed the natural background by several orders of magnitude. Exposure of employees to airborne microorganisms in these settings is very high and may cause adverse health effects if precautionary measures are not taken. Exposed persons may suffer from infections, allergic reactions, breathing problems or irritation symptoms. For the protection of health and safety at work the European directive 2000/54/EC which in Germany is implemented by the "*Biostoffverordnung*" states that nature, degree, and duration of workers' exposure to microorganisms must be assessed in relation to potential health risks in a risk assessment. Nonetheless, these legal regulations do not contain any specification on exposure measurements at workplaces or threshold limit values. Therefore, the bioaerosol assessment at workplaces is still rather a difficult task.
In contrast to this and accelerated by increasing numbers of bioaerosol emitting facilities, concerns among residents about health risks have become explicit. This has led to interest in emissions and immissions particularly in environmental politics, and questions arose how such bioaerosols should be assessed. In contrast to workplace regulations, major efforts have been done to standardize methods and analytical procedures for bioaerosol determination in the last decade.
Based on selected results gained from poultry houses (stocked with up to 40.000 animals) from which about 10^{16} cells are emitted per fattening period, standardized methods, new analytical approaches and limitations in bioaerosol analyses will be presented.

QDV-FG02
Novel microfluidic biosensor for online monitoring of biofilm formation
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Controlling and monitoring of biofilm formation are still demanding tasks. Established biofilm research methods mainly provide destructive end-point analysis. Therefore, we developed a new sensor system for characterizing biofilm formation online in a microfluidic flow system by two parameters: attached biomass and biofilm activity. Our newly developed microfluidic biosensor is based on electrical impedance spectroscopy and parallel measurement of amperometric current which allows the real-time monitoring of biofilm formation processes. Biofilm biomass and activity are recorded in a non-destructive manner. Thereby increasing impedance correlates with an increase in biomass attached to the electrode and increase in amperometric measured current corresponds to a higher respiratory activity of the biofilm. These features were proven by microscopic time lapse experiments and exo-enzymatic activity measurements. Integration of a reference channel allows minimizing environmental oscillations. The microfluidic properties of the senor enable parallel screening of different bacteria as well as biofilm affecting substances by providing 48 parallel flow channels with each containing two electrodes. A direct RNA extraction out of the channels allows transcriptome analysis on desired time-points. Using this setup we were able to monitor biofilm development of different Gram-positive and Gram-negative bacterial species including *Pseudomonas aeruginosa, Stenotrophomonas maltophilia, Staphylococcus aureus, Bacillus subtilis*, and even complex waste water biofilms. Screening of strain collections identified different biofilm formation potentials of the strains. Furthermore, the device enables monitoring of agents with biocidal or biofilm destabilizing effects. Loosening of the EPS matrix leads to a destabilized biofilm which can be monitored online by a decrease in impedance signal. Toxicity effects of biocides cause inactivation of the biofilm and therefore a decrease in the amperometric signal is observed. Regrowth after treatment reveals the presence of persister cells and is indicated by a recovery of both signals. Overall, this sensor provides a tool for real-time monitoring biofilm formation and allows a rapid screening of biofilm influencing substances in a microfluidic system. Ongoing development of the sensor targets the implementation into technical systems, where biofilm formation is a demanding problem and early on monitoring is necessary.

QDV-FG03
Solutions for overcoming testing challenges with disinfectant coupon studies
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The industry has seen an increase in the number and species of bacterial endospores and mold spores found in many facilities. This presentation will focus on ways to proactively limit bacterial and mold spore contamination from incoming items into cleanrooms and limit other sources of spore contamination. Current industry regulations in the US and Europe will be discussed related to sporicides and disinfectants. Additionally, the presentation will convey a robust approach to addressing bacterial and fungal spores by covering personnel practices, incoming items into cleanrooms, facility design and conditions, and the products used to address bacterial and mold spores as well as more resistant bacterial spore species such as Bacillus cereus. Case studies will be covered which present coupon studies and cover some of the intricacies of the testing such as porosity of surfaces, surface sterilization, and pooling on surfaces. The attendees will ascertain a better approach to coupon testing and determining sources of spore contamination in their cleanroom and controlled facilities.

QDV-FG04
Blood Platelet transfusion relevant bacteria reference strains – suitability test of different Morganella morganii strains
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Introduction: Blood platelet concentrates (BPC) are stored at 22°C ± 2°C for optimal platelet function. This temperature favours the growth of microorganisms. The mortality rate for platelet related sepsis ranges from 1 in 20.000 to 1 in 100.000 recipients. [1,2,3,4,5] Blood Bacteria Reference Strains (BBRS) are a tool for objective validation and assessment of microbiological methods for blood safety [6].
Objectives: Bacteria in BPC can be inactivated by self-sterilisation effects of blood, may persist or grow in PCs during storage. Different isolates of the same bacteria species may vary in their behaviour in BPC. The growth potential of the bacteria is supposed to be on strain level not on species level.
Materials and Methods: Three different Morganella morganii morganii isolates were evaluated in an international study under "real life" and routine conditions (inoculated in PC-bags) regarding their ability to proliferate in platelet concentrates after low spiking (< 1 Colony Forming Unit per millilitre). Microbial count was performed at day 1, 2, 4 and 7.
Results: Whereas one isolate showed a logarithmic growth in PCs, two other isolates remained on a low microbial count / were not detected at all during 7 days. The data provide indication that the potential of growth in PCs is on strain level.
Conclusion: Blood Bacteria Standards (BBS) are a feasible tool for objective validation and assessment of detection methods for contamination in blood components. The example of Morganella morganii morganii showed that growth ability may vary even on strain level.

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QDV-FG05
Regulatory Aspects of Microbial Quality of Cell Based Medicinal Products

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The EMA "Guideline on human cell-based medicinal products", Directives 2004/23/EC and 2006/17/EC and the "Rules Governing Medicinal Products in the European Union" (EudraLex) are the regulatory background for cell-based preparations. In addition, monographs and chapters of the European Pharmacopoeia have legal force and define the conditions of manufacturing and quality control. Cell-based preparations are a very heterogeneous group and there is high variety of the source and sampling material. The influences on microbiological quality are versatile, and microbiological testing is only one aspect of a sufficient strategy to ensure microbial safety. The main elements of the microbiological concept are safeguarding of an appropriate quality of the source material, validation of the manufacturing process and application of suitable microbiological methods. Influences related to the donor, the procurement of the cells, the manufacturing process, sampling and testing methods play an important role and constitute several risk factors for an insufficient quality and possible adverse events in the recipient. Considering the different types of samples like transport or rinsing solutions, cell culturing media, cell suspensions or solid tissue samples, questions arise about representative sampling as well as adequate testing strategy and methods. Due to the specific nature of many of those preparations and their short shelf-life, alternative approaches for testing must be considered. They include an intermediate readout of the tests for product release ("negative-to-date" result) and the use of rapid microbiological methods on final product. Revisions of several regulatory documents like *Ph. Eur.* Chapter 2.6.27 and the "Guide to the quality and safety of tissues and cells for human application" are in progress in order to provide a more flexible and reliable basis for microbiological control.

RSV-FG01
Extracytoplasmic function (ECF) σ factors – the third pillar of bacterial signal transduction

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Question: The bacterial world is full of uncertainties and surprises to which microorganisms have to respond with efficiency, speed and precision. Such quick reflexes require molecular mechanisms that connect an (extra)cellular stimulus with an intracellular response. Next to one- and two-component systems, alternative σ factors of the extracytoplasmic function family provide bacterial cells with a third fundamental means to adapt to their environment.
Methods: Comparative genomics was applied to identify and classify ECF σ factors, based on sequence similarity of the σ factor, its cognate anti-σ factor (if present), the target promoter motif and overall genomic context conservation of ECF-encoding loci.
Results: ECF σ factors represent the third most abundant signaling principle in the bacterial world, with about 6 ECF-encoding genes per bacterial genome on average. By now, almost 100 distinct ECF subgroups have been identified. Our work not only demonstrates that ECF σ factors represent a rather widespread signaling principle, but also provides clear evidence for a regulatory diversity that goes far beyond what has so been known and appreciated. Many novel groups with potentially unique regulatory features now await experimental verification, which can be guided by the data stored in the ECF classification system.
Conclusions: This talk will provide a brief overview on the characteristic features and regulatory diversity of ECF σ factors, as derived from comprehensive comparative genomics studies. Its goal is to set the stage for this minisymposium.

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RSV-FG02
Physiological role and regulatory crosstalk of ECF sigma factors in *Bacillus subtilis*

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Bacillus subtilis responds to a variety of stresses affecting the cell wall and membrane by activation of one or more of seven extracytoplasmic function (ECF) sigma factors. While some stimuli very specifically activate only a single ECF sigma factor and its target genes, other stimuli may activate several sigma factor regulons. Moreover, many genes have promoters that are activated by more than one ECF sigma factor (cross-talk).
Question: How are promoter sequences optimized to enable highly selective recognition by one ECF sigma factor, or to enable cross-regulation by more than one ECF sigma factor?
Methods: We have characterized the target genes (regulons) controlled by ECF sigma factors using transcriptomics (in vivo and in vitro) and bioinformatic-based approaches using strains in which individual ECF sigma factors are activated in either wild-type cells or cells containing only a single ECF sigma factor.
Results: Our results reveal specific sequence elements that allow promoters to be recognized by specific ECF sigma factors. For example, the -10 element plays a key role in the divergent evolution of ECF sigma regulons such that one or two base changes can affect the set of sigma factors that can recognize a target promoter. In the specific case of the SigV regulon, which is very specifically activated by lysozyme, a T-rich sequence in the promoter spacer region is critical for activation and distinguishes those promoters activated by SigV from those that are otherwise similar in sequence, but not activated.
Conclusions: ECF sigma factors display very high promoter selectivity in vivo, and may therefore be of use in synthetic biology. However, we are still learning the rules that allow promoters to be recognized selectively (or not) by one or more ECF sigma factors in those organisms that contain multiple (and in some cases very numerous) paralogs.

RSV-FG03
General stress response in alphaproteobacteria

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Question: Bacteria employ many regulatory systems to sense and to respond to environmental changes. In contrast to specialized systems that perceive only one type of signal and trigger an adequate response, the general stress response is stimulated by a broad range of stress factors and controls diverse mechanisms of protection. These facilitate survival of environmental challenges and prepare the cell for subsequent stresses. Alphaproteobacteria lack orthologues of *rpoS and sigB* encoding alternative sigma factors that are the central regulators of the general stress response in *Escherichia coli* and *Bacillus subtilis*.
Methods: Comparative genomics and RNAseq were applied to identify and assign promoters to sigma factor regulons and to characterize the general stress response regulon in *Sinorhizobium meliloti*.
Results: An introduction to the general stress response in alphaproteobacteria will be given. It, is controlled by alternative extracytoplasmic function (ECF) sigma factors belonging to the ECF15 (EcfG-like) family. Their activity is regulated by a conserved partner-switching mechanism involving the anti-sigma factor NepR and the anti-anti-sigma factor PhyR. The latter is a reponse regulator whose activity is controlled by phosphorylation (Kaczmarczyk *et al.* 2014). In the symbiotic nitrogen fixing alpha-rhizobium *S. meliloti* the ECF15 family sigma factor RpoE2 regulates the general stress response. RNAseq approaches to characterization of sigma factor regulons will be exemplified for RpoE2 and its integration in the regulatory network will be outlined.
Conclusions: This talk will provide an overview of the general stress response in alphaproteobacteria, describe RNAseq approaches to unravel sigma factor regulons and introduce specific features of the general stress response regulon in *S. meliloti*.

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RSV-FG04
Metal sensing and transmembrane signal transduction by the CnrYXH complex from *Cupriavidus metallidurans*
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In *Cupriavidus metallidurans*, the Cnr regulation system that allows the set up of the cobalt and nickel resistance, is composed at the resting state of an inner-membrane anchored sensor (CnrX) protruding in the periplasm, a very short bitopic anti-sigma factor (CnrY), and an ECF-sigma factor (CnrH) sequestered at the membrane by CnrY. Upon sensing of increasing amount of Ni or Co in the environment by CnrX, CnrH is made available in the cytoplasm to activate RNA-polymerase *via* an as-yet uncharacterized signaling cascade, promoting thus transcription initiation at *cnr* promoters.
To characterize the signaling cascade that leads to CnrH release, we are collecting precise structural data of the different proteins, isolated or in complex, and are delineating the dynamics of the conformational rearrangements that participate in the critical steps of the signaling cascade.
On the periplasmic side, the recruitment of the Met123 side chain in the coordination sphere of Ni or Co is the key event of both the sensing mechanism [1,2] and the first step of signal propagation [3,4].
On the cytoplasmic side, the crystal structure of a complex between CnrH and the cytoplasmic domain of CnrY (CnrYc) showed how the minimal-size anti-sigma CnrYc achieves function and displays a new mode of ECFs inhibitory binding by stabilizing a conformation of CnrH that cannot bind the *cnr* promoter -10 element [5].
On the membrane side, a combination of X-ray structure determination, molecular dynamics simulations, and *in vivo* assays was used to evaluate the contribution of the transmembrane segments of CnrX [6] and CnrY in the whole mechanism. The characterization of the interactions between these two partners both in the membrane and the periplasm is in progress.

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SIV-FG01
Metabolic Coevolution between Symbiotic Bacteria and their Insect Hosts
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Various bacteria have adopted a cooperative lifestyle with animals. Many of these associations have a nutritional basis. The bacteria may variously modify the animal diet, overproduce specific metabolites that are valuable to the host, and modulate the animal signaling circuits that regulate nutrient acquisition and allocation. Our research concerns the metabolic traits of bacteria that live in the gut and cells of insects. Genomic approaches, including genome analysis of obligate intracellular bacteria in plant sap feeding insects and metagenome-wide association studies of the gut microbiota in drosophilid flies, are revealing the bacterial genes and metabolic pathways that mediate these nutritional interactions. The underlying evolutionary processes include selection for specific bacterial traits and genomic decay, together with co-evolved metabolic adaptations in the host. These diverse instances of metabolic coevolution mediate bacterial amelioration of the insect diet and provisioning of supplementary nutrients, contributing to the ecological success of the insect and, by extension, the fitness of their bacterial symbionts.

SIV-FG02
Diversity and functions of the ecto- and endosymbionts of cellulolytic protists in the gut of termites
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Termites harbor diverse gut microbes, which comprise protists, bacteria, and archaea. The majority of these microbes are specific to termites, and the microbiota is essential for the host to thrive on recalcitrant, nitrogen poor dead plant matter. One of the prominent features of this complex microbiota is cellular association between the cellulolytic protists and diverse prokaryotic assemblages. The parabasalid and/or oxymonad gut protists generally accommodate prokaryotes on their cell surface as well as within the cytoplasm, and occasionally even within the nucleus (*1*,*2*). The relationship between the protists and the symbiotic prokaryotes is basically species-specific, and at least 9 prokaryotic phyla are involved.
Because these symbiotic prokaryotes are as yet unculturable, our research team has been trying to predict their functions by analyzing their genome sequences. In 2008, we succeeded in acquisition of the complete genome sequences from two uncultured intracellular bacteria, Rs-D17 (*Candidatus* Endomicrobium sp.; phylum *Elusimicrobia* or formerly Termite Group 1) and CfPt1-2 (*Candidatus* Azobacteroides pseudotrichonymphae; order *Bacteroidales*), which are found exclusively within the cytoplasm of the parabasalid protists *Trichonympha agilis* and *Pseudotrichonympha grassii*, respectively (*3*,*4*). The genome analysis of these endosymbionts suggested that their primary role in the symbiotic system is to upgrade and provide nitrogenous compounds such as amino acids and vitamins, and in case of *A. pseudotrichonymphae*, it also can fix atmospheric dinitrogen.
Currently, our team has been analyzing the genomes of additional symbiont species, and I will present our recent results and future perspectives.

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SIV-FG03
The gut microbiome of phytopathogenic root fly larvae – insights into the detoxification of plant secondary metabolites by insect-associated microbes
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Brassica plants produce various toxic compounds such as isothiocyanates in response to herbivore damage. Despite their toxicity, some insects cope well with these compounds. One example is the cabbage root fly (*Delia radicum*) which is a serious agricultural pest. The mechanism by which these root feeding insects detoxify isothiocyanates has not yet been explored. Our hypothesis is that microorganisms residing in the gut of *D. radicum* contain enzymes that break down the isothiocyanates and are thus crucial for survival and phytopathogenicity.
We substantiated this hypothesis by isolating several microbial species from the *D. radicum* gut that were highly resistant to isothiocyanates as judged by disc diffusion assays with the root volatile 2-phenylethyl isothiocyanate. A subset of these microorganisms was also able to break down isothiocyanates. When we isolated and sequenced plasmid DNA of those strains it became apparent that they shared several genes which are prime candidates to encode enzyme systems that break down toxins. One of the interesting shared genes encoded a hydrolase of the beta lactamase family that may be involved in the breakdown of isothiocyanates. This protein (SaxA) was produced in *Escherichia coli*, purified and subjected to enzyme activity assays where it catalysed the breakdown of 2-phenylethyl- and allyl-isothiocyanate.
When we compared the *saxA* gene sequence to the metagenome of the *D. radicum* microbial gut community, it became apparent that on average about 5 % of the community members contained one copy of the hydrolase SaxA which matches well with the number of 8 % of 16S rRNA reads of the metagenome that mapped best to the microbial strains that showed to break down 2-phenylethyl isothiocyanate.
Taken together, the results of this study demonstrate for the first time that microorganisms are important for the breakdown of isothiocyanates in the root fly larval gut. Both the metagenome analyses and the isolation experiments indicate clearly that the *D. radicum* gut contains a highly specialized microbiota that helps the root fly larvae to survive on their toxic host plants utilizing the plasmid-encoded hydrolase SaxA.
This work was supported by a fellowship within the Postdoc-Program of the German Academic Exchange Service (DAAD) and the Soehngen Institute of Anaerobic Microbiology (OCW 024002002)

SIV-FG04
A case of bacteria-mediated egg defense – the *Burkholderia:* Lagriid symbiosis
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Introduction: While many of the insect-bacteria symbioses described so far have a nutritional basis, an increasing number of defensive alliances are being discovered. However, to date there is no direct evidence of a bacteria-mediated protective role at the egg stage in an insect. Beetles within the Lagriinae subfamily live in association with *Burkholderia* (ß-proteobacteria), a genus which exhibits extraordinary metabolic and ecological versatility including a number of plant-pathogenic traits. The high potential of *Burkholderia* for producing bioactive metabolites [1], in combination with its transmission via egg-smearing and specialized localization in the host, drove our attention to the possibility of a previously unknown strategy for egg defense.
Objectives: (1) Assess the functional role of the symbiotic *Burkholderia* in Lagriid beetles and (2) evaluate the potential for transmission of the bacterial symbiont to the insect's food plant, soybean.
Methods or Materials & Methods: Symbiont-free *Lagria villosa* beetles were generated via egg-surface sterilization and after one generation the eggs were used for *in vivo* assays testing fungal inhibition. Additional *in vitro* tests were carried out against three different fungal species. Finally, the possibility of an insect-mediated infection of the soybean plants (Glycine max) with *Burkholderia* bacteria was tested by exposing the plants to symbiotic beetles and quantifying *Burkholderia* in the leaves via qPCR.
Results: We could show that the presence of *Burkholderia gladioli* on the surface of *L.villosa* eggs significantly inhibits fungal growth as compared to symbiont-free eggs, suggesting a defensive function. Furthermore, *in vitro* bioassays confirmed growth inhibition caused by the symbiotic *B.gladioli.* First experiments also demonstrate the plausibility of host-mediated transmission of *Burkholderia* to soybean plants.
Conclusion: This egg defense strategy may reveal an unexplored component of symbiotic functions in insects, in which metabolites produced by associated bacteria result in effective chemical protection at the nutrient-rich and immobile egg stage of insects. From the microbe's perspective, the advantage of dispersal to plant hosts via the insect might be a major factor promoting the symbiotic association.

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SIV-FG05
Beggars can be choosers – host selection of gut microbiota in cockroaches
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Introduction: Both termites and their closest relatives, the cockroaches, harbor a diverse gut microbiota that plays important roles in symbiotic digestion. While the structure of their intestinal communities has been intensively studied, the factors shaping their composition remain unclear. Termites are obligately dependent on their gut community for lignocellulose digestion, and vertical transmission seems to play an important role in its maintenance across generations. However, the elaborate social system of termites makes it difficult to test the influence of the host on the uptake of bacterial lineages from foreign inocula and the maintenance of bacterial community structure. To investigate these aspects, we developed a gnotobiotic model of the cockroach *Shelfordella lateralis*.
Objective: To assess if the host plays a role in the determination of gut community structure.
Methods: Germ-free cockroaches were inoculated with a full complement of gut microbiota from three termite species, and the composition of the resulting bacterial assemblages was determined by 454 pyrosequencing of their 16S rRNA genes.
Results: Although the bacterial lineages in the inocula strongly influenced the composition of the artificial communities, gut community structure resembled that in conventional cockroaches. Bacterial lineages abundant in conventional cockroaches were selectively enriched from the termite inocula, whereas lineages abundant only in termites were unable to colonize germ-free cockroaches. Exposure of foreign-inoculated cockroaches to conventional ones restored their normal microbiota, indicating that autochthonous lineages are more competitive.
Conclusions: Our results document that selection by the host environment (habitat filtration) is an important factor shaping microbial community structure in insect guts. We expect that the gnotobiotic cockroach model will provide further insights also into the digestive symbiosis.

SIV-FG06
Vitamin supplementation by gut symbionts ensures metabolic homeostasis in an insect host
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Introduction: The gut microbiota of firebugs (Hemiptera: Pyrrhocoridae) is dominated by two actinobacterial taxa (*Coriobacterium glomerans* and *Gordonibacter* spp.) that are vertically transmitted from mother to offspring via the smearing of egg surfaces [1]. Egg surface sterilization results in symbiont-deprived individuals (aposymbionts) that suffer lower fitness and higher mortality [2].
Objectives: In our study, we examined the functional importance of these symbionts, and their effect on the host's metabolism.
Methods and Results: Findings from artificial diet experiments demonstrate that the symbionts contribute towards their host through the provisioning of B-vitamins; a condition further supported by our discovery of complete pathways for the biosynthesis of five B vitamins in the sequenced genome of *C. glomerans* [3].
Comparative transcriptomic analyses of insect genes expressed in the midgut regions of firebugs revealed differential metabolic properties across symbiotic and aposymbiotic states.
Genes involved in the import and processing of B-vitamins were universally up-regulated among aposymbionts, consistent with how animals regulate the expression of these genes under conditions of B-vitamin starvation. Normal expression levels, however, can be restored either through the artificial supplementation of B-vitamins into the insect's diet or re-infection with the actinobacterial symbionts [3].
We also observe a significant upregulation in expression of a single antimicrobial peptide, C-type lysozyme, in the midgut region of symbiotic firebugs, which in turn, may implicate the enzyme in mediating the release of vitamins into the gut lumen through the immunoharvesting of symbiont cells [4].
Conclusions: Collectively, our findings demonstrate that - despite an extracellular localization - beneficial gut microbes can be integral to an insect's metabolic homeostasis, reminiscent of bacteriome-localized intracellular mutualists.

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IMV01
Change the way you think about Cloning: Discover In-Fusion®
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Cloning is essential to most experiments carried out in molecular biology laboratories today. However, it is usually not the main focus, but is rather an essential first step on the way to running the actual experiment and meeting specific research goals. A fast and accurate cloning method would thus be advantageous to minimize the time and resources spent on this initial cloning stage. The majority of researchers still use traditional ligation based cloning which can be time consuming and cumbersome due to several inherent limitations.
The availability of specific restriction sites is a major limitation as this often narrows the cloning strategies possible for the generation of specific constructs. Another drawback is that researchers often need to screen a considerable number of colonies before obtaining positive clones with the insert in the correct orientation. The potential need for sub-cloning is another big limitation since it is not always possible to directly clone into the final destination vector. However, Clontech-Takara provides In-Fusion® HD Cloning Plus, a faster and more highly efficient alternative cloning technology to traditional ligation based cloning.
In-Fusion is sequence independent, seamless, directional, and allows over 95% cloning efficiency to be consistently achieved. This method enables the cloning of any PCR insert into any vector at any locus. Therefore, researchers are not limited by restriction site availability as the In-Fusion

Enzyme fuses PCR-generated insert sequences and linearized vectors efficiently and precisely, utilizing a 15 bp overlap at their ends. PCR carried out with custom primers is used to add the 15 bases found at either end of the linearized vector to the ends of the insert. No additional treatment of the PCR insert is required such as restriction digestion, ligation, phosphorylation, or blunt-end polishing.
A simple 15-30 minute In-Fusion reaction results in the creation of seamless and precisely engineered constructs, where no extra bases of vector or restriction-site-derived DNA is added. This is a crucial advantage when generating protein expression constructs as researchers usually avoid adding extra bases/amino acids to their target protein as this could result in altered target protein function. The high cloning efficiency (> 95%) of In-Fusion also means that far less colonies need to be screened before obtaining positive clones. Finally, In-Fusion has also been successfully used in many applications such as multiple fragment cloning, site directed mutagenesis (insertions, deletions and substitutions) and high throughput cloning.

BTP01
Characterization of a bioflocculant produced from the consortium of three marine bacteria of the genera *Cobetia* and *Bacillus* and its application for wastewater treatment
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In this study, we purified and characterized bioflocculant from a mixed culture of three marine bacteria (*Cobetia* sp. OAUIFE, *Bacillus* sp. MAYA, and *Bacillus* sp. Gilbert with accession number JF799092, JF799093, and HQ537128 respectively) isolated from the sediment sample of Algoa Bay South Africa. The bioflocculant from the consortium showed high flocculation of river water, brewery waste water, and dairy waste water, with resultant flocculating activities of 96.4 %, 93.7 %, and 82.2 % respectively. The applicability of the bioflocculant for removing turbidity and reducing chemical oxygen demand (COD) from these real waste waters was excellent. The bioflocculant turbidity removal efficiency for brewery waste water was 92 %, while its COD reduction efficiency for dairy waste water was 99 %. Scanning electron microscopy (SEM) image of the purified bioflocculant revealed an amorphous morphology, while Fourier Transform Infra-Red (FTIR) analysis also revealed the presences of amino, hydroxyl, and carboxyl functional groups amongst others which could be responsible for the high flocculating activity of the bioflocculant. This environmentally friendly bioflocculant holds promise for application in the field of biotechnology.

BTP02
T7 RNA polymerase-dependent gene expression system for *Corynebacterium glutamicum* – construction and comparative evaluation at the single-cell level
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Introduction: *Corynebacterium glutamicum* is an important model organism in white biotechnology used for production of amino acids and proteins [1]. Currently, only few systems for regulatable (over)expression of homologous and heterologous genes are available, all of which are based on transcription by the endogenous RNA polymerase.
Objectives: The aim of this study was the development of an IPTG-inducible expression system in *C. glutamicum* based on T7 RNA polymerase (T7-RNAP).
Methods: *C. glutamicum* MB001(DE3) carries a chromosomally integrated 4.5-kb fragment of *E. coli* BL21(DE3) including *lacI* and the T7-RNAP gene *1* under control of the *lac*UV5 promoter. The expression vector pMKEx2 was constructed for cloning target genes under control of the T7*lac* promoter. System properties were evaluated using *eyfp* (enhanced yellow fluorescent protein) as reporter gene at the population level and at the single cell level using fluorescence microscopy and flow cytometry.
Results: After maximal induction with 250 µM IPTG, the specific eYFP fluorescence increased 450-fold compared to the uninduced state and was about 3.5-times higher than in control strains expressing *eyfp* via the IPTG-induced *tac* promoter with endogenous RNA polymerase. Flow cytometry revealed that T7-based *eyfp* expression resulted in a highly uniform population with 99% of all cells showing high fluorescence. Functionality of the corynebacterial T7 expression system was also demonstrated by overexpression of the *C. glutamicum pyk* gene for pyruvate kinase, which led to an increase of the specific activity from 2.6 to 135 U/mg.
Conclusion: The newly developed T7 expression system allows very efficient and controllable protein overproduction in *C. glutamicum* to levels that outperform other available systems. It thus presents an efficient new tool for protein overproduction, metabolic engineering, and synthetic biology approaches with *C. glutamicum* [3].

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BTP03
Isolation and Characterization of Sulfur Oxidizing Bacteria involved in the Biogenic Sulfuric Acid Corrosion from different Full-Scale Digesters
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Concrete corrosion due to biogenic sulfuric acid production is a serious problem affecting both sewerage infrastructure and sludge handling facilities. For the biogenic sulfuric acid corrosion (BSA), aerobic sulfur/sulfide oxidizing bacteria (SOB) are required converting reduced sulfur compounds to sulfuric acid. BSA is well studied in sewer pipes, but a lack of understanding exists regarding this process in sludge digesters. Due to the predominant anaerobic conditions in a digester, no sulfuric acid production by SOB is expected, since oxygen is necessary for their growth. Nevertheless, typical BSA damage patterns were observed in various full-scale digesters. Therefore, the aim of this study was to reveal BSA in digesters by isolating and characterizing the relevant SOB communities.
For SOB analysis, biofilm was scratched off from the concrete surface within six different full-scale digesters. Since the focus was on the isolation of active sulfuric acid producing bacteria, specific liquid media, differing in pH and energy source, were used for SOB enrichment and cultivation. By cultivation of SOB on the corresponding agar media, pure cultures were obtained and identified by polymerase-chain-reaction (PCR) and sequence analysis. SOB diversity studies within the enriched SOB cultures were performed using PCR, denaturing gradient gel electrophoresis (DGGE) and sequencing.
With cultivation techniques four different pure SOB cultures were obtained: *Acidithiobacillus thiooxidans, Acidithiobacillus ferrooxidans, Thiomonas intermedia* and *Thiomonas perometabolis*. Since every SOB is characterized by a certain pH optimum statements about the progression of microbial corrosion can be made. *A. thiooxidans*, the key organism within the BSA process, as well as *A. ferrooxidans* are known to be acidophilc and provide evidence of a progressed BSA attack. The neutrophilic *Thiomonas* species, however, indicate a lower extent of corrosion. All pure SOB cultures could be also confirmed by PCR-DGGE and even additional sulfur oxidizing genera could be identified: *Alicyclobacillus*, *Ancylobacter*, *Delftia*, *Hyphomicrobium*, *Mesorhizobium*, *Paracoccus* and *Thiobacillus*.
Finally, the identification of many SOB communities demonstrates that BSA is not only a problem occurring in sewer pipes, but also in sludge digesters.

BTP04
Synthesis, separation and modification of lysine-rich cyanophycin
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The biopolymer cyanophycin (CGP) has been studied for several years due to its unique properties, easy purification and putative use as a precursor for biodegradable chemicals. CGP and CGP dipeptides are also interesting for applications in agriculture, food supplementation, medical and cosmetic purposes. Therefore an efficient production method is key for commercial use of CGP and new compositions and variations are desirable to increase putative applications. We recently demonstrated the synthesis of soluble and insoluble CGP in *E. coli* up to the 30 L-scale. Using an optimized cultivation method and a plasmid-based addiction system we were able to achieve a cell density of 10.2 g/L and a CGP content of 36.2% wt/wt of the CDM (25.1% insoluble and 11.1% soluble CGP). Both forms of the polymer showed high amounts of lysine

replacing the arginine residues. The lysine content was identified as a critical factor for the solubility behavior of the polymer and by using a temperature based separation method it was possible to fractionate insoluble CGP in dependence of its specific lysine content. Insoluble CGP with a lysine content of less than 4 mol% was not soluble even at 90°C, while e.g. CGP containing 31 mol% lysine was soluble at 30°C. Higher lysine contents would result in CGP soluble at even lower temperatures explaining the occurrence of soluble CGP. In our case the isolated soluble CGP showed a lysine content of over 36 mol%, proving the relation of lysine content and solubility behavior. Using this soluble CGP we established a chemical procedure to modify the polymer. *In vitro* guanidination with *o*-methylisourea converted 100% of the lysine side chains to the nonproteinogenic amino acid homoarginine. The conversion also caused a solubility change of the modified CGP, which now resembled the behavior of insoluble CGP showing again the influence of lysine on the solubility of CGP. By establishing a chemical procedure to modify CGP we added another dipeptide to the spectrum of possible applications, and also showed for the first time that modification of CGP after its synthesis is feasible and not only limited to the digestion into dipeptides or a total hydrolysis of the polymer to its basic amino acids. Based on this results we opened a whole new range of possible modifications for further research.

BTP05

Exploration of natural TALE flexibility for biotechnology and synthetic biology

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Introduction: Plant pathogenic *Xanthomonas* bacteria translocate transcription activator like-effectors (TALEs) into host cells to specifically induce transcription of target genes by direct binding to promoter sequences. TALEs bind via tandem 34-amino acid-repeats, which recognise the DNA in a consecutive one repeat to one base pair manner. The repeats vary especially in two amino acid positions, the repeat variable diresidue (RVD), which determine the base specificity of each repeat for one distinct or several alternative nucleotides. In biotechnology, this modular DNA binding mode is used to create DNA-specific tools for desired functions like gene regulation and genome editing. A few natural TALEs contain single repeats that differ by several amino acids in length from standard repeats. These aberrant repeat variants possess new opportunities for molecular TALE tools.
Objectives: We investigate how single or multiple aberrant repeats influence the DNA-binding behaviour of TALEs and how this expands the possibilities to apply TALE derivatives in biotechnology.
Methods: The target sequence specificity of artificial TALEs with aberrant repeats is revealed by *Agrobacterium*-mediated expression *in planta* and beta-glucuronidase (GUS) reporter induction. Transcribed and translated TALE nucleases (TALEN) are used in cleavage assays with putative target DNA sequences to test DNA recognition activity *in vitro* and to study possible applications.
Results: The experiments revealed that TALE derivatives with a single aberrant repeat activate transcription at the optimal target sequence but also at target sequences with a -1 nucleotide frameshift, which abolishes recognition by conventional TALE proteins [1]. Surprisingly, TALEs with tandem aberrant repeats behave differently and allow recognition of target sequences with larger frameshifts, but with less flexibility.
Conclusions: Aberrant repeats break the strict consecutiveness of TALE-DNA binding, presumably by conditionally looping out of the repeat array. This has implications for the development of molecular tools as master-regulators in synthetic circuits and opens the possibility to insert functional domains into the repeat array itself.

BTP06

Immobilization of an integral membrane protein for biotechnological application

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Numerous soil bacteria are able to metabolize styrene via side-chain oxygenation. The pathway mentioned comprises a styrene monooxygenase (SMO), which oxidizes styrene to (*S*)-styrene oxide, a styrene oxide isomerase (SOI), which subsequently converts the epoxide into phenylacetaldehyde, and a phenylacetaldehyde dehydrogenase (PAD). The latter one oxidizes the aldehyde to phenylacetic acid. The acid formed can subsequently be degraded to intermediates of the tricarboxylic acid cycle [1,2].
The SOI has been identified to be an integral membrane protein [3]. It performs a highly selective isomerization reaction of epoxides to yield pure aldehydes [3]. But, a previous study has also shown a high sensitivity of the SOI toward its product resulting in an irreversible inhibition [3]. In our present study [4] we report on the covalent immobilization of an SOI from *Rhodococcus opacus* 1CP on SBA-15 silica carriers. The enzyme was immobilized and finally applied in aqueous as well as in two-phase systems. While the linkage of the protein to the SBA-based carriers resulted only in a poor stabilization of the enzyme in an aqueous system, an improved stability was observed toward organic phases like the non-toxic 1,2-cyclohexane dicarboxylic acid diisononyl ester (Hexamol DINCH). With this two-phase system and the immobilized SOI, 1.6-2.0x higher product yields were reached compared to the non-immobilized catalyst. Furthermore, Hexamol DINCH was used to our knowledge for the first time in a bioprocess and offers a promising alternative to other solvents for biotechnology.

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BTP07

Detection of *Helicobacter pylori* in sample of Iraqi patients by some invasive and noninvasive methods

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Introduction: *Helicobacter pylori* is one of the most common chronic bacterial infection in human. It has been estimated that more than half of the adult population in the world is infected with this organism (1)
Objective: This study aim to detect H.pylori by invasive and noninvasive methods.
Material and method: Seventy four patients (40 male and 34 female) also 40 (20 male and 20 female) apparently healthy as a control group collectively with age (21-80 year) were enrolled in this study. Samples of patients were collected from different hospitals in Baghdad city/ Iraq, from November 2012 till November 2013,Three specimens were taken from each patients, biopsy for invasive diagnosis, salvia and blood, for non-invasive diagnosis.The molecular analysis conducted to detect H.pylori DNA in saliva and biopsy using PCR technique with primer for ureC and 16 srRNA of gene specific for H.pylori.
Result: The result revealed that ureC and 16 srRNA were positive for all the patients (100%) in the biopsy samples while only one specimen of saliva was positive for ures (1.35%) and six (6.19%) were positive for 16srRNA.The immunological result by indirect immuonoflurecen (IFT) revealed that IgG appeared in 91.89% of patients. Depending on endoscopic diagnosis, the patients were grouped into: sever antral gastritis (66.22 %) prepyloric ulcer (9.46%) duodenum ulcer (6.76%) sever prolapse gastropathy (5.41%) reflex esophagitis (5.41%) hemorrhagic gastritis (2.7%) gastric cancer (2.7%) multiple ulceration(1.35%)

Conclusion: It was concluded that the estimation of anti H.pylori IgG in the serum was very effective as noninvasive method and should be taken into account for the diagnosis of the H.pylori and molecular analysis by using conventional PCR presented high efficiency to detect the ureC and 16 srRNA genes specific for H. pylori from biopsy sample as invasive method

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BTP08

Influence of promoter and replicon on phenotypic heterogeneity during plasmid-based recombinant protein production in *Bacillus megaterium*

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Introduction: The Gram positive *Bacillus megaterium* was systemically developed as an expression system for the production of recombinant proteins using the strong xylose-inducible promoter system within the last years. This system is based on a multicopy plasmid containing the functional elements of the system namely the gene encoding the xylose repressor XylR and the optimized promoter P_{xylA}. For deeper analyses, P_{xylA} was fused to the coding sequence of an enhanced form of the green fluorescent protein (GFP). In the absence of xylose *gfp* expression is repressed by the repressor XylR while in the presence of xylose the expression is derepressed.
Objectives: Although GFP is recombinantly produced in high yields the culture showed a significant level of heterogeneity with up to 30% of low-producing cells at the single cell level analyzed by fluorescence microscopy and flow cytometry. To figure out the influence of the regulation of P_{xylA} on this phenomenon, XylR was investigated in more detail.
Results: DNA/XylR (+/- bound xylose) binding studies showed the occurrence of the postulated two different conformation and oligomerization states of XylR surprisingly both binding to the repressor binding site diminishing the existing regulation model. On the other site *in vivo* studies of the XylR-free *B. megaterium* carrying the *xylR* free multicopy plasmid gave some hints that the phenomenon of phenotypic heterogeneity is more related to different plasmid copy numbers or plasmid distribution within the producing cells. This was further confirmed by analyzing plasmid-based *gfp*-expression by the control of the constitutive promoter of the gene encoding the pyruvate dehydrogenase complex which resulted in a similar portion of low and high GFP-producing cells as caused by the xylose-inducible promoter. For investigating the influence of plasmid copy number, distribution and stability on culture heterogeneity, different replicons were tested by individually replacing the existing one keeping P_{xylA}-*gfp*. These new plasmids were used for recombinant xylose-induced GFP-production in *B. megaterium*. Compared to the original plasmid these plasmids caused at least 50 % less recombinantly produced GFP. Further on, culture heterogeneity differs between less than 60 % and more than 92 % of GFP-producing single cells analyzed by flow cytometry.

BTP09

Salt bridges affect the temperature stability of the ene reductase OYERo2 from *Rhodococcus opacus* 1CP

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Introduction: The rising demand for enantiopure molecules in the fine-chemical and pharmaceutical industry requires the availability of well-characterized and stable enzymes. Recently, we discovered a new member of the Old Yellow Enzyme (OYE) family by genome analysis of the actinomycete *Rhodococcus opacus* 1CP. OYERo2 belongs to the thermophilic-like subclass showing highest sequence identity to a thermostable OYE from *Thermus scotoductus* SA-01 [1]. However, OYERo2 possesses no thermostable properties and showed a rapid activity decrease at 37 °C within a few minutes.
Objectives: Structural studies of thermostable OYEs suggest that there is a relation between complex salt networks found at the dimerization interface of the enzymes and its temperature stability [2]. In this study we focussed on the creation and characterisation of a mutant of OYERo2 including characteristic complex salt bridges in order enhance the temperature stability.
Methods: The mutant was created by an exchange of five amino acids on the surface of OYERo2. The *oyeRo2-mut* gene was recombinantly expressed in E. coli BL21 and purified by IMAC as an N-terminal his-tagged protein. For characterization enzyme activity was measured via NADPH consumption followed at 340 nm absorption.
Results: Not otherwise than OYERo2 the mutant showed high specific activity and catalytic efficiency with maleimide (v_{max}=46,4 U/mg, k_{cat}/KM=10 658 $s^{-1}\cdot mM^{-1}$). In comparison to the original enzyme we measured higher enzyme activities at temperatures between 40 and 50 °C. OYERo2-mut showed also an enhanced stability. After 2-hour incubation at 30 °C OYERo2-mut had at least 80 % of initial activity, whereas we observed an activity degrease to 20 % for OYERo2. At 37 °C OYERo2 was completely inactivated within 10 min, OYERo2-mut showed activity over 30 min.
Conclusion: The addition of a complex salt bridge network into the dimerization interface of OYERo2 led to an improvement of temperature stability. This provides an insight into structural stabilization methods for this industrially important family of biocatalysts.

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BTP10

Systems biology and Engineering of *Clostridium pasteurianum* for new bioproduction processes

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Introduction: *Clostridium pasteurianum* is a new and promising producer of chemicals and fules[1]. For example, it can produce 1,3-propanediol (1,3-PDO) and *n*-butanol with completely different patterns from the well-studied *C. butyricum* for 1,3-PDO production or *C. acetobutyricum* for the classic aceton-butanol-ethanol process.
Objectives: Within two EU 7th FR projects (PROPANERGY and EUROBIOREF), we aimed to understand and engineer different *C. pasteurianum* strains for the production of *n*-butanol and 1,3-PDO[2,3]. More recently, we set out to explore electricity-driven fermentation with *C. pasteurianum.*
Materials & Methods: DSMZ 525 and a new isolate of *C. pasteurianum* were comparatively studied at physiological, genomic, proteomic and process levels.
Results: Optimization of medium and culture conditions for the two *C. pasteurianum* strains resulted in the production of ~70 g/L PDO in one case, and 21 g/L *n*-butanol in another case. A process with simultaneous production of both products at high concentrations was also developed. For further improving the processes, physiology and regulation of metabolic pathways in both strains were systematically studied. Among others, iron availability was found to play essential roles in shifting the metabolism. Genome sequencing and proteomic analysis revealed difference and importance of several pathways and enzymes.
For the optimization of *n*-butanol and 1,3-PDO production, which are both NADH-dependent, the supply and regeneration of reducing power is a key issue. Here, the use of electricity for biosynthesis is of particular interest and incorporated into our study. Exogenous electron supply in electrochemical bioreactors clearly reveal an electrically-driven metabolic shift to the reduction pathways and hence a better product formation.
Conclusion: The systematical study of cell biology and functional genomics of two *C. pasteurianum* strains led to the development of new bioproduction processes and advanced our knowledge of this promising industrial microorganism. The electricity-driven fermentation with this microorganism has high potential for completely new bioprocesses.

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BTP11
Electrokinetic control of bacterial deposition and transport
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Introduction: Microbial biofouling causes significant problems in medical and technical applications. It leads to biocorrosion, clogging of filters and membranes or affects human safety in water treatment processes. There is hence high interest in novel approaches preventing microbial adhesion as the initial step of biofilm formation.
Objectives: We tested the hypothesis wether the shear force induced by an electroosmotic water flow (EOF) over a collector allows overcoming the attractive interaction energy of initial cell adhesion. Different to a parabolic profile of pressure driven hydraulic flows, the plug-shaped velocity profile 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 or Materials & Methods: By applying EOF of varying flow strengths (as induced by varying electric fields and ionic strength of the percolation buffer) we quantified the deposition of bacteria (e.g. *Pseudomonas fluorescens* Lp6a; *Mycobacterium frederiksbergense* LB501T) in percolation column experiments. Deposition data were discussed by the extended Derjaguin, Landau, Verwey, and Overbeek (XDLVO) theory of colloid stability and by clean bed filtration theory.
Results: We found that the presence of DC may reduce bacterial surface coverage and initial adhesion efficiency of the cells by > 90 %. Based on the data we present a model to predict EOF-induced prevention of bacterial adhesion as based on XDLVO theory and the EOF-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, to prevent initial adhesion and biofouling in technical applications.

BTP12
Expression and purification of biopharmaceuticals in *Ustilago maydis*
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Introduction: Many proteinaceous medicals are generated by microbial expression systems linking industrial biotechnology closely to the pharmaceutical industry. Since every protein has specific requirements to its expression host there is a need for a broad variety of production systems, especially with regard to posttranslational modifications such as N-glycosylation or disulfide bonds. The phytopathogenic fungus *U. maydis* represents a recent member of these organisms. It uses an unconventional secretion pathway which circumvents N-glycosylation to export the endochitinase Cts1. To establish *U. maydis* as a protein expression system proteins of interest are fused to the Cts1 protein which is hence mediating its co-export.
Objectives: For the expression and unconventional secretion of heterologous proteins in *U. maydis* several potential bottlenecks such as proteolytic degradation, protein stability or growth behavior in large scale cultivations need to be investigated. Interesting targets are different antibody formats like nanobodies, potential antigens or other valuable therapeutic proteins.
Material & Methods: The focus of this study lies on the strain optimization with regard to proteolytic degradation using protease deletion approaches, on the optimization of expression constructs and on the scale up of cultivation.
Results: The expression and export of a variety of interesting proteins could be shown. The sequential deletion of proteases has led to a strong increase in protein yields. Furthermore, the expression construct design and the cultivation conditions have drastic effects on protein stability and activity.
Conclusions: The expression and unconventional secretion of target proteins in *U. maydis* shows highly promising results. Several potential proteins could successfully be expressed. Strain as well as expression construct optimization coupled with careful cultivation adjustments pledge good results regarding future industrial applications.

BTP13
Identification of a novel itaconic acid biosynthesis pathway in *Ustilago maydis*
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The unsaturated dicarboxylic acid itaconate is a bio-based chemical building block for production of plastics, paints and cosmetics. Currently, itaconic acid is produced by fermentation of *Aspergillus terreus*. In this organism, biosynthesis occurs by decarboxylation of *cis*-aconitate. The reaction is catalyzed by *cis*-aconitate decarboxylase (CAD), which belongs to the PrpD protein family and is similar to methylcitrate dehydratases. Itaconate production has also been observed in *Ustilago maydis* and some related *Pseudozyma* species. However, the biosynthetic route for itaconate production has not yet been characterized in these fungi.
Here, we report that *U. maydis* uses an alternative biosynthesis pathway for itaconic acid production. In this fungus, all genes required for itaconate biosynthesis are organized in a gene cluster, which also contains a pathway-specific transcriptional regulator. Within this gene cluster we could identify two genes coding for enzymes critical for itaconate production. Both enzymes were characterized by deletion analysis and their biochemical activity was confirmed by *in vitro* assays with proteins purified from *E. coli.* This allowed us to propose an alternative pathway for itaconate biosynthesis. In *U. maydis,* itaconate is produced by decarboxylation of trans-aconitate which is generated by isomerization of *cis*-aconitate. We were able to achieve itaconic acid production in the heterologous host *Saccharomyces cerevisiae* by expression of the respective *U. maydis* genes.
The identification of an alternative itaconic acid biosynthetic pathway and the detailed characterization of enzymes will be used to improve biotechnological production of this interesting chemical building block in *U. maydis* and thus highlights the high biotechnological potential of this fungus.

BTP14
Biodegradation of crude oil under salt stress using *Penicillium sp* UTMC1843
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High salinity and crude oil pollution are two stress conditions that are common in the oil rich areas in the Middle East. Numerous researchers have evaluated the ability of bacteria to degrade petroleum hydrocarbons in saline condition, but there are few studies about role of fungal strain in such areas. The aim of the present study is evaluating of indigenous fungal strains that are capable to grow and degrade the crude oil under salt stress.
For the fungal strain isolation, different contaminated saline soil samples were prepared from different habitats of Iran. The salinity of collected soil was measured and the fungal strains were isolated by two techniques: spread plate method in PDA (supplemented with 1% crude oil, 10% NaCl and 50 mg/l chloramphenicol); and enrichment technique in Minimal Salt Medium (MSM) (supplemented with 1% crude oil, 10% NaCl and 50 mg/l chloramphenicol). Afterward, isolation and purification of isolates were carried out on PDA media. Then, each of pure fungal isolates were evaluated for growth rate and degradation ability after 21 days under 1% crude oil and 10% NaCl in MSM, by measuring the dry weight of fungal biomass and total petroleum hydrocarbons (TPH) assay. The best isolate was selected base on the maximum growth rate and crude oil degradation. The salt tolerance assay (0, 2.5, 5, 7.5, 10, 15 and 20 % NaCl) and the best combination of oil and salt for getting the maximum oil degradation were studied in liquid PDB medium. Finally, the selected isolate was identified based on its morphology of colony and microscopic examination with the reference to identification keys.
In this study, twenty five fungi isolates with different morphological characteristics have been isolated from different saline oil polluted soil. All fungal isolates were screened for their ability to degrade the crude oil and average growth rate. Among the isolated strains, SP-08, with 70% removal of crude oil under saline stress, showed the highest potential for oil biodegradation in saline soil. Salt tolerance assay of the SP-08 illustrated that crude oil degradation was not significantly affected in different salt stress until 15% salt, but for the higher amount of salt, negative effect on degradation activity was observed. Morphological identification showed that isolated strain belongs to *Penicillium sp*.
These results show that *Penicilliun* sp UTMC1843 as hallotolerant fungi can be extensively degrade crude oil under salt stress and will be beneficial strain for bioremediation of petroleum polluted environments.

BTP15
Evaluation of immune response to Hepatitis B vaccine in Egypt and generation of the corresponding antibodies against the recombinant HBV vaccines
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The study aimed to evaluate immune responses to recombinant hepatitis B vaccine in Egypt after 6-8 weeks of completing vaccine by measuring the generation of the corresponding hepatitis B antibody titer.**So** a total of two 215 healthy volunteers divided into two groups. First group (135) received a full dose of recombinant hepatitis B vaccine. Only 100 chosen for the study and the other where excluded. Second group (80) was considered as control. Only (50) was chosen for the study others where excluded.HBsAb level was measured after 6-8 weeks of completing vaccination course. The non responders where 18%. The moderate responders where 30%. The high responders 52%. The immune response to Hepatitis B Vaccine in Egypt is only 82%.After vaccination the ones who have immune moderate response 30% may have a risk of infection.

BTP16
Intermediates of bacterial fermentation indicate the performance of a full operating biogas plant
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One of the most important renewable energy sources is biomass from energy crops and organic waste that can be used for the production of bioethanol or biogas. The production of biogas, which mainly consists of CH_4 and CO_2, is based on anaerobic fermentation of organic matter and is performed in biogas plants that generate electric power and heat. For a more precise and targeted optimization, it is crucial to understand the biological processes during biogas production. Emphasis should therefore be placed on the development of fast and reliable test systems for the analysis of metabolic processes of the organisms involved in biomethanation and in the identification of metabolic bottlenecks.
Here we show that the addition of intermediates of the fermentative processes in biogas plants can be used to detect the metabolic activity of microorganisms involved in the different steps of substrate degradation. Using authentic samples from full operating agricultural biogas plants and by addition of substrates for acidogenic (e.g. sugars) and syntrophic bacteria (butyrate, propionate) as well as for methanogenic archaea (acetate, H_2 and CO_2) we could monitor the efficiency of the different steps involved in the anaerobic degradation process. Most of the analyzed biogas sludge samples were stimulated by the addition of ethanol or acetate indicating that syntrophic ethanol oxidation and aceticlastic methanogenesis were not the rate limiting steps in routinely operating agricultural biogas plants. The rate of biogas formation was rather limited by the activity of syntrophic butyrate/ propionate oxidizers. Biogas sludge from plants with low organic dry mass was stimulated by all metabolic intermediates, indicating that all levels of degradation suffered from substrate limitation. In rare cases we observed stimulation by the addition of propionate and butyrate indicating an impaired hydrolytic activity in the first step of biomethanation. Furthermore, we found that elevated concentrations of NH_4^+ resulted in a severe reduction of the stimulatory effect of acetate on methane formation. In combination with 16S rDNA and metagenomic analysis it became evident that the number of aceticlastic methanogens of the family Methanosarcinaceae dramatically decreased by increasing NH_4^+ concentrations.

BTP17
Identification of new CALB-type lipases within the genus *Pseudozyma*
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Introduction: Lipase B (CALB) from *Pseudozyma antarctica* (formerly *Candida*) is one of the most widely applied enzymes in industrial biocatalysis especially in regio- and enantioselective synthesis. Additionally to its main reactions CALB is able to catalyze the lipase-unconventional formation of peracids. This phenomenon named enzymatic promiscuity can be found in the enzyme evolution and is based on a common ancestor. Hence, further residual side reactions of enzymes inside the genus *Pseudozyma* are expected. Furthermore CALB features lipase-unusual properties such as no interfacial activation and a modified consensus sequence of Thr-X-Ser-X-Gly (Thr instead of Gly).
Objectives: Our aim was the identification of new lipases homologue to lipase B (CALB) from *P. antarctica* with similar or novel enzymatic properties for biotechnological applications.
Methods: Using degenerated primers partial sequences homologue to CALB from *P. antarctica* were identified in different strains of the genus *Pseudozyma*. Full sequences were isolated from generated genomic libraries by genome walking. Selected lipases were recombinantly expressed in *Pichia pastoris* for further investigation.
Results: With nucleotide similarities between 60 and 99 % the isolated sequences showed high homology to CALB. The heterologous expression in *P. pastoris*, purification, and enzymatic activity of the novel CALB-type lipases are currently under investigation.
Conclusion: CALB-type lipases are highly conserved within the genus *Pseudozyma*. With the identification of further CALB variants we hope to identify common structural features to explore the enzymatic promiscuity of CALB.

BTP18
Diatoms as bioreactors for the synthesis of therapeutics and biopolymers
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Recombinant proteins are used in various industrial, therapeutic and diagnostic applications and consequently there is a great demand for expression systems involving low production cost. Well established systems like bacteria, yeast, mammalian or insect cells all depend on external carbon sources, which is quite an important cost factor for large-scale expression. In the 1980s plants came into focus as photosynthesis fuelled expression system, however, long growth rates and low expression levels still hinder the establishment as biotechnical expression system. Microalgae are like land plants powered by sunlight, but exhibit very rapid growth rates, are easy to cultivate and do not compete for important agricultural area or resources, which makes them very attractive as biotechnical protein expression system. However, research on that topic is still very rare calling for further investigations in this highly promising field.
Here we will present data on the expression of human IgG antibodies against a Hepatitis B Virus surface protein and a nucleoprotein of Marburg Virus in the diatom *Phaeodactylum tricornutum*. The antibodies are fully-assembled and functional and get efficiently secreted into the culture medium. Furthermore, we show that the bio-plastic polyhydroxybutyrate (PHB) can be synthesized very efficiently in a microalgal system. PHB levels of up to 10% of algal dry weight were obtained by introducing the PHB-pathway of the bacterium *Ralstonia eutropha*. Altogether, our studies reveal the great potential of diatoms as efficient factory for protein and biopolymer production.

BTP19
Improvement of butanol production in *C. beijerinckii* and *C. saccharobutylicum* via genetic engineering
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Introduction: The importance of butanol as new biofuel has led to numerous efforts in order to establish an economic biotechnological production process with clostridia [1].
Objectives: The aim of this work is to improve the butanol production in selected strains of *C. beijerinckii* and *C. saccharobutylicum* via genetic engineering.
Methods: Mutations were found in commercial strains of these two species by comparative genome analysis and their metabolic influence was verified by enzyme tests. Mutations showing a positive influence on fermentation performance will be transferred to target strains using the "allelic exchange" (AE) system [2, 3].
Results: A mutation found in the *ptb* gene of *C. beijerinckii* NCP260 leads to a 57 % lower phosphotransbutyrylase activity compared to the parental strain *C. beijerinckii* BAS/B3/I/124, resulting in more substrate being used for butanol production. Also, a mutation found in the *bdh* gene of *C. saccharobutylicum* NCP200 leads to 150 % higher butanol dehydrogenase

activity than in the parental strain *C. saccharobutylicum* BAS/B3/SW/136, suggesting the upregulation of an alternative alcohol dehydrogenase. The mutated alleles *ptb* from *C. beijerinckii* NCP260 or *bdh* from *C. saccharobutylicum* NCP200 will be transferred into parental strains of the same species via AE. Therefore, the knockout-plasmids pMTL-Em-ptb and pMTL-Em-bdh were constructed.
Conclusion: AE could be achieved by conjugating the plasmid pMTL-Em-ptb into *C. beijerinckii* BAS/B3/I/124 [4], as well as by transforming the plasmid pMTL-SC-bdh into *C. saccharobutylicum*BAS/B3/SW/136 via triparental mating [5].

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BTP20
Characterization of a novel PQQ-dependent ketoaldehyde dehydrogenase isolated from *Sphingomonas wittichii* RW1 that belongs to a novel class of quinoproteins
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Quinoproteins play an important role in the energy metabolism of many gram-negative bacteria and are involved in the oxidation of a wide range of substrates such as alcohols, aldehydes and sugars [1]. They are membrane integrated or located in the periplasm and are linked to the respiratory chain.
The genome of the α-proteobacterium *Sphinghomonas wittichii* RW1 is completely sequenced [2]. Bioinformatic analysis revealed that this organism possesses a multitude of PQQ-dependent uncharacterized dehydrogenases. One of them is encoded by the gene *swit_1323* and is annotated as polyvinyl alcohol (PVA) dehydrogenase. The enzyme is localized in the periplasm and contains PQQ and heme *c* as prosthetic groups. The gene *swit_1323* was amplified, fused to a streptag coding sequence and cloned into the broad-host-range expression vector pBBR1p264 [3]. Since the heterologous expression of this gene in *E. coli* failed, the expression vector was transferred into *S. wittichii* by electroporation. The corresponding protein was expressed homologously in the native host and purified via Strep-Tactin affinity chromatography. The protein was detected as a distinct band at 66 kDa in a SDS-PAGE and could also be identified by protein immunoblotting. Enzymatic assays showed that Swit_1323 is a ketoaldehyde dehydrogenase and is active with substrates such as methylglyoxal (12 U/mg), phenylglyoxal (1,5 U/mg) and 2-keto-D-glucose (13 U/mg). Contradictory to the prediction, Swit_1323 showed no specific activity with PVA or similar compounds. Therefore, the quinohemoprotein Swit_1323 is a ketoaldehyde dehydrogenase, which belongs to a novel class of PQQ-depending enzymes.

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BTP21
Structural Characterization of the two Siderophores from *Rhodococcus* genus and Elucidation of their biosynthetic machinery
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Introduction: *Rhodococcus* species are extensively studied as extraordinary biocatalysts. However, only very few natural products have been isolated from this genus.
Objectives: Exploring the chemical structures of the two siderophore from *Rhodococcus* strains, identification of the gene cluster encoding the siderophores biosynthesis enzymes and the biochemical characterization of the enzymes.
Methods: The structure elucidation of the two siderophores were accomplished via MS^n analysis and NMR spectroscopy. For the isolation of the siderophores, The *Rhodococcus* strains were grown for two days in LB medium. Cells were harvested, and resuspended in M9 medium. Polycarbonate flasks containing fresh minimal medium supplemented with trace elements and 4 g/L glucose. Cultures were grown for two days until a CAS-positive reaction of the supernatant was observed. The culture supernatant was extracted with 5 g/L of XAD-16 resin, the adsorbed compounds were eluted with MeOH and concentrated under reduced pressure at 30 °C to dryness.
Results: In this work, the isolation, the structural characterization, and the elucidation of the biosynthetic machinery of Rhodochelin and heterobactin mixed-type siderophopres isolated from *Rhodococcus jostii* RHA1 and *Rhodococcus erythropolis* PR4 respectively are reported.
Conclusion: The Rhodochelin siderophore structural elucidation revealed the tetrapeptide to contain an unusual ester bond between an L-δ-N-formyl-δ-N-hydroxyornithine moiety and the side chain of a threonine residue. On the other hand the heterobactin siderophore structural characterization display the noteworthy presence of a peptide bond between the guanidine group of an arginine residue and a 2,3-dihydroxybenzoate moiety.

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BTP22
Electrode mediated unbalanced fermentation by heterologous production of an electron transport chain in *Escherichia coli*
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The realization of unbalanced fermentation processes is a challenging task whereby microbial electrochemical cells (MEC) might open new perspectives. However, the amount of exoelectrogenic organisms acting as potential biocatalysts for this kind of application is rather limited due to their narrow substrate spectrum. In contrast *Escherichia coli* is metabolically versatile and genetically easily tractable since it is the best understood microorganism so far.
This study describes the process of reprogramming *E. coli* for efficient use of anodes as electron acceptor. Electron transfer into the periplasm of *E. coli* was accelerated by 89% via heterologous expression of the three *c*-type cytochromes CymA, MtrA and STC from the exoelectrogenic organism *Shewanella oneidensis*. STC was identified as a target for heterologous expression by a two stage screening approach. First, mass spectrometric analysis was conducted to identify natively expressed periplasmic cytochromes in *S. oneidensis* under conditions of extracellular respiration. Corresponding genes were cloned and tested for activity in *E. coli* using a novel assay that is based on the continuous quantification of methylene blue reduction in cell suspensions. Periplasmic electron transfer could be extended to a carbon electrode surface using methylene blue as redox shuttle. Results from first MEC experiments revealed a shift in the fermentation product spectrum towards more oxidized end-products. In this context a new reactor setup was designed to optimize the analysis of volatile fermentation products. Previous experiments demonstrated that glycerol fermentation of *E. coli* can be improved by co-cultivation with *Methanobacterium formicicum* [1]. Although methanogens are undesirable in most MEC applications it was shown that the glycerol consumption during the described unbalanced fermentation process could be improved by co-cultivation. Furthermore relevant amounts of current and methane were produced.
This study clearly demonstrates that the production of a new electron transport chain enables *E. coli* to perform an unbalanced glycerol fermentation which could offer new opportunities for biotechnological applications.

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BTP23
Transcriptional analysis of genes putatively coding for carbonic anhydrase in *Corynebacterium glutamicum*
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Introduction: *Corynebacterium glutamicum* is a non-pathogenic, Gram-positive organism used as industrial workhorse for the production of amino acids, e.g. L-lysine and L-glutamate. The biosynthetic pathways of amino acids involve several steps using different carboxylase/decarboxylase enzymes, highlighting the importance of CO_2 homeostasis, in production of amino acids in *C. glutamicum*. The PEP-pyruvate-oxaloacetate node, important for oxaloacetate anaplerosis, has a

key role in production of L-lysine. Several enzymes like PEP-carboxylase, pyruvate carboxylase, PEP-carboxykinase and oxaloacetate decarboxlase are among the key enzymes in this node and play crucial role in oxaloacetate anaplerosis, needed for L-lysine biosynthesis. The homeostasis of CO_2 in the intracellular environment is maintained by carbonic anhydrase enzyme, catalyzing the reversible hydration of carbon dioxide to carbonic acid.
Objective: The aim of the present work was to study the genetic organization as well as the expression profile and regulation of the genes *bca* and *gca*, putatively coding for carbonic anhydrase enzyme in *C. glutamicum*.
Methods: Determination of transcriptional start sites (TSS) by 5'-RACE, reporter gene assays using chloramphenicol acetyltransferase, determination of DNA/protein interaction using electrophoretic mobility shift assays (EMSAs)
Results: The TSSs of *bca* and *gca* were identified. For both genes, comparison of the activities of their corresponding promoter regions on different carbon sources in wild-type background in *C. glutamicum* showed carbon-source dependent variation in expression. Furthermore, comparison of activity of the promoter in the wild-type and a regulator-deficient mutant followed by retardation of the promoter region with purified regulator protein, as observed in EMSAs, showed regulation for one of the putative genes by that regulator in *C. glutamicum*.
Conclusion: The transcriptional organization of two putative carbonic anhydrase genes in *C. glutamicum* was analyzed and the expression profiles showed carbon source-dependent regulation of both genes. Mutational analysis and EMSAs showed that the expression of one of the two genes is under the control of the regulator.

BTP24
Techniques for the diagnosis of American foulbrood (*Paenibacillus larvae*) in honeybee colony
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Introduction: American foulbrood is a common bacterial disease in bees (*Apis mellifera* L.) It is found on all continents where beekeeping is practiced, and considered as the most contagious brood disease of the honey bee, which can destroy an entire colony. The causative agent of American foulbrood is a Gram-positive bacterium *Paenibacillus larvae*.
Objectives: This work proposes to compare the methods of detection of *Paenibacillus larvae* in bee L depending on the nature of léchantillon (honey bees, pollen, wax and debris from the hive) colonies.
Materials & Methods: Sampling was carried out on two apiaries:
- An AFB contaminated apiary located in the region of Bougara (Blida), which is used to isolate sick hives in this area.
- A healthy supposed apiary located in the region of Baba Ali (wilaya of Algiers)
Samples were taken during the spring period of 10 colonies at both apiaries. The samples were taken on:
- The worker bees collected directly from frames.
- Honey stored in cells.
- Wax taken directly from frames.
- From Pollen collected within the colony.
- The remains of the hive collected at the bottom of the hive.
MYPGP the culture medium is used during all the identifications of the bacterium.
Results: For the contaminated apiary, the detection rate of AFB obtained in samples of bees, honey and debris from the hive is 80% and 70% contamination. For debris from the hive, the detection rate is 70%. For pollen, the rate of contamination by bacteria is 60%. In the wax, the average obtained is lower at 40%. The apiary considered to be healthy. One positive sample is detected on bees and debris from the hive of honey and two (10 and 20% contamination). For other categories (wax, pollen) no positive samples were detected
Conclusion: In light of the results, several conclusions can be drawn:
Even in the absence of symptoms of the disease, the bacteria can be detected in honey or bees. Detection methods on honey bees are the most effective in the diagnosis of pathology techniques

BTP25
Enzymatic *N*- and *O*-alkylation/benzylation of tyrosine and derivatives
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Introduction: Prenyltransferases (PTs) play an important role for creation of structural diversity and bioactivities in natural products (*1*). The *O*-prenyltransferases TyrPT and SirD from the dimethylallyl tryptophan synthase (DMATS) superfamily catalyze the transfer of dimethylallyl moiety from dimethylallyl diphosphate (DMAPP) to tyrosine and derivatives (*2-4*).
Objectives: To study the substrate promiscuity of TyrPT and SirD towards unnatural DMAPP analogues, we test their acceptance for methylallyl, 2-pentenyl and benzyl diphosphate, which were well accepted by indole prenyltransferases (*5-7*).
Methods: The overproduced and purified TyrPT and SirD were incubated with DMAPP analogues and aromatic substrates. The reaction mixtures were analyzed on HPLC and the structures of the isolated enzyme products were elucidated by NMR and MS analysis.
Results: TyrPT and SirD also accepted DMAPP analogues and catalyzed alkylation/benzylation of hydroxyl or amino group at the *para*-position of the side chain of the aromatic ring.
Conclusions: TyrPT and SirD also showed substrate promiscuity towards prenyl donors, which expands the usage of these enzymes as biocatalysts in chemoenzymatic synthesis and contributes significantly to structural diversity of alkylated compounds.

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BTP26
High efficient cyanophycin production in cyanobacteria using the P_{II} (I86N) Variant
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Introduction: Cyanophycin or multi-L-arginyl-poly-L-aspartate is a non-ribosomally synthesized biopolymer which can be found in many cyanobacteria and a few heterotrophic bacteria. It can be used as an amino acid source in the food industry and as a source of polyaspartic acid in the raw material industry.
The accumulation of cyanophycin is triggerd by high cellular arginin levels, which depends on the interaction between the P_{II} protein and the N-acetylglutamate kinase (NAGK). P_{II} activates NAGK under nitrogen-excess conditions, which leads to increased arginine biosynthesis [1]. A signal transduction variant of P_{II} (I86N) leads to a permanent activation of NAGK [2].
Objectives: In this study we explored the possibility of metabolic pathway engineering *Synechocystis sp.* PCC 6803 using the P_{II} (I86N) variant as a test case. Due to overactivation of NAGK, we expected significant effects on arginin levels and cyanophycin accumulation.
Methodes: To characterize cyanophycin accumulation and the optimal production conditions, we used cyanophycin quantification methods under several growth conditions. To get a deeper insight of the behavior of the cyanophycin production strain, microscopic and metabolomic studies were performed.
Results: The P_{II} (I86N) variant in *Synechocystis* strongly increases the arginin and cyanophycin amount in the cell. The arginin amount during exponential growth is 12 fold higher compared to the wildtyp. Under optimal production condition, the cells were able to accumulate up to 50% cyanophycin at the cell dry mass.
Conclusion: The P_{II} (I86N) variant of *Synechocystis* represents the most efficient cyanophycin production stain described so far. The P_{II} (I86N) variant of *Synechocystis* represents a low nutrient requiring option for biotechnological production of Cyanophycin.

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BTP27

Enhancing the detection rate of functional metagenome screens using an *E. coli* host strain carrying the *C. cellulolyticum rpoD* gene

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The functional detection of enzymes from metagenomes is in general limited since only few reliable screening procedures are available and detection frequencies are often poor. Employing RNA-seq for the analysis of the expression profile of 19 fosmid clones in the background of *E. coli* EPI300 suggested that transcription of metagenome-derived genes is a limiting factor in functional metagenome searches using *E. coli* as a general host strain. As a first step towards overcoming this limitation we have constructed an *E. coli* strain harboring the *Clostridium cellulolyticum rpoD* gene. The *C. cellulolyticum rpoD* gene was chromosomally integrated into the *bioF* gene of the *E. coli* EPI300 strain commonly used for the construction of large insert libraries and the resulted strain was designated UHH01. UHH01 was not impaired in growth or in transduction frequencies. Function-based screenings for amylase encoding clones in large libraries with approximately 10,000 clones suggested that the strain UHH01 was superior to the parent strain EPI300 for the detection of amylolytic activities. While UHH01 was superior for the isolation of amylase-encoding clones it showed only minimal improvement for the detection of lipase- and esterase-encoding clones. However, the combined use of EPI300 and UHH01 as screening hosts resulted in general in an increased detection frequency.

BTP28

Exploring the chemoecology of Planctomycetes – a putative source for novel antibiotic compounds

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Planctomycetes are environmentally important ubiquitous bacteria, which comprise a complex cell biology and divide FtsZ independent through budding. They attach to surfaces and form biofilms. Some species only divide once attached and their unique division mechanism is associated with slow growth. Despite this putative disadvantage we found Planctomycetes to be associated with phototrophic organisms, where they can account for up to 50% of the biofilm forming bacterial population. Consequently, an important role in carbon remineralization after phototrophic blooms was suggested for Planctomycetes. Employing high throughput phenotypic MicroArrays we determined Planctomycetes utilize compounds of phototrophic origin such as algal polysaccharides. We suspect that those compounds serve as trigger signals for the secretion of small molecules from Planctomycetes. This is in good accordance with our recent discovery of many secondary metabolite related gene clusters among the already sequenced planctomycetal genomes. Since Planctomycetes differ in so many ways from other bacteria, it is interesting to investigate whether secondary metabolites from this phylum are different from those produced by the "usual suspects" such as *Streptomycetes* or Myxobacteria. We describe the development of tools to screen Planctomycetes for the production of antimicrobial compounds, involving chemical and biological methods. In order to investigate the chemoecology of Planctomycetes, we follow en ecomimetic approach. We developed a chemically defined maintain medium, allowing Planctomycetes only to survive. Adding carbon sources derived from phototrophs, we could induce alterations in the production of secondary metabolites as detected by HPLC. To further characterize such metabolites we developed a protocol for planctomycetal cultivation in computer - controlled bioreactors. In addition, we constructed genetic tools to allow heterologous expression of identified secondary metabolite gene clusters in our model organism *Planctomyces limnophilus*. As proof of principle, we demonstrate antimicrobial activities of selected planctomycetal extracts. Our findings might have implications on future drug development since novel antibiotics are urgently required.

BTP29

Generation of Catabolite-Repression negative *Clostridium acetobutylicum* strains using continuous cultures

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Introduction: The Gram-positive, obligate anaerobic bacterium *C. acetobutylicum* is widely used for the production of acetone, butanol and ethanol from renewable resources. In order to circumvent competition of such fermentations with food production, substrates containing a large share of pentoses and hexoses, such as media produced from lignocellulosic material come into focus.
Objectives: Catabolite repression by glucose prevents usage of pentoses[1]. While catabolite repression is well studied in the Gram-positive model organism *B. subtilis,* very little is known about catabolite repression in Clostridia. Therefore, generation of new strains circumventing catabolite repression is required.
Methods: Continuous cultivation of *C. acetobutylicum* ATCC 824 in a chemostat on phosphate-limited medium[2] with a minimum concentration of glucose and excess xylose was used to create enough selective pressure on the organism to select for mutations that enable high xylose consumption in the presence of glucose. Transcriptional analysis and sequencing approaches were used to characterise those mutants.
Results: We identified two operons of *C. acetobutylicum* ATCC 824 that are induced during growth on xylose, glucose mixtures and characterized mutants in those operons. Furthermore, we isolated mutants that are able to ferment xylose in the presence of glucose by enrichment in a carbon-limited chemostat. We characterized those mutants towards their substrate usage, solvent formation and the locus of the mutations.
Conclusion: Mutagenesis by creating selective pressure in a continuous culture could be a promising approach for creating new strains of *C. acetobutylicum* without catabolite repression.

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BTP30

Characterization of novel thermostable α-galactosidases

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Introduction: The oligosaccharides raffinose and stachyose are composed of one or two terminal galactose residues linked with sucrose. These α-galactosides function as storage polysaccharides in many plants, especially legumes and grains. α-Galactosidases catalyze the removal of the galactose moieties. Due to the lack of α-galactosidases in the human digestive tract, these carbohydrates are fermented by the gut microbiome that leads to gas formation. In order to improve digestibility α-galactosidases are used for food treatment. Additionally, α-galactosidases are applied in the sugar industry to improve the crystallization efficiency in sugar beet molasses by raffinose elimination. Especially thermostable α-galactosidases are of great interest due to their applicability in industrial high-temperature processes.
Results: A metagenome from an environmental sample derived from a hot spring (Azores, Portugal) was sequenced and screened for the presence of α-galactosidase-encoding genes. One gene coding for a putative α-galactosidase (*agal1*) was identified. Another gene (*agal2*) was discovered by screening available whole-genome sequences. According to structural motifs, Agal1 and Agal2 were classified as bacterial family 36 glycoside hydrolases. Both enzymes were produced recombinantly in *E. coli*. Agal1 and Agal2 were active towards *p*NP-α-galactopyranoside and raffinose. High activities were observed at pH 6.0-7.0 at 80 °C for Agal1 and 65 °C for Agal2.
Conclusion: The degradation of raffinose at high temperatures by Agal1 and Agal2 demonstrates a high potential of α-galactosidases for the application in industrial processes that run at elevated temperatures.

BTP31
Purification and characterization of novel membrane bound dehydrogenases from the metagenome of Acetic Acid Bacteria
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Introduction: Various genera from the family of Acetobacteraceae are well known for their ability to perform rapid, regio- and stereo-selective incomplete oxidations. Because of this characteristic, acetic acid bacteria are used in several biotechnological processes.
Objectives: Many of these incomplete oxidations are difficult or even impossible to accomplish using organic chemistry. On the other hand many strains of acetic acid bacteria cannot be cultivated in the laboratory. Therefore, there is a large biotechnological interest in the development of strains that can be used as specific oxidative biocatalysts.
Materials and Methods: We used a metagenomic approach to identify new membrane-bound dehydrogenases of potential value for biotechnology. The expression took place in specially designed *Gluconobacter oxydans* strains, in which all existing membrane bound dehydrogenases were deleted using a markerless deletion system [1]. In order to characterize the activity and substrate specificity of those metagenomic membrane bound dehydrogenases we utilize a high throughput in vivo assay [2]. In a second step we purify interesting unknown dehydrogenases for detailed characterization.
Results: An uncharacterized membrane bound dehydrogenase showing a hitherto unknown substrate spectrum was identified, purified and characterized.
Conclusion: With the described approach it is possible to screen, express, purify and characterize new metagenomic membrane bound dehydrogenases from habitats rich in acetic acid bacteria.

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BTP32
Cultivation of the basidiomycete *Agrocybe aegerita* on different residues of the food industry and their impact on mushroom yield and aroma profile
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Due to their pleasant flavour and high nutritional value, mushrooms are valued food all over the world since thousands of years. Surprisingly, the secondary metabolism of volatiles of these mushrooms, and especially its induction by different growth substrates has been only marginally investigated so far. According to their aroma profile, three classes of edible mushrooms are distinguished: fungi rich in carbon-eight flavours, such as oct-1-en-3-ol and oct-1-en-3-one, fungi containing high concentrations of terpenoid volatiles, and fungi mainly containing sulphurous odours [1]. Although the quality of mushrooms depends essentially on the aroma of the fruiting bodies, traditional strategies in strain and substrate development have been focused on production yields only [2]. In the present study, we intended to improve the aroma profile and the fruiting body yield of the mushroom *Agrocybe aegerita*. *A. aegerita* was grown on wheat straw which was supplemented with different residues of the food industry. The effect of the supplements on growth and yields, but particularly on the volatile secondary compounds formed by the fruiting bodies of *A. aegerita* was determined. Therefore, the secondary metabolites of the fruiting bodies of *A. aegerita* were extracted, and analysed by GC-MS/MS-O. Key aroma compounds were identified by means of aroma extract dilution analysis. The fruiting body yield was increased by supplementation with black tea pomace, whereas cocoa shells led to an altered growth of the fungus.

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BTP33
Production and characterization of novel cold-active α-amylases
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Introduction: α-Amylases catalyze the hydrolysis of α-1,4-glycosidic linkages in starch resulting in oligosaccharides. These enzymes account for a significant part of the overall enzyme market and find applications for example in the detergent, food, paper and textile industry. Due to energy limitation and climate change the development of environmentally friendly processes is in focus. Cold active α-amylases can be used in laundry and dish-washing detergents and therefore enable lower washing temperatures. Furthermore, they are promising supplements for food processing because they can be inactivated at elevated temperatures. Further possible applications for cold active α-amylases are waste water treatment and environmental bioremediation in cold environments.
Objective: Aiming at the characterization of new cold-active α-amylases whole genome sequences were screened for appropriate genes. Identified genes were cloned and the recombinant proteins were characterized.
Results: One α-amylase encoding gene was identified from *Pseudoalteromonas arctica* (*amypa*), a second one from *Vibrio fisheri* (*amyvf*). Both genes were successfully cloned and expressed in *E. coli* C43 and the recombinant proteins were purified. Amypa displayed highest activities in the range of 25-40 °C at pH 7.0 and Amyvf was optimally active at 15-25 °C and pH 7.5. Both enzymes were activated in presence of bivalent cations such as Ca^{2+}, Mg^{2+}, Mn^{2+} and Sr^{2+} and several reagents like DTT, urea and β-mercaptoethanol. The α-amylases were inhibited by $Cu^{2+}$and Zn^{2+}. Kinetic and physiochemical properties of the recombinant enzymes including pH- and temperature-stabilities, as well as the influence of detergents will be presented.
Conclusion: Due to the low reaction temperatures both α-amylases show high potential for application in food processing or bioremediation in cold environments.

BTP34
Identification and characterization of a thermophilic multidomain cellulase from *Thermococcus spp.*
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Introduction: Biomass hydrolyzing extremophilic enzymes, particularly glycoside hydrolases like cellulases cleaving β-1,4-glycosidic bonds, are advantageous for industrial production of fermentable sugars from lignocellulosic biomass. In course of the EU funded Hotzyme project aiming at the identification of novel hydrolases, a 3912 bp gene of thermococcal origin encoding an unusual five domain protein (1303 amino acids, 143 kDa) has been identified from a metagenomic sample. It consists of three glycoside hydrolase (GH) domains and two carbohydrate binding modules (CBM) (domain order GH5-GH12-GH12-CBM-CBM, N- to C-terminal direction). Although the single domains of this putative multidomain cellulase (MDC) showed significant similarities to data base entries (e.g. GH5 79% identical to characterized *Pyrococcus horikoshii* endocellulase) no homologs with similar domain architecture could be identified.
Objectives: The MDC should be recombinantly produced and enzymatically characterized along with the functional analyses of the single domains.
Materials & Methods: The full length *mdc* gene was cloned and expressed. The His-tagged MDC was partially purified and characterized using carboxymethylcellulose (CMC), dinitrosalicylic acid and azurin-crosslinked substrates. To elucidate the function associated with the single domains the full length gene was truncated domainwise from the C-terminus and the resulting proteins were purified and analyzed for activity.
Results: Only a small portion of the full length MDC was obtained in the soluble fraction. The partially purified MDC was characterized as β-endoglucanase with higher activity for β-glucan (1.7 U m^{-1}) than for CMC (1.2 U m^{-1}). Using zymogram gels the activity could be directly linked to a 143 kDa protein. The temperature optimum was between 60°C and 70°C and the pH optimum in the alkaline range (pH 7-9). The truncated versions were also recombinantly produced. The proteins showed increased solubility with decreasing protein size and for all of them cellulolytic activity could be shown.
Conclusion: The unusual MDC with novel 5 domain architecture was characterized as β-Endoglucanase. The cellulolytic activity appears to be associated with the N-terminal GH5 domain whereas the C-terminal domains were shown to be dispensable for CMC cleavage.

BTP35

Testing of Model Systems for Growth and Disinfection of Bacterial Biofilms

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Introduction: Disinfectants are commonly tested on planktonic bacteria. However, most bacteria in the environment live in slime-enchased, surface adhered microbial communities called biofilms. Biofilms were found to have a higher tolerance against antimicrobials like antibiotics and disinfectants compared to their planktonic counterparts. Hence, they represent a permanent challenge for standard disinfection protocols in hygiene-sensitive areas like hospitals. Especially the recent outbreaks of recalcitrant biofilm-forming strains have underlined the necessity to re-evaluate existing disinfection protocols. The assessment of disinfectants and disinfectant protocols for the Federal Republic of Germany resides within the duties of the Robert Koch Institute, which is obliged to update its methods periodically to meet the current state of knowledge.
Objectives: The goal of this study was to establish methods at the Robert Koch Institute for the standardized testing of disinfectants on bacterial biofilms.
Methods: Standard reference strains as well as strains that were involved in clinical outbreaks were cultivated in the so-called CDC-biofilm reactor and/or in a special 96-well biofilm test plate to form biofilms. All biofilms were treated with varying concentrations of glutaraldehyde. The disinfectant was neutralized and the biofilm removed from the surface. Finally, the amount of remaining vital bacteria was established by determination of colony forming units.
Results: Both cultivation methods allowed to produce biofilm and to measure the efficacy of disinfectants by using the same procedures as employed for testing planktonic cells. Pros and cons of the individual methods will be discussed.
Conclusion: Standardized testing of disinfectant efficacy on biofilm is possible by using the CDC reactor or the particular 96-well test plate system.

BTP36

Providing α-ketoglutarate from pentose *in vitro* – one milestone for hydroxyamino acids production *in vivo*

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Introduction: Hydroxyamino acids (HAs) are important precursors for the pharmaceutical and other industrial applications. However, so far HAs are mainly extracted from natural raw materials in complex and cost intensive processes which hampers the broader application of these compounds. A sustainable alternative is the enzymatic production of HAs via dioxygenases converting amino acids by molecular oxygen, using α-ketoglutarate (αKG) as electron donor.
The thermophilic crenarchaeon *Sulfolobus solfataricus* [1] and mesophilic bacteria *Caulobacter crescentus* [2] have been demonstrated to degrade pentoses, 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: Genes encoding the enzymes for pentose conversion to αKG from *S. solfataricus* and *C. crescentus* were cloned and recombinantly expressed. 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 pentoses (D-xylose and L-arabinose) was analyzed.
Results: Both enzyme cascades from *S. solfataricus* and *C. crescentus*, respectively, were successfully reconstituted from the recombinant enzymes indicated by the αKG production from pentoses.
Conclusion: αKG could successfully be produced by enzyme cascades derived from *S. solfataricus* and *C. crescentus*. Optimization of reaction conditions, combination with the dioxygenases and analyses of HA production are currently undergoing.

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BTP37

Laccase-mediated elimination of water micropollutants by cleavage or polymerization reactions

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Introduction: Pharmaceutical residues are repeatedly detected in the aquatic environment [1]. Among the most frequently found compounds are diclofenac (DCF) or bisphenol A (BPA). Pharmaceuticals are insufficiently degraded in municipal sewage treatment plants (STPs). Thus, only 17-69% of DCF was removed in STPs and was also found in groundwater [2]. The compounds are recalcitrant and may cause toxic effects such as induced feminization of aquatic organisms [3].
Objectives: To reduce the concentration of micropollutants two reaction possibilities are conceivable. The first one is their degradation and the second involves their binding and immobilization on other compounds [4]. The enzyme laccase [E.C. 1.10.3.2] was chosen to catalyze both options. Laccases need atmospheric oxygen as its only co-substrate and possess a broad substrate spectrum (>100 compounds). The pharmaceuticals are oxidized by laccase to reactive radicals that undergo cleavage and/or coupling/binding reactions.
We used laccase for the elimination of micropollutants as a prerequisite for the development of a bioreactor.
Methods: The laccase C of *Trametes* spec. was used for degradation of micropollutants. The removal of substrates as well as product formation was determined by HPLC analyses. The structural characterization of products was performed by GC/MS and LC/MS.
Results: The laccase-mediated reactions resulted in the elimination of pollutants such as BPA, antibiotics, β-estradiol or chlorinated compounds (DCF). Combinations of the substances were tested. The reactions resulted in cleavage or polymerization products. The binding of substances in polymers is particularly useful for substances such as sulfamethoxazole, which are not oxidizable by laccase.
Conclusion: The dual reaction possibility of laccase allows the cleavage of pollutants to less toxic low molecular weight products or to their polymerization which thereby excludes further reaction with the biotic environment. The results of this study will be used for the development of an enzyme-based bioreactor designated as additional treatment stage in STPs.

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BTP38

Metagenomics of microbial communities in agricultural production scale biogas fermenters

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Microbial communities in agricultural biogas fermenters are optimized for the anaerobic digestion of plant biomass to methane. A prerequisite for this conversion is the efficient primary degradation of highly recalcitrant lignocellulosic plant biomass to fermentable oligo- and monomers. Identifying crucial bacterial groups involved in this process and tapping into their genomic potential provides a basis for the discovery of potential novel and highly active hydrolytic enzymes. Here we report on the construction of a metagenomic fosmid library encompassing approximately 275 Mbp of genetic information derived from an agricultural biogas fermenter. Functional screenings of this library led to

the discovery of several feruloyl esterases, a group of accessory enzymes involved in the degradation of plant cell wall polysaccharides. Some of the identified feruloyl esterases exhibit only low sequence similarities to known enzymes and are currently characterized. In addition to the construction of the library, the extracted DNA was sequenced using Illumina HiSeq technology, resulting in 1.2 gigabases of assembled metagenomic DNA. This dataset allows sequence based analyses to verify and extend hits derived from functional screens. It further allows a comparison of this artificial habitat with natural occurring systems (e.g. animal rumen or intestines) with regard to their hydrolytic capabilities. Moreover, a phylogenetic analysis of the microbial community based on 16S rRNA gene amplicons was conducted. This analysis suggests a highly diverse community (>1150 OTUs) in the analyzed fermenter. About 70% of the bacteria are affiliated with the phyla of *Firmicutes* and *Bacteriodetes* while the majority of archaea belong to the genus *Methanoculleus* followed by *Methanosarcina* and *Methanomassiliicoccus*.

BTP39
Improving an esterase-based assay for studying molecular biology in extremophiles
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Quantification is of key importance for understanding molecular processes in biological systems, e.g. analyzing gene expression profiles during different stages of bacterial growth or establishing heterologous biosynthetic pathways. The standard reporter protein GFP however has substantial shortcomings when used in extremophile hosts which grow at elevated temperatures or under oxygen limitation. By utilizing an esterase from a thermophilic *Bacillus* we were able to establish an *in vivo* reporter which circumvents these issues. Furthermore, it offers the advantage of signal amplification due to its enzymatic activity, which results in a low limit of detection. In order to further improve the signal-to-noise ratio different substrates were tested including derivatives of coumarin and fluorescein. The resulting fluorescence was quantified in bulk using a multiwell plate reader and on the individual cell level using fluorescence microscopy. Since esterases display a distinct substrate preference in terms of substrate size, especially the chain length of the acyl modification on the fluorogenic substrate was optimized. The esterase was also used as a fusion partner to monitor the synthesis of other heterologous proteins in the extremophile *Geobacillus thermoglucosidasius*.
The esterase assay and its optimization presented here are at the foundation of any research that wants to understand and engineer extremophiles, where GFP and variants have limited use. It is essential in order to establish them as 'chassis' for synthetic biology and biotechnology. Using the assay, different processes on the molecular level and inside the cell can now be monitored and quantified in these extremophile host systems. In general the proposed reporter protein will enable the molecular analysis of fundamental biological questions regarding their lifestyle and open the way to utilizing these organisms as whole cell catalysts (Examples will be shown).

BTP40
Biogas plant performance boost by implementation of the MFC technology
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Simultaneous generation of combustible gas and electricity from anaerobic biomass digestion has been well known for a long time. Up to 8000 biogas power plants are operated in Germany nowadays [1]. Taken together with gaseous, solid and liquid biomass, electricity from biomass totaled more than 44 bn kWh [2]. This defines biomass besides wind power as the most important branch of the renewable energies.
Nevertheless due to the amendment to the "Erneuerbare-Energien-Gesetz" (EEG 2012) the construction of new biogas plants has decreased drastically [1]. In this manner it is necessary to increase biogas plant efficiency and thereby make it more attractive for the plant manufacturer. This can be achieved by identifying the limiting factors in the fermentation process and overpass them. These efficiency limitations are mainly caused by imbalances during the fermentation process. The indicators of a process imbalance can appear for instance as increasing of the hydrogen partial pressure and the acidification of the medium due to accumulation of volatile fatty acids (VFA) resulting in the inhibition of acetogenesis and methanogenesis. In order to increase the stability and efficiency of the microbial processes in biogas plants two new approaches were investigated in this study. Firstly the process imbalances are monitored via a microbial fuel cell (MFC)-based biosensor by detecting VFA, which are accumulated during interferences. Secondly we want to control the hydrogen partial pressure and the VFA concentrations using the principle of MFC. For this purpose an anode will be installed directly into the biogas fermenter. The exoelectrogenic consortium on the anode is supposed to minimize the VFA concentrations by electrogenesis and to support the acetogenesis by electrosyntrophy. By applying different potentials the activity of these organisms could be regulated. Exoelectrogens and methanogens are examined during these processes to check their stability via barcodes using quantitative PCR (qPCR) and fluorescence *in situ* hybridization (FISH).

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[2]BMUB: Entwicklung der erneuerbaren Energien in Deutschland im Jahr 2012

BTP41
Creation of Mutations in Cellulose Metabolism of *Gluconacetobacter hansenii*
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Introduction: *G. hansenii* is a model system for cellulose biosynthesis and produces extracellular cellulose. This organism is an excellent source for pure cellulose production that is devoid of lignin and hemicellulose. This kind of cellulose, also called bacterial cellulose (BC), has many unique properties e.g. high purity and high crystallinity. These properties of BC allow special applications in the biomedical field, as reinforcing agent for paper or a food additives [1, 2].
Objectives: The physiological effects of mutations in cellulose metabolism or rather production of cellulose of *G. hansenii* are investigated in order to detailed analyse the molecular biology of cellulose production.
Methods: In this study mutations were generated by gene deletion via a *codAB*-based markerless deletion system developed by our group as described by Kostner *et al.* (2013) [3]. The obtained mutant strains were verified via PCR and Southern Blot. The mutant phenotype and BC production respectively were analysed visually on solid medium plates and in Fernbach flasks.
Results: All genes of the main cellulose synthesis operon (*acsABCD, CMCase, ccp, bgl*) were separately deleted also the identified genes for a 2nd and a 3nd cellulose synthase (*acsAB*2, *acsAB*3). Furthermore two of five genes for diguanylate cyclases, which could be involved in the regulation of cellulose production were deleted as well. Every deletion resulted in a different consequence for cellulose or rather biofilm production as compared to the wild type. The mutants were no longer able to produce a tightly arranged cellulose biofilm. However, some kind of biofilm formation was observed for each mutant. These were more fluffily, thin or otherwise different as compared to the biofilm of the wild type strain.
Conclusion: The first results of this study revealed that three operons for cellulose metabolism seem to be important for the production of a tightly arranged cellulose biofilm. Any single mutation or deletion in those operons lead to an inefficient cellulose production. This shows the need for further studies on cellulose metabolism in *G. hansenii*.

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BTP42
A concept for enzyme - based detection of acetate in biogas processes
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Introduction: In order to ensure optimum conditions for the formation of methane from biomass, biogas plants need to be constantly monitored. The concentration of acetate and other volatile fatty acids is an excellent indicator to capture the current state of the system, since they are accumulated in cases of process disturbances[1]. Due to the fact that these process parameters can only be monitored by time-consuming offline analysis up to now, a promptly intervention in imbalanced biogas reactors is often not possible.

Objectives: The aim of the project is to develop an enzyme-based amperometric sensor chip for rapid on-site monitoring of several key parameters in the biogas process, including organic acids and volatile fatty acids. We present the principle structure of a biosensor for the detection of acetate, using a new method of measurement.
Materials and Methods: Three different enzymes from *E. coli* and *B. subtilis* were combined for the development of the enzymatic assay.
Results: For the quantitative determination of acetate a new enzymatic assay is established, using an acetyl-CoA synthetase, coupled with a malate synthase and malate dehydrogenase. Subsequently an immobilized diaphorase is used to recycle NADH in the presence of the redox mediator hexacyanoferrate(III), resulting in the formation of hexacyanoferrate(II). The amperometric detection of acetate is then realized by the oxidation of hexacyanoferrate(II).
Conclusion:
The represented concept for an enzym-based amperometric acetate sensor chip might be a cost-effective alternative for established acetate measurement methods, which allows an early detection and elimination of potential process imbalances. The use of the diaphorase also provides the possibility to analyze other crucial parameters such as lactate and formiate, each in combination with a corresponding NAD^+ - dependent dehydrogenase. Based on this principle, the construction of a multi-parameter sensor chip is planned, to offer a complex and rapid analysis of biogas samples in the future.

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BTP43
Identification of cultivated ink caps (hed-muek) in Thailand as *Coprinopsis cinerea* (hed-cone-noi)
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Introduction: About 20 different edible mushroom species are cultivated in Thailand with local market prizes between 20-350 Thai Baht (0.6-9.1 US $) per kg fresh weight, in addition to some higher-priced medicinal mushrooms. Amongst the edible mushrooms are inky caps (hed-muek) which sale with prizes of 120-160 Baht.
Objective: Different ink cap species are claimed to be presented under the mushrooms cultivated in small farms in Thailand such as *Coprinus comatus*, *Coprinus atramentarius* (new name *Coprinellus atramentarius*) and *Coprinus fimentarius* (new name *Coprinopsis cinerea*). Some names given in the literature are clearly species misidentifications. Coprenoid species are generally difficult to distinguish purely by mushroom morphology and are thus commonly easily be mixed up. Molecular data should be used in addition for reliable species identification beyond doubt.
Methods: Spawn for the mushrooms were bought on two occasions from local Thai markets and fungal mycelium was isolated. Isolates were cultivated on different substrates (artificial medium, straw, horse dung) and development of mycelium and fruiting bodies was observed. DNA was isolated from mycelium for ITS-sequencing.
Results: As to be expected for mushroom production, cultures had clamps and were dikaryons. Fruiting bodies were induced on artificial medium as well as horsedung. The mushrooms had the typical morphology of *C. cinerea* (hed-con-noi) fruiting bodies. ITS sequences confirmed the species identification. A sporeless mutant was coincidently obtained from one commercial strain from mated basidiospores that may be favorable for mushroom cultivation.
Conclusion: *C. cinerea* has been undoubtedly identified as species used in Thailand for mushroom cultivation. The fungus easily fruits on different plant wastes and dungs at warm temperatures (25-28°C). A sporeless white mushroom might have advantages for mushroom production by possible longer shelf-life and avoidance of induction of any spore allergies.

BTP44
Community characterizations of *Bdellovibrio*-and-Like-Organisms (BALOs) in aerated and non-aerated constructed wetlands treating domestic wastewater
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Constructed wetlands (CWs) are engineered near-natural wastewater treatment systems that have been shown to achieve several log unit removal of fecal indicators, *i.e*. surrogates for pathogenic microbes, from domestic wastewater. While micro-ecological interactions such as predation are of likely significant importance for pathogen elimination in CW systems, the actual removal pathways are still obscure. As a first step towards a better understanding of the fate of pathogens in CWs, we characterized and quantified the populations of bacterial predators, *Bdellovibrio*-and-Like-Organisms (BALOs), in two reference constructed wetland types over the course of four seasons. The first system was a conventional horizontal subsurface flow CW and the second one a horizontal subsurface flow CW with an integrated aeration system. Both CWs were planted with *Phragmites australis* and received the same primary settled domestic wastewater. BALOs communities were characterized in samples from the influent, discrete points along the flow path, and the effluent of the two wetlands by DGGE combined with DNA sequencing. We targeted the four groups, *Bdellovibrionaceae*, *Bacteriovoraceae*, *Peredibacter*, and *Micavibrio*. Furthermore, *Bdellovibrionaceae* were quantified by real-time PCR and compared with total 16S rRNA gene and faecal indicator abundance, as well as with conventional physicochemical wastewater quality parameters. The aerated CW, which was shown to provide significantly better fecal indicator inactivation, had substantially higher abundances of *Bdellovibrionaceae* in all seasons. The DGGE-based survey revealed a shift in the community composition of BALOs in the two CWs that may have been also a direct result of aeration. In conclusion, this first investigation of predatorial communities in CWs highlights the effect of design and operational parameters for sanitation of domestic wastewater via these ecological treatment systems.

BTP45
Chemical and enzymatic decomposition of wood fibers for upgrading of lignocellulose into value-added biochemicals
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Introduction: Bioethanol is currently produced by fermentation of sugars derived from annual crops. Usage of wood lignocellulose for bioethanol production appears to be energetically more favorable, with positive ecological and socio-economic aspects. Important steps in such processes are suitable pretreatment technologies to unlock cellulose from the lignocellulose matrix, hydrolysis of cellulose into glucose and fermentation of the sugars into ethanol.
Objectives: We applied a moderate temperature acetosolv process at ambient pressure to produce fibers suitable for highly efficient enzymatic hydrolysis of cellulose to glucose without any inhibition effects in further fermentation.
Methods: Poplar, willow, beech and spruce wood and wheat-straw were subjected to an acetosolv pretreatment. The produced fibers were mildly washed with NaOH before enzymatic hydrolysis for transforming the cellulose into fermentable glucose.
Results: Optimal acetosolv pretreatment parameters (115°C/60 min/0.2% HCl) for wood were determined that preserved nearly all cellulose (~ 90%) at highest purity (~ 93%) in the solids except for spruce which failed in the pretreatment. In the poplar fibers, the process reduced the lignin content from 23-25% to about 5%. Cellulose in the fibers was to 33% acetylated by the HCl-catalyzed acetic acid treatment. Full cellulose to glucose conversion by enzymatic hydrolysis was possible after mild NaOH-washing (50°C/120 min) of the pretreated fibers. NaOH-washing deacetylated the cellulose, eliminated lignin re-depositions from the fibers, and further increased the cellulose purity. Lignin as valuable co-product was found in the spent-liquor easy to precipitate in water. Moreover, furfural in the spent-liquor can be recovered as further high value co-product.

Conclusion: An efficient laboratory acetosolv pretreatment run at low temperature and ambient pressure is presented that highly enriches clean cellulose fibers from wood in nearly total original amounts of the cellulose. After mild alkaline washing, the cellulose is easily converted by hydrolytic enzymes into glucose. The crude glucose is free of any inhibitors and can be used in fermentations.

BTP46

Development of new biocatalysts and process optimization for the production of long-chain α,ω-dicarboxylic acids

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Introduction: α,ω-dicarboxylic acids (DCA) are versatile chemical intermediates of different chain length used as raw materials for the preparation of parfums, polymers, or adhesives. The majority of industrial short chained DCA production is based on chemical conversion from petrochemical raw materials. However, chemical synthesis of long-chain DCA (>13 C atoms) is challenging and expensive. An alternative to chemical synthesis is the biotechnological production of DCA from renewable resources. Some microorganisms, like yeasts of the genus *Candida*, are able to oxidize long-chain, unsaturated alkanes or saturated and unsaturated fatty acids at the terminus and convert them selectively to α,ω-dicarboxylic acids.
Objectives: Our aim is the process optimization for the production of long-chain DCA with *Candida tropicalis* and the development of new biocatalysts via metabolic engineering.
Methods: We investigated growth and production parameters for the bio-conversion of fatty acids into diacids with *C. tropicalis*. In parallel, we screened for alternative microbial biocatalysts and have started with targeted metabolic engineering.
Results: We could improve the fermentative production process of 1,18-octadecenoic diacid with *C. tropicalis*. In addition we demonstrated the conversion of several renewable resources (fatty acids or fatty acid methylesters) into diacids. Also, we identified *Pichia guilliermondii* as potential new DCA-producer.
Conclusion: *C. tropicalis* is an optimized biocatalyst for DCA production with a broad substrate spectrum and high production yields. However, industrial usage of *C. tropicalis* is hindered because of its BSL-2 status in Europe. New BSL-1 biocatalysts like *P. guilliermondii* have to be genetically improved via metabolic engineering.

BTP47

Bisphenol A and its analogues as environmental pollutants with estrogenic activity – Novel mechanisms of microbial biotransformation, detoxification and reduction of estrogenicity

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Introduction: Bisphenol A (BPA) is a high production volume chemical used for manufacturing polycarbonate plastics and epoxy resins and enters the environment with input quantities of more than one million pounds per year [1]. Thus, bisphenols pervade almost every sphere of life and can be found in most consumer products and even as developer in thermal paper used for receipts and as dental sealant. Migration from food beverage containers and inhalation of bisphenol-containing dust contributes to daily human exposure as well. Furthermore, BPA and analogues are endocrine disruptors and interfere with the hormonal system. But little is known about the environmental fate. Additionally, there is a broad range of different bisphenol analogues used in industry often lacking information on environmental degradability and toxic properties.
Objectives: We investigated the transformation of BPA and four analogues BPC, BPE, BPF und BPZ by the environmental bacterial strain *Cupriavidus basilensis* and compared toxicity and estrogenicity of the bisphenols and the transformation products formed.
Methods: Analysis comprised high performance liquid chromatography (HPLC), liquid chromatography /mass spectrometry (LC/MS) and nuclear magnetic resonance spectroscopy (NMR) for structure elucidation of the products formed as well as a yeast-estrogen-screen-assay.
Results: The initial oxidation step is the hydroxylation of BPA in *ortho*-position to the hydroxy group. This dihydroxylated intermediate is substrate for ring fission resulting in a lactone with pyrane structure. Furthermore, one hydroxy group could be replaced by an acetamide group. The arrangement of functional groups on the parent bisphenol is decisively for its degradability: substituents in *ortho*-position to the hydroxy group inhibited ring cleavage but offered new targets for a microbial attack.
Microbial biotransformation led to a detoxification and reduction of estrogenicity of all bisphenols tested.
Conclusion: Naturally occurring microorganisms are able to transform bisphenols either by using them as growth substrate [2] or by oxidation resulting in products with less toxicity and estrogenicity.

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BTP48

Improving a squalene-hopene cyclase for the conversion of non-natural terpenoid substrates

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Introduction: Bacterial squalene-hopene cyclases (SHCs) catalyze in nature the cyclization of squalene to hopene and hopanol. This Brønsted acid-driven polycyclization is initiated by a conserved aspartic acid rich network that delivers the proton for substrate activation. During the subsequent reaction cascade, several carbocationic species are generated and guided by bordering active site amino acids through the reaction mechanism, giving in one step a pentacyclic ring-system with several new stereo-centers. To accomplish that the enzyme's active site cavity serves as the ideal template to force the substrate into its reactive conformation including the stabilization of emerging cationic intermediates during the course of reaction.
Objectives: Our major objective is the investigation of novel proton-initiated chemistry. Therefore, through engineering, the catalytic reactivity of the SHC from *Alicyclobacillus acidocaldarius* (*Aac*SHC) should be extended towards new biocatalytic conversions, which are not yet addressable with SHCs.
Methods: In previous biotransformations applying SHCs, a range of non-natural substrates with different chain lengths as well as functional groups could already be converted into the corresponding cyclic terpenoid compounds, which often display valuable substances or building blocks for further reactions. To exploit the scope of SHC-accomplished reactions and thereby gain access to completely new enzymatic proton-initiated catalysis, we make use of a rationally designed *Aac*SHC mutant library possessing diverse spacially reshaped active sites [1].
Results: This strategy already delivered several enzyme mutants that enabled the productive binding and cyclization of non-natural substrates and also allowed new insights about how such modifications can influence *Aac*SHC's (stereo)selectivity.
Conclusion: To further expand the reactive diversity of *Aac*SHC, upcoming investigations aim at the improvement of this cyclase through engineering for novel Brønsted-acid catalysis e.g., in enzymatic pathways or additional monocyclizations.

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BTP49

Resolving single steps of InhA catalysis: Identifying the Achilles heel of *Mycobacterium tuberculosis*.

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Introduction: The enoyl-ACP reductase from *Mycobacterium tuberculosis*, InhA, catalyzes the NADH dependent reduction of enoyl-ACP intermediates in fatty acid biosynthesis. The enzyme is important for the biosynthesis of mycolic acid which is thought to be essential for *M. tuberculosis* pathogenicity. As such it has become the target of many clinically relevant drugs *e.g.* Isoniazid and Triclosan. Despite the numerous efforts to study the mechanism of InhA its mechanism is still not fully resolved[1].
Objectives: To study the mechanistic details of InhA by combining kinetic isotope effects and UV-Vis spectroscopy to facilitate rational drug design for this enzyme.
Methods: We developed a novel method to quantify the solvent kinetic isotope effect in InhA WT and tyrosine 158 mutants. The method is based on performing the enzyme reaction at increasing D_2O concentrations and measuring deuterium incorporation by mass spectrometry. The obtained kinetic isotope effects reflect the intrinsic isotope effect and are not

masked by pre-equilibria and intermediates. Additional characterization of the enzymatic reaction were performed with UV-Vis and NMR spectroscopy in WT and mutants.
Results: The intrinsic kinetic isotope effects are significantly different from previously measured apparent kinetic isotope effects suggesting that tyrosine 158 in InhA is the active site acid, contrary to previous results. When tyrosine 158 is mutated to phenylalanine a novel catalytic intermediate is observed by UV-Vis spectroscopy indicating an enzyme mechanism similar to Etr1p, a mitochondrial enoyl-CoA reductase[2].
Conclusion: The mechanism of InhA proceeds through a catalytic intermediate that has gone unnoticed so far. This discovery provides the basis for the rational development of new antibiotic strategies to target InhA catalysis.

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BTP50

Preparative-scale enzymatic synthesis of D-sedoheptulose 7-phosphate and its cleavage with an engineered transaldolase B from *Escherichia coli*

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Introduction: Erythrose 4-phosphate (E4P) and sedoheptulose 7-phosphate (S7P) are metabolites of the pentose-phosphate pathway and essential for the biosynthesis of amino acids or LPS. Commercial availability of E4P and S7P, however, is not reliable. A preparative synthesis is therefore desirable. Traditional chemical synthesis of both sugar phosphates is still challenging due to the high demands in chirality.
Objectives: This work aims on the enzymatic synthesis of S7P and E4P in a g - scale preparative process.
Materials and methods: S7P was formed in a carboligation reaction (donor: β-hydroxy pyruvic acid; acceptor: ribose 5-phosphate) by the *E. coli* transketolase A (TKT). The synthesis was performed in a pH-stat apparatus in order to avoid the usage of buffer substances which might hamper product recovery. The progress of S7P formation was followed by enzymatic assays, HPLC, MS, and $^{13}C/^{1}H$ NMR. S7P was isolated as barium salt and converted to its sodium salt (IEX resin). S7P was then cleaved into E4P and dihydroxyacetone by the retro-aldol activity of an engineered *E. coli* transaldolase B (TalB) [1].
Results: In the TKT reaction, conversions of 100 % and yields of more than 80 %, were achieved. The product was validated to be S7P by its relative mass of 229.01 m/z (in perfect agreement with the theoretical m/z value). The product was a donor substrate for TalB and the NMR spectra were in good agreement with published data for S7P [2]. The cleavage of S7P was shown to form E4P (E4P-DH reaction). Currently, maximum E4P yields of 35 % and 18 % have been achieved at S7P concentrations of 5 mM and 50 mM, respectively.
Conclusion: A protocol for the enzymatic synthesis of S7P with TKT from *E. coli* was established, conforming the requirements for a preparative scale synthesis. In a further reaction, S7P could be cleaved to E4P. Limitations as the inherent instability of E4P need to be overcome.

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BTP51

Improving the regioselective terminal hydroxylation of fatty acids by engineering CYP153A chimera enzyme

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Oxygenated fatty acids have a great interest for industrial applications: in the area of high end polymers, in fine chemicals and in the cosmetic and fragrance industry. The regioselective hydroxylation of fatty acids, remaining chemically challenging, can be accomplished by cytochrome P450 enzymes. The bacterial monooxygenase CYP153A from *Marinobacter aquaeolei* fused to the reductase domain of $P450_{BM3}$ from *Bacillus megaterium* (termed CYP153A-CPR) is able to selectively hydroxylate medium chain-length fatty acids at the terminal position.[1,2]
To achieve efficient whole cell biotransformations and to increase the yield of bioconversion of fatty acids into the ω-hydroxy products, we have identified present limitations including low enzyme activity and stability. Our strategy to overcome existing bottlenecks is the generation of a focused library to screen and to characterize improved versions of the current chimera biocatalyst.
The small library involves mutations closed to the active site, at the entrance of the substrate channel or located at the interface between the heme domain and the substrate entrance tunnel. The screening of a large variety of mutants is performed by using a well-established assay dependent on the production of ω-hydroxy fatty acids.
We present the work on the generation, the identification, the screening and the characterization of improved mutants of CYP153A-CPR to catalyse the terminal hydroxylation of medium chain-length fatty acids.

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BTP52

Novel donor compounds for 1,2- and 1,4-addition reactions of the ThDP-dependent enzyme MenD from *Escherichia coli*

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Introduction: The ThDP-dependent enzyme MenD (SEPHCHC synthase) from *E. coli* performs a unique Stetter-like 1,4-addition reaction to an α,β-unsaturated carboxylic acid (isochorismate, 2,3-*trans*-CHD) [1,2]. Its physiological donor substrate is 2-ketoglutarate which is bound to ThDP and decarboxylated. MenD is also able to perform 1,2-addition reactions with 2-ketoglutarate, and a variety of aliphatic or benzylic aldehydes as acceptors to form stereospecific R-hydroxyketones [2,3,4]. This makes MenD a promising novel biocatalyst for carboligation reactions.
Objectives: We are studying the acceptor and donor substrate range of recombinant MenD from *E. coli* to extend the donor substrate range. Based on crystal structures of MenD from *E. coli* and *B. subtilis,* enzyme variants of *E. coli* MenD were created and assayed for their substrate ranges.
Methods: Overexpression of *menD* in *E. coli*; purification with IMAC; detection of carboligation products by uv-spectroscopy, GC and HPLC.
Results: Here we report that MenD is able to utilize various keto acids like pyruvate, 2-ketobutyrate, or 2-ketoisovalerate as donors. We studied utilization of these donors with 2,3-CHD and with 2-fluorobenzaldehyde as acceptor compounds. We established a cascade reaction starting synthesis of the diacid 2-oxo-4-hydroxyglutarate (KHG) either by a chemical reaction (product: D,L-KHG) or by an aldolase reaction (EDA from *E. coli*) of glyoxylate and pyruvate [5]. In the next step KHG served as a novel donor compound for carboligation catalyzed by MenD as shown by GC and HPLC.
Conclusion: We showed activity of MenD with novel donor compounds. This allows access to functionalized products for consecutive synthesis reactions.

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BTP53

Bentazon Transformation by Representative Soil-Derived Fungal Isolates of the *Ascomycota, Basidiomycota, and Zygomycota*

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Bentazon (3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide) is a widely used herbicide since the 70's. Bentazon is mobile and susceptible to leaching into groundwater, and consequently of environmental concern. Information on the fate of bentazon in soils are scarce and microorganisms associated with bentazon degradation in soils are essentially unknown. Therefore, microorganisms that are potentially linked to bentazon degradation in soil were enriched and isolated from agricultural soil of Scheyern. 25 bacterial and four fungal isolates were obtained after a total bentazon consumption of 0.65 mg. Bentazon degradation capabilities of four fungal isolates and three reference cultures

from a culture collection were tested under oxic conditions in the presence and absence of additional carbon sources. 0.01 % (w/v) bentazon did not affect growth. A pure culture of *Paecilomyces lilacinus* (*Trichocomaceae*, *Ascomycota*) and isolate AD13 [(closely related to *Mortierella elongata* (*Mortierellaceae*, *Zygomycota*)] transformed bentazon to 6-hydroxybentazon (6-OH-bentazon). The detected concentrations of 6-OH-bentazon after seven days of incubation were in the range of 6 % to 20 % of the initial applied bentazon. Transformation of bentazon mainly occurred in the presence of an additional carbon source, indicating a co-metabolic process. Cells of *Trametes versicolor*, a laccase- producing fungus affiliated to *Polyporaceae* (*Basidiomycota*) transformed 50 % of the initial bentazon. However, transformation products remained undetected by HPLC. Cell suspensions of the other fungi showed no decrease in bentazon concentration. Thus, 40% of tested fungi scored positive for bentazon transformation. Investigation of the bacterial bentazon degradation potential is still in progress, however preliminary tests suggest at least a tolerance towards an elevated bentazon concentration. The collective data indicate that bentazon transformation capabilities are (i) widespread among fungi, (ii) associated with laccase producing fungi, and (iii) co-metabolic.

CCP01
How to generate 3D images by light- and scanning electron microscopy using PICOLAY
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Focus stacking is a technique that allows for production of micrographs with enhanced sharpness and depth of field. It is based on the analysis of image stacks taken at sequential focus levels and does not require technical modifications of the microscope. A depth map generated during the stacking procedure allows for three-dimensional visualization of the specimen [(1)]. The method can often replace confocal scanning microscopy. However, it is usable with any microscopic technique, and is not restricted to fluorescence microscopy. Here we demonstrate application of the freeware stacking program PICOLAY (www.picolay.de) for light and scanning electron microscopy. We compared 3D images generated with a field emission scanning electron microscope (SEM) by focus stacking with those obtained using a tilted stage, and found the stacking method easier to apply and often giving better results. A very simple way of generating a 3D image from a single SEM picture is its use as a depth map by PICOLAY. The performance of the method will be demonstrated in a stereoscopic slide show for which anaglyph or polarizing glasses will be provided.

[(1)] Raap E, Cypionka H (2011) Vom Bilderstapel in die dritte Dimension: 3D-Mikroaufnahmen mit PICOLAY. Mikrokosmos 100:140-144

CCP02
Coming close to the edge: cellular organization of *Myxococcus xanthus* by bactofilins
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Introduction: Cytoskeletons are essential mediators of cellular organization in all three domains of life. One of their functions is to establish landmark structures that recruit target proteins to defined locations within the cell, thereby promoting the assembly of multi-protein complexes and compartmentalizing biological processes within the cell. A recently identified class of bacterial cytoskeletal proteins are the so-called bactofilins. They are widespread across various phylogenetic lineages and polymerize into higher-order structures without the need for nucleotide cofactors. Previous work has shown that bactofilins can act as localization factors for proteins involved cell wall biogenesis and motility, but overall, their biological functions are still poorly defined.
Objectives: This work aims to explore the roles of bactofilins in the delta-proteobacterium *Myxococcus xanthus*, a model organism commonly studied for its social behavior and gliding motility.
Methods: *M. xanthus* produces four bactofilin homologs, one of which (BacM) has previously been implicated in cell shape maintenance. In this study, we have applied a combination of genetic, cell biological and biochemical approaches to clarify the function of the other three homologs (BacNOP).
Results: Our results reveal a novel mechanism in which BacNOP filaments extend throughout the subpolar regions of the cell, thereby immobilizing the chromosomal replication origin regions at a defined distance from the cell poles. This activity involves interactions with the chromosome segregation proteins ParA and ParB, which associate with the pole-distal tips or the whole length of the filaments, respectively. The interaction with ParA is mediated by a newly identified protein, BadA, which is conserved among *Myxococcales*. Defects in the formation of this subpolar complex result in chromosome segregation defects, indicating that the proper arrangement of the DNA segregation machinery by BacNOP is critical for optimal fitness.
Conclusion: Combined with our previous finding that BacP also mediates the subpolar localization of a GTPase regulating gliding motility, these results indicate that in *M. xanthus* bactofilins form multi-purpose scaffolds that position functionally diverse proteins in the subpolar regions of the cell.

CCP03
Lipid diversity and heterogeneity
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Lipids are a poorly defined group of compounds essential for life that are divided into two fundamentally different groups; those based on isoprenoids (branched C5 units) or carboxylic acids (C2 units). Their common feature is that the side chains form a hydrophobic layer that separates the aqueous extracellular environment from the intercellular cytoplasm. Based on a limited number of "model" organisms the impression is often given that all organisms contain the same lipids or that the lipid diversity is limited. Nothing could be further from the truth and an appreciation of the diversity of lipids is inherently linked to understanding their role in the biology of the cell, elucidating the underlying enzymatic pathways, the mechanisms by which they are regulated as well as documenting the genes underlying these processes. The large diversity of membrane lipids present in prokaryotes also needs to be considered in the light of their interactions with one another as well as with other integral parts of the membrane, such as proteins. We know that the distribution of lipids in membranes is not homogeneous both across the membrane leaflets as well as within a leaflet, adding two additional degrees of difficulty to understanding the function of individual lipids in the system, which is further complicated by the significant differences in lipid components to be found in different prokaryotic evolutionary lineages.

CCP04
Localisation studies of archaeal chemoreceptors
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Introduction: Prokaryotes possess a remarkable system to sense environmental stimuli which is coupled to the motility apparatus directing their movement towards more favourable locations. Signal recognition is achieved by methyl-accepting chemotaxis proteins (MCPs or chemoreceptors) which are typically transmembrane proteins with a periplasmic ligand binding region and a C-terminal cytoplasmic signalling and adaptation domain.
Objectives: The subcellular localisation of MCPs should be analysed by immuno-labelling studies using recombinant antibodies generated against chemoreceptors of the Euryarchaeota *Methanocaldococcus villosus* and *Thermococcus kodakarensis*.
Methods: Annotated chemoreceptors of both organisms were searched for conserved/unconserved parts. Whole proteins or specific regions were expressed recombinantly and used for generation of polyclonal antibodies; the specificity of antibodies was determined by Western Blotting. Cells of *M. villosus* and *T. kodakarensis* were either high pressure frozen/freeze-substituted or chemically fixed and resin-embedded for ultrathin sectioning [1]. Sections were analysed by transmission electron microscopy with/without prior immuno-labelling.

Results: Both organisms possess five chemoreceptors, four classical transmembrane receptors and one cytoplasmatic receptor. Bioinformatic analyses of the former type showed that the C-terminal part is highly conserved. Antibodies generated against the C-terminus of an *M. villosus* chemoreceptor were found to label not only *M. villosus* MCPs but also those of *T. kodakarensis* in Western Blots whereas antibodies against N-terminal parts of MCPs were highly specific. Electron microscopic analyses of ultrathin sections identified a complex submembraneous structure at one cell pole which resembled bacterial chemoreceptor arrays. The suggested function of this complex was proven by immuno-labelling.
Conclusion: Analyses of *M. villosus* and *T. kodakarensis* suggest that archaeal transmembrane chemoreceptors are arranged in a polar complex as known for bacterial chemoreceptor arrays.

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CCP05

Listeria monocytogenes GpsB is a functional paralogue of the cell division protein DivIVA

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Introduction: DivIVA proteins are essential for cell division, growth or virulence of many Gram-positive bacteria. Firmicutes contain a DivIVA paralogue, named GpsB, which bears a shorter C-terminal part. GpsB is implicated in the coordination of cell wall biosynthesis in *Bacillus subtilis* and is essential for septal ring closure in *Streptococcus pneumoniae* (1, 2). While DivIVA of *L. monocytogenes* is essential for cell division and virulence (3, 4), listerial GpsB is uncharacterized.
Objective: The aim of this study was to elucidate the function of *L. monocytogenes* GpsB.
Methods: *L. monocytogenes* GpsB and its truncations were purified to analyze their oligomerization. Subcellular localization of GpsB was determined by fluorescence microscopy and cell fractionation. The *gpsB* gene was deleted and the Δ*gpsB* phenotype was determined via growth and infection experiments as well as using various genetic and microscopic assays.
Results: GpsB was found to be a tetrameric lipid binding protein, presumably sensitive to membrane curvature. Its deletion conferred a growth defect and heat sensitivity to *L. monocytogenes*. Δ*gpsB* mutants were attenuated in virulence, prone to cell lysis and impaired in peptidoglycan biosynthesis. Cells deleted for both, *divIVA* and *gpsB*, formed extremely long filaments, indicating a role of GpsB in cell division. Bacterial two hybrid and genetic experiments indicated a putative control of the enzymatic activity of penicillin binding protein PBP A1 by GpsB.
Conclusion: Our results ascribe a function to listerial GpsB, which behaves like DivIVA in many aspects. Interestingly, attenuation of *L. monocytogenes* virulence upon *gpsB* deletion is as strong as upon the deletion of known virulence factors. This further strengthens the idea that DivIVA/GpsB proteins might be useful targets for novel chemotherapeutics.

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CCP06

Unconventional role of the MinD-type ATPase, FlhG, during flagellar assembly

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Introduction: Flagella are bacterial organelles of locomotion. Number and location of flagella are species-specific and appear in regular patterns that represent one of the earliest taxonomic criteria in microbiology (1). However, the molecular mechanisms that ensure the correct reproduction of the flagellation pattern during each round of bacterial cell division are only poorly understood. The putative nucleotide-binding protein FlhG (2) is essential for the peritrichious flagellation pattern of the gram-positive bacterium *Bacillus subtilis* (3).
Objectives: Here we aimed at the structural and functional characterization of FlhG.
Methods: X-ray crystallography, biochemical assays, hydrogen-deuterium mass-spectrometry (HDX), ATPase activity assays, high-resolution fluorescence microscopy, identification of novel binding partners and characterization of their interaction with FlhG, pulldown assays
Results: Here, we show by biochemical and structural analysis that FlhG is a *bona-fide* MinD ATPase. Like MinD; FlhG forms ATP-dependent homodimers that interact with lipids through a C-terminal membrane targeting sequence (MTS). While MinD interacts with MinC, FlhG underwent a molecular evolution allowing its interaction with a complex of the flagellar C-ring proteins FliM/FliY. FlhG assists in the assembly of nascent flagellar C-ring in a nucleotide- and lipid dependent manner.
Conclusion: FlhG assists in coordinating the assembly of the flagellar C-ring. However, pattern specific information may be encoded in an underappreciated diversity of the C-ring proteins FliY/FliN.

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CCP07

Measurement of *E. coli* Growth Characteristics in Microfluidic Segments using Impedance Spectroscopy

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Introduction: Droplet-based microfluidics enables the discrete analysis of fluids as individual droplets. This method is relevant to biology especially to high-throughput analysis in microbiology. This paper presents the detection of bacterial growth in microfluidic segments by means of sensing impedance changes [1]. High-throughput analysis can be used to study the bacterial evolution.
Objective: To study how culturing *E. Coli* in microfluidic segments affects its growth characteristics.
Materials & Methods: *E. Coli* (K12) was cultivated in LB medium with 0.5 g/l NaCl and from this medium segments are generated using a two-fluid probe patented at the authors institute [2]. These segments are pumped through PTFE tubing to a measuring chip made of polycarbonate with titanium electrodes for impedance measurement. The chip and electrodes are coated with a hydrophobic layer to prevent from the wetting of the surface. High-throughput measurement of impedance spectra between 10 kHz and 10 MHz was achieved by the design of an impedance spectrometer based on a high-speed USB oscilloscope and a related front-end amplifier. The reference measurement of optical density (OD,) pH and conductivity were measured externally using other standard devices.
Results: The segments were measured every half hour to monitor the growth of *E. Coli* inside the segments. In LB media *E. Coli* initially metabolizes the available sugars to acetic acid resulting in a slight decrease in pH and then oligopeptides are catabolized to ammonium resulting in an increase in pH. The acetic acid and ammonium increase the conductivity of the growth medium. In the first 6 hours of growth an impedance change of around 22% was observed. A comparable variation was also observed in the measurement of OD and conductivity.
Conclusion: The measurement of bacterial growth inside the fluid segments using EIS is a promising technique for the development of high-throughput sensors for the food industry (e.g. pathogen detection in milk, juice etc.), and bioprocessing industries and for studying bacterial evolution.

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CCP08

Cell cycle control during the developmental program of *Myxococcus xanthus*

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M. xanthus initiates a complex developmental program upon nutrient starvation. During this program cells aggregate, resulting in the formation of fruiting bodies that contain environmentally resistant myxospores. In addition, some cells, named peripheral rods, remain as rod-shaped cells outside of fruiting bodies. Fruiting body formation and sporulation depends on replication early during starvation[1]. Moreover, spores contain two chromosomes whereas peripheral rods contain one chromosome[2], suggesting that cell division is inhibited in cells that differentiate into myxospores. Recently, we generated a range of tools to investigate chromosome organization during replication and segregation in vegetative growing cells[3]. In addition, we have evidence that *M. xanthus* uses the regulators PomXYZ to recruit the essential cell division protein FtsZ to the future cell division site. Currently, we hypothesize that the completion of replication is closely connected to the initiation of cell division in vegetative cells[4/5]. Using our tools to investigate chromosome organization, we were able to support previous results of two chromosomes in myxospores. The lack of any of the cell division regulators PomX, PomY or PomZ did not cause defects in fruiting body formation and sporulation. Moreover, preliminary data suggest that the level of FtsZ is not important for *M. xanthus* to build fruiting bodies but for correct timing. In total, these data match the idea that key players of cell division are dispensable for fruiting body formation. Furthermore, the levels of FtsZ and PomZ decrease during development in total cells. Experiments in which future myxospores and future peripheral rods were separated demonstrated that FtsZ does not accumulate in future spores. One possibility to control protein levels is regulated proteolysis. In a mutant lacking the ATP-dependent protease LonD, the levels of FtsZ and PomZ in total cells are stable during the developmental program, which make LonD a putative candidate involved in regulating the cell cycle of *M. xanthus* during development.

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CCP09

Analysis of novel *Myxococcus xanthus* motility regulatory proteins RomX and RomY

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Introduction: The rod-shaped *Myxococcus xanthus* cells move on surfaces with defined leading and lagging cell poles using two distinct systems. Occasionally, cells undergo reversals, which correspond to an inversion of the leading-lagging polarity axis. Reversals are induced by the Frz chemosensory system and depend on relocalization of polarly localized motility proteins between the poles. The Ras-like GTPase MglA localizes to and defines the leading cell pole in the GTP-bound form while MglB, the cognate MglA GTPase activating protein, localizes to and defines the lagging pole. MglA and MglB depend on the polarly localized RomR response regulator for correct polar targeting. During reversals, MglA, MglB and RomR switch poles [1, 2].
Objectives: To understand how MglA, MglB and RomR localize dynamically to the cell poles, we performed a bioinformatics analysis of 1609 prokaryotic genomes. These analyses, identified two uncharacterized proteins, RomX and RomY, that co-occur with RomR. The aim of this project is to determine the function of RomX and RomY in motility.
Methods: Fluorescence microscopy, motility assays, bacterial two hybrid (BTH).
Results: Genetic analyses revealed that RomX and RomY are both important for the two motility systems. Also, the reversal frequency is decreased in the absence of RomY. Furthermore, both proteins localize in polar clusters that relocate during reversals. BTH studies revealed interactions between RomX and RomR as well as between RomX and MglA. Additionally, RomY in BTH interacts with FrzZ, the output protein of the Frz chemosensory system. Using fluorescent fusion proteins, we found that RomR recruits RomX to the poles and that RomX, in turn, recruits MglA to the leading cell pole.
Conclusion: We have identified two proteins that are involved in regulation of *M. xanthus* polarity. RomX acts as an adaptor protein between MglA and RomR to recruit MglA to the leading cell pole. RomY may connect the Frz system to the MglA/MglB/RomR polarity module.

Keilberg at al. 2012
Zhang at al., 2012

CCP10

Construction of a data-independent acquisition pipeline for the investigation of *Staphylococcus aureus* specific host-pathogen interactions

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Introduction: *S. aureus* related diseases range from mild skin infections to severe infections like endocarditis or systemic syndromes (*1*). Proteome analyses can help to identify key components important for disease pathophysiology. However, it is know that data-dependent acquisition (DDA) display lower reproducibility and comprehensiveness compared to data-independent acquisition (DIA) (*2*) and this benefit of the latter can be essential for a deeper understanding of the infection-related adaptations of the pathogen.
Objectives: From 144 data-dependent acquisition MS runs an analysis pipeline was established to generate a spectral library which is suitable for the analysis of DIA MS data. This library was benchmarked with a well-characterized biological standard data set (exp. vs. stat. growth phase) and used for the analysis of an *in vivo* murine infection experiment.
Materials & Methods: *S. aureus* proteome samples were extensively fractionated to obtain a comprehensive proteome map for routine proteomics approaches. The DDA data were integrated to a spectral library which is suitable for DIA analysis. *S. aureus* grown in TSB medium and harvested during exponential and stationary phase was used for the library benchmarking. A murine pneumonia model was further used to test the library to gather infection-related knowledge.
Results: The library covers 72% of the *S. aureus* proteome assigning most of physiological relevant proteins. The benchmarking revealed a very high reproducibility (majority of CVs < 10%) and cross-MS compatibility.
DIA analysis of lavage from 8 infected mice revealed an up-regulation of proteins involved in oxidative stress, dNTP synthesis and protein biosynthetic activity after 24h.
Our peptide blast analyses vs. 47 *Staphylococcus* strains showed that the library mainly covers > 80% of peptides from each strain and can be used as a global *Staphylococcal* DIA library.
Conclusion: The new spectral library generated and successfully tested represents a valuable resource for the whole *Staphylococcus* community by providing the basis for large scale and high-quality data generation via DIA in the future.

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CCP11

Methicilin Resistant Staphylococcus aureus and its associated factors among HIV positive Paedatric Patients in Amhara National Regional State, Ethiopia – A cross Sectional Study Design

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Background: Increasing evidence suggests that MRSA infections are becoming more prevalent throughout the HIV community. MRSA infections are a challenge to physicians when treating the condition because of the limited choice of therapeutic options available. They are also a challenge to patients for infections are associated with increased cost of care.
Objectives: this study was aimed to determine the prevalence of colonization by Methicillin resistant Staphylococcus aureus species among HIV positive pediatric patients in the Amhara National Regional State, North West Ethiopia.
Methods: Eligible participants were HIV-infected, Data was analyzed by descriptive and logistic regression model using SPSS version 16.
Results: MRSA colonization was detected in 67 (16.8%) of the 400 participants, as computed from counts of MRSA at any one of the specimens collected from each patient. There were no significant associations between MRSA colonization and the independent variables including the use of antibiotics in the previous 3 months or hospitalization in the past year either. Concomitant resistance of MRSA to clindamycin, chloramphenicol, co-trimoxazole, ceftriaxone, erythromycin and tetracycline was 7.6%, 6%, 5.25%, 20.9%, 23.9% and 73.1% respectively.
Conclusion and Recommendation: High rates of colonization by pathogenic MRSA strains is observed among HIV positive pediatric patients in the Amhara National Regional state. Further studies on the molecular types of the isolated MRSA strains are recommended.
Key words: MRSA, Pediatrics, HIV, Ethiopia

CCP12

Identification of novel FtsH substrates by a trapping approach

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Regulated proteolysis adapts the cellular proteome to the environment. Among the five AAA^+ proteases in *E. coli* FtsH is the only membrane-bound and essential one. Besides the quality control of membrane proteins and SsrA-tagged polypeptides, FtsH is responsible for the degradation of enzymes and regulatory proteins like the transcription factor RpoH and LpxC, the key enzyme of lipopolysaccharide biosynthesis [1, 2]. Compared to other proteases, the number of known FtsH substrates is limited.

To find new FtsH substrates we recently designed a proteolytically deficient FtsH variant ($FtsH^{trap}$), which is still able to unfold and translocate substrates into the inactive proteolytic chamber. Proteins trapped by this variant were purified, separated by 2D-PAGE und subsequently identified by mass spectrometry. By this approach we were able to identify and validate four new FtsH substrates (YfgM, DadA, IscS, FdoH), which are involved in various biological functions [3].

Because of the limited suitability for hydrophobic and low abundant proteins, we modified the trapping approach to identify purified protease-substrate complexes directly by LC-MS/MS. Furthermore, we looked for substrates under specific growth conditions like stationary phase and anaerobiosis. Among the more than 100 putative substrates were the already known substrates RpoH, LpxC, SecY and YfgM, which validated our strategy. Further studies on these trapped proteins are expected to provide insights into new physiological roles of FtsH, the most important AAA^+ protease in *E. coli*.

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CCP13

Comparative Proteome Analysis Reveals Four Novel PHB Granule Associated Proteins in *Ralstonia eutropha* H16

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Introduction: *Ralstonia eutropha* H16 is a model organism of Polyhydroxybutyrate (PHB) research and is also used in industrial processes to produce PHB as a biodegradable polymer with plastic like properties (1). Multiple evidence has accumulated that PHB granules are not only simply storage tanks but represent well-defined subcellular organelles that consist of a polymer core and a surface layer to which many proteins with specific functions are attached. A surprisingly high number of PHB granule associated proteins (PGAP) with proven or postulated function in PHB metabolism had been previously described (2-4).
Objectives: The aim of this work was to verify the true in vivo number of PGAPs in *R. eutropha*.
Methods: Comparative proteome analysis, LC-MS/MS, fluorescence microscopy, knock-out mutants and quantitative GC analysis of PHB.
Results: Four subcellular fractions (soluble, membrane, membrane-associated and PHB granules) were prepared and the proteomes of all fractions were determined and compared. Identification of proteins that were present in the PHB granule fraction but were absent in the other fractions, revealed the presence of only twelve polypeptides with PHB-specific location plus four previously known PHB associated proteins with multiple locations. Four novel polypeptides were determined that had not yet been identified in PHB granules. The localization of the four new proteins at the PHB granule surface was confirmed by fluorescence microscopy and the phenotypes of deletion mutants will be shown. None of the previously postulated PHB depolymerase isoenzymes except PhaZa1 and none of the two known 3-hydroxybutyrate oligomer hydrolases were significantly present in isolated PHB granules.
Conclusion: Comparative proteome analysis is a powerful tool for the determination of the "true" PHB granule proteome. Possible functions of the four novel PHB granule associated proteins will be discussed.

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CCP14

Characterization of a putative lipid binding protein 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 and possesses a 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 of 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.

The codon-optimized PA3911 protein was fused to a thioredoxin-His_6-S-tag, heterologously overproduced in *E. coli* and subsequently purified *via* affinity chromatography. The specific lipid-binding capacity was analyzed using commercially available membrane lipid strips™ (Echelon® Biosciences Incorporated). This experiment revealed the binding of phosphatidic acid which is a central precursor of phospholipid biosynthesis [2].

To further analyze the lipid-binding characteristics of PA3911 the three-dimensional protein structure was theoretically modeled using the SWISS-MODEL server [3] (template model: rabbit sterol carrier protein-2, PDB code 1C44). Five amino acid residues of the potential lipid binding pocket were substituted by arginine or tryptophan in a site-directed mutagenesis approach. The lipid-binding capability of ten mutant proteins was further investigated with lipid binding experiments. In all cases amino acid replacement by arginine resulted in a mutant protein with significantly reduced phosphatidic acid binding capacity. These results allow for the partial characterization of the proposed lipid binding pocket of PA3911.

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CCP15

Fast, high-throughput measurement of collective bacterial chemotaxis

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Introduction: Population-level measurements of the chemotactic behavior of microorganisms have been limited to semi-quantitative (agar plate assay [1]) or indirect, poorly time-resolved (cell density profile responses) techniques. Recent developments in Fourier image analysis have significantly improved the accuracy and processing time in measuring the dynamics of microorganisms [2].
Objectives: We designed a new method (φDM) which allows the fast accurate measurement of collective drifts using video-microscopy [3]. As a proof of principle, we investigated the chemotaxis of *E coli* populations.
Methods: φDM was tested using computer simulations and then applied to experiments. Populations of planktonic bacteria are subjected to steady gradients of attractants, created using home-made millifluidic devices. The motion of thousands of cells is recorded using low-magnification video-microscopy. A subsequent computer analysis of the temporal evolution of the Fourier components of the image intensities enables to measure the collective drift velocity of the population of cells, without actually tracking them.
Results: The technique is found to measure collective drifts with a precision of 50 nm/s and a temporal resolution set by the camera (down to 2 ms in our case). The response to gradients of methylaspartate is found to display a regime of absolute gradient sensing at low background concentrations of attractants, followed by a relative gradient sensing at higher concentration. A model for the collective chemotactic velocity was derived based on the classical single cell models, and found to be in excellent agreement with our data. We also quantified the reduction in chemotactic efficiency of strains with modified chemoreceptors, with an increasing number of impaired methylation sites.
Conclusion: φDM is a robust technique for measuring chemotactic drifts, with unprecedented precision and excellent time resolution, making it fit to investigate weak responses and time varying stimuli. This population-level assay has a clear interpretation: only the chemotactic efficiency is probed, independently of other factors like swimming efficiency or growth rate.

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CCP16

Uptake and Incorporation of Exogenous fatty acids with lipid portion of lipoprotein in *S. aureus*

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Staphylococcus aureus was firstly discovered in the 1880s and commonly caused the skin infection, respiratory diseases and food poisoning. Since the 1940s, the medical treatment for *S. aureus* has been successful established with the introduction of antibiotics, such as penicillin. However, methicillin resistant *S. aureus* strains (MRSA) have subsequently developed resistance to almost antibiotics. Especially, MRSA USA300 spread more easily among humans and to household contacts compared to other *S. aureus* strains. Therefore, the USA300 strain is selected for the studies focusing on the development of novel antimicrobial agents.
Fatty acids are believed to possess antimicrobial activity. Human skin contains about 28% free fatty acids that possess potent antimicrobial activity against *S. aureus*. The major unsaturated free fatty acids in human nasal secretion are palmitoleic acid (C16:1D6), and linoleic acid (C18:2). However, the understanding of functional roles of these fatty acids in *S. aureus* infection is still limited. In addition, the mechanisms by which the fatty acids affect the human cell signaling during immune response against *S. aureus* infection are not investigated.
In this study, we aim to investigate the functional role of fatty acids by using USA300 strain as a model. The new findings significant contribute to understand the incorporation of fatty acid with lipoprotein, the influence of fatty acid in peptidoglycan synthesis and the effect of fatty acids on the immune response during *S. aureus* infection.

CCP17

Gene knock-outs of putative ATPase *traE* and further key factors to decipher the *Enterococcus* pIP501 Type IV Secretion System

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Multidrug resistant pathogens present a serious threat for human health. Exchange of antibiotic resistance genes often occurs through conjugative type IV secretion systems (T4SSs). We investigate the putative key factors of the T4SS from the broad-host range plasmid pIP501 present in nosocomial pathogens, such as *Enterococcus faecalis* and *Enterococcus faecium* strains. pIP501 encodes 15 putative transfer genes in a single operon. The 15 corresponding proteins are proposed to form a T4SS multiprotein complex (TraA-TraO). TraA has been characterized as a relaxase which autoregulates the pIP501 *tra* operon. Based on protein-protein interaction studies we proposed a first model of the pIP501 T4SS. Key factors are two putative ATPases, TraE and TraI/TraJ, the first putative two-protein T4S coupling protein and the muramidase TraG. Other putative essential members are the channel components, TraL and TraM and the surface factor TraO. The function of most of the Tra proteins is not fully understood. Our goal is to knock-out putative key factors in the $T4SS_{pIP501}$ to elucidate their role in T4S in Gram-positive pathogens. To make pIP501 T4S gene knock-outs we use a homologous recombination method [1]. We generated the knock-out mutants *E. faecalis* JH2-2 pIP501Δ*traG* [2] and *E. faecalis* JH2-2 pIP501Δ*traE* [3]. Biparental matings demonstrated that the muramidase TraG and the putative ATPase TraE are essential for pIP501 conjugative transfer. Complementation of the knock-out mutants with the wild type gene in trans showed recovery of the wild type transfer rate. We also analysed whether one of the peptidoglycan-degrading domains (CHAP or SLT) of TraG is sufficient to complement the *E. faecalis* JH2-2 pIP501Δ*traG* mutant. Further knock-outs are in progress which will help decipher the pIP501 conjugative transfer machinery which would represent the first solved T4SS from a Gram-positive pathogen.

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CCP18

Differential gene expression of *Enterococcus faecalis* and *Staphylococcus aureus* subjected to metal stress

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E. faecalis and *S. aureus* are nosocomial pathogens and strong biofilm formers on medical devices, making them more resistant to antibiotic treatment, and physical and chemical stresses. Together with the problem of emerging antibiotic resistance among pathogens this gives rise to a need for novel antimicrobial materials. Here we present the gene expression of *E. faecalis* 12030 and *S. aureus* ATCC 29213 exposed to AgXX®, a novel antimicrobial surface coating based on micro galvanic elements of silver and ruthenium. For *E. faecalis* next generation RNA sequencing, for *S. aureus* RT quantitative real time PCR (qPCR) was performed to study a putative mode of action of the antimicrobial surface coating.
E. faecalis was subjected to metal stress by exposure to stainless steel meshes covered with Ag or AgXX®. As control, cells were grown without metal mesh. Different exposition times allowed covering a possible quick and transient metal stress response and a possible adaption reaction. Total RNA was isolated and enriched, the remaining (m)RNA was fragmented and used as template for whole transcriptome cDNA libraries. RNA sequencing was carried out in an Ion Proton™ Sequencer. A correlation between exposition time and gene expression intensity was observed. Gene products of top up-regulated genes are involved in heat shock, oxidative, metal and general stress response. *S. aureus* was also subjected

to metal stress by exposure to the same materials. As control, cells were grown without metal mesh. During mid exponential growth phase upon contact with AgXX®, samples were taken, RNA was isolated, transcribed into cDNA and used for qPCR. In comparison to the control, AgXX® treated samples showed 100 × upregulation of *copA* and *copZ* encoded by the copper transport operon and 13 × upregulation of *katA* encoding catalase. A putative mechanism of action of the novel antimicrobial substance will be presented.

CCP20

Expression and isolation of subcomplexes of the *Enterococcus faecalis* Type IV Secretion System

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Horizontal gene transfer is one of the major mechanisms spreading antibiotic resistances even between distantly related bacteria. Most important vehicles of resistance transfer are conjugative plasmids. They encode so-called conjugative type IV secretion systems (T4SS). T4SSs of Gram-negative bacteria are well studied, information on T4SSs in Gram-positive bacteria is scarce and mainly based on *in silico* comparisons with Gram-negative T4SSs. Our working model is the broad-host-range conjugative plasmid pIP501 which is frequently found in nosocomial *E. faecalis* and *E. faecium* strains. $T4SS_{pIP501}$ encodes the genes, *traA* to *traO* in one operon which is negatively autoregulated by the first gene product, the TraA relaxase.
TraE is the putative motor ATPase and TraI/TraJ the putative first two-protein coupling protein found in a T4SS. TraN was postulated to be a DNA-binding accessory protein of the TraA relaxase and TraO a surface factor involved in establishing the contact with the recipient cell.[1]
To solve the structure of the first T4SS from a Gram-positive bacterium we cloned *traB-traO* and *traB-traN* of the $T4SS_{pIP501}$ under control of a tetracycline-inducible promoter in the *E. coli* expression vector pASK-IBA3c. This should result in overexpression of the respective 14 or 13 T4SS proteins, with TraO and TraN, respectively, tagged with a C-terminal Strep-tag. *E. coli* (BL21 [DE3]) cells were transformed with pASK-IBA3c-*traB-traO* and pASK-IBA3c-*traB-traN*, respectively. To verify expression of the T4SS proteins immunoblotting with Strep-tactin and T4SS protein-specific antibodies was performed. To isolate stable $T4SS_{pIP501}$ (sub)complexes from the *E. coli* membrane different membrane protein extraction protocols will be tested. Stable $T4SS_{pIP501}$ (sub)complexes will be analyzed for the presence of the respective T4SS proteins by immunoblotting. When sufficient quantities of the (sub)complexes are available they will be applied to electron cryo-microscopy and crystallization trials.

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CCP21

Awakening of the Undead – regeneration of chlorotic *Synechocystis sp.* PCC 6803 cells

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Introduction: Chlorosis is a process that describes the depigmentation of cyanobacterial cells triggered by differentenvironmentalinfluences. 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.
Objectives: To gain deeper insight in this process, we examined the physiological and morphological 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. Furthermore transmission electron microscopy was performed to describe the morphological changes during nitrogen starvation and the regeneration.
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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CCP22

Analysis of morphogenesis and cell division in *Hyphomonas neptunium*

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Introduction: Bacteria have evolved a variety of different cell shapes and reproduction strategies, but the underlying molecular processes have so far been mainly studied in bacteria that divide by binary fission. Hence, the mechanisms mediating alternative reproduction modes such as the formation of multiple offspring or budding are largely unknown. To further our understanding of bacterial growth, we have started to study the dimorphic alpha-proteobacterium *Hyphomonas neptunium* as a new, alternative model organism. *H. neptunium* is a close relative of the well-studied species *Caulobacter crescentus*, but it proliferates by a unique budding mechanism whereby new offspring emerges at the tip of a stalk emanating from the mother cell body.
Objectives: Although the two species share a similar set of cell division and morphogenetic proteins, their distinct modes of propagation likely go along with differences in the assembly and regulation of critical components. We have therefore set out to characterize the spatiotemporal dynamics and functions of key factors mediating budding and cell division in *H. neptunium*.
Methods: After developing methods to genetically manipulate this species[1], we performed comparative localization studies and in-depth functional analysis of selected proteins. Furthermore, we conducted a comprehensive analysis of deletion and depletion phenotypes.
Results: Our results show that several components of the bacterial cytoskeleton and cell wall-remodeling machinery localize in a cell cycle-dependent manner to the division site and to distinct areas of cell growth. Notably, several important cell division proteins exhibit an unusual localization pattern, forming complexes at locations that differ from the final cell division site.
Conclusion: This study for the first time provides insight into the mechanisms of morphological differentiation and asymmetric cell division in a stalked budding bacterium. Our data indicate that although many cell division and cell wall biogenetic proteins are conserved among species, their spatiotemporal regulation can strongly differ to fit the needs of distinct lifestyles.

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CCP23

A prophage encoded actin-like protein required for efficient phage replication

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Introduction: Virus replication is localized at specific subcellular sites of the host cell. Viruses that infect eukaryotic cells often use host-derived cytoskeletal structures, such as the actin skeleton, for intracellular positioning. The actinobacterium *Corynebacterium glutamicum* harbours three prophages. Upon induction of the SOS response, the CGP3 prophage excises from the chromosome, circularizes and replicates. The *C. glutamicum* temperate phage CGP3 encodes a novel actin-like protein (AlpC) and a small protein (AlpA) of unknown function.

Objectives: Our aim was to understand the molecular role of the novel actin-like protein AlpC and the co-transcribed AlpA protein and their influence on the CGP3 phage.
Methods: *In vivo* co-localization, time lapse, FRAP, DNA-protein interaction studies, nucleotide hydrolysis, nucleotide dependent protein sedimentation and co-sedimentation, EMSA assays.
Results: In the absence of *alpC* or *alpA*, replication of the circular CGP3 DNA is significantly reduced. AlpC polymerizes into dynamic filamentous structures that interact directly with AlpA, which forms compact foci *in vivo*. In addition, AlpA binds to a consensus sequence (*alpS*) in the upstream promoter region of the *alpAC* operon, thus connecting excised, circular phage DNA to the actin-like filaments.
Conclusion: We show that the AlpACS system is required to transport the CGP3 phage particle to the correct subcellular position, likely the cell membrane, where phage replication occurs. The AlpACS system of *C. glutamicum* is remarkably similar to actin-assisted membrane localization of eukaryotic viruses that use the actin cytoskeleton to concentrate virus particles at the egress sites and provides a link of evolutionary conserved interactions between intracellular virus transport and actin.

CCP24
Activity of proteins mediating DNA repair via homologous recombination at the single molecule level
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DNA double strand breaks (DSBs) are the most dangerous lesions in the genome, and a variety of proteins is dedicated to repair such threats. The major repair route in bacteria is that of homologous recombination (HR), with ATPase RecA as a key player. This process is regulated by a plethora of Rec-proteins (Rec NJORAFX) that are orchestrated in a cascade like manner. In *Bacillus subtilis* this process starts with RecN forming foci 15 min after DSB induction and is finished when RecA filaments disassemble and cell growth resumes after 3 h.
We wished to obtain a more detailed view on the dynamics of these proteins, and employed single molecule fluorescence microscopy in live cells. Using 20 ms stream acquisition, the movement of single Rec proteins was monitored and analyzed mathematically. We observed in exponentially growing cells, that RecN continuously scans the nucleoid, only pausing stochastically. In contrast, exonuclease RecJ seems to be retained at the replication machinery. Upon induction of DSBs, RecN arrest at several sites on the DNA, usually stopping up to a few hundred milliseconds. RecO and RecR behave similarly. In contrast to RecNOR exonuclease RecJ becomes more dynamic upon induction of DSBs, being released from the replication machinery and searching for DNA ends to mediate strand resection. Trapping times of RecJ range in a few hundred of milliseconds, showing that this step is highly efficient. This data suggests that initial detection of a DSB, processing of free DNA ends and loading of RecA on the generated ssDNA site takes place in a very short time frame.
When RecA is monitored in untreated cells movement is restrained to the nucleoid with only short stochastic stops. Upon induction of DSBs the average trapping time increases, however a molecule never stops longer than a few hundred milliseconds, suggesting that RecA filaments are highly dynamic structures with a high turnover. The chromosomal arrangement does not change during HR, indicating that RecA transports ssDNA between distinct sites on the nucleoid.
In toto, our work shows that Rec proteins respond differently to the generation of DSBs in terms of their kinetics at a single molecule level, with residence time below a second. This reveals that HR can be achieved in very short time frames in vivo and is thus much more efficient than previously anticipated.

CCP25
***In vivo* and *in vitro* characterization of *Corynebacterium glutamicum* DivIVA**
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Introduction: DivIVA is a bacterial scaffold protein conserved among Gram-positive bacteria. A distinguishing feature of DivIVA is its ability to use membrane curvature as a cue for localization. In the absence of organelles, the ability of proteins, such as DivIVA, to recognize basic biophysical features is fundamental for bacteria to spatially organize cell functions. DivIVA has been proposed to recognize negative curvature, found at cell poles and septa, by means of molecular bridging. This is a localization process based on the ability of a protein to form large assemblies and to bind membranes.
Objectives: This study focuses on the *Corynebacterium glutamicum* encoded DivIVA. *C. glutamicum* is a member of the *Actinobacteria* phylum and this study aims towards the understanding of the localization mechanisms and function that characterize DivIVA in *Actinobacteria*.
Methods: *In vivo* dynamics of a fluorescently tagged DivIVA was analysed by mean of fluorescence recovery after photobleaching and time lapse microscopy. *In vitro* characterization of DivIVA protein purified from its native host includes analysis of its oligomeric state by gel filtration and native-PAGE. Further, its post translational modification state is analysed by mass spectrometry.
Results: Absence of the known interaction partner, ParB, caused a decrease in the fluorescence recovery rate during FRAP experiments, while disruption of the membrane potential did not translate into an alteration of the rate. Time lapse microscopy suggested that the *de novo* clusterization of DivIVA at the septum act as a single clusterization process where the cluster increases in size until every suitable area is occupied. When run on a SDS-PAGE, DivIVA presents two protein bands of different molecular weight. The two species are differently enriched between cytosolic and membrane fractions. When analysed by mass spectrometry, the two bands show a different phosphorylation status.
Conclusion: DivIVA oligomerization is driven by binding-energy and does not require energy coupling. The dynamic exchange of subunits can be influenced by interaction partners. DivIVA presenting high levels of post translational modification is enriched in the membrane fraction suggesting that these modifications influence DivIVA-partner interactions and cluster stability.

CCP26
Planctomycetes – no fungi, no eukaryotes, but remarkable bacteria
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Planctomycetes were first mistakenly described as fungi in 1924. 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, FtsZ-independent cell division, a condensed nucleoid and an endocytosis-like uptake of proteins. Despite these eukaryotic-like traits, Planctomycetes were thought to lack the otherwise universal bacterial cell wall component peptidoglycan.
However, many of these features are currently under debate, while some turned out to be misinterpretations.
Here, we revisit the controversy question of the planctomycetal cell architecture, employing genetic- and microscopic techniques. Since among Planctomycetes, only *Planctomyces limnophilus* is genetically accessible, we focused our studies on this organism. First we developed additional tools that allow the constitutive expression of GFP under the transcriptional control of the endogenous GAPDH promoter in *P. limnophilus*. We found mut2GFP to localize in the cytosol of *P. limnophilus*. 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. After observing literally thousands of individual cells, three basic phenotypes emerged: *P. limnophilus* could equal similar stained like *E. coli,* that served as control (type 1). Others showed single (type 2) or multiple (type 3) membrane invaginations into the cytosol. The morphotype 2 and 3 equal *E. coli* cells after a treatment with high sucrose concentrations and similar staining. 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. This hypothesis was further supported by freeze fractioning and cryo-electron-tomography of planctomycetal cells.
Our findings point towards a more gram-negative like cell plan of *P. limnophilus*, that might lack an eukaryote-like compartmentalization. However, the periplasm is very unusual and seems to be dynamic.

CCP27

Study of the interactions between the cell division regulator MipZ and its binding partners

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Introduction: In most bacteria, the site of cell division is determined by the position of the cytokinetic Z-ring, a structure formed by polymers of the tubulin homolog FtsZ. Whereas the cell division machinery itself is relatively conserved, the mechanisms controlling its localization are highly diverse in different bacterial lineages. The alpha-proteobacterium *Caulobacter crescentus* rergulates cell division by unique mechanisms that directly couples Z-ring positioning to chromosome segregation. This regulatory system is based on the P-loop ATPase MipZ, which interacts with the polarly localized chromosome segregation protein ParB and non-specific chromomsomal DNA to establish bipolar gradients within the cell. MipZ concomitantly interferes with FtsZ polymerization, thereby inhibiting FtsZ polymerization close to the cell poles and restricting Z-ring assembly to midcell. The dynamic interaction of MipZ with FtsZ, ParB, and DNA is dependent on its nucleotide-dependent alternation between a monomeric and dimeric state with distinct interaction networks.
Objective: In this study, we have investigated the molecular basis of this regulated change in binding specificities by mapping the contact sites between MipZ and its different binding partners.
Methods: We systematically exchanged surface-exposed residues of MipZ by alanine-scanning mutagenesis. Analyzing the subcellular distribution of the mutant proteins and their ability to support division site placement, we then identified mutations that likely affect the interaction with FtsZ, DNA or ParB, respectively. The different MipZ variants were purified and further characterized *in vitro* to verify the initial phenotype-based categorization. Moreover, we performed ChIP-seq analyses to probe the DNA-binding properties of MipZ.
Results: We have identified three clusters of residues that are critical for MipZ function. Two of them are involved in contacting FtsZ, DNA and ParB, respectively. Moreover, the ChIP-seq data revealed that MipZ preferentially binds to DNA sequences of extremely high GC-content.
Conclusion: These results further clarify the role of ATP binding and hydrolysis in controlling the subcellular localization and FtsZ-inhibitory activity of MipZ.

CCP28

A cell wall amidase enables cell-cell communication in the multicellular cyanobacterium *Nostoc punctiforme* ATCC 29133

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Introduction: *Nostoc punctiforme* is a multicellular cyanobacterium growing in long filaments of vegetative cells with elaborate cell communication. To cope with changing environments, cells with special tasks are differentiated. Our previous studies revealed the importance of a cell wall lytic enzyme, amidase AmiC2, in cell differentiation and cell-cell communication. It drills pores of 20 nm in diameter in the newly formed septal peptidoglycan (PG) to form a nanopore array, a prerequisite to build communication structures between the cells. Hence, in the *amiC2* knockout mutant, cell-cell-communication is abolished, filaments show abnormal morphology and cells cannot differentiate [1, 2].
Objectives: Our main objective is to elucidate how the amidase AmiC2 transforms the septal peptidoglycan into a cell-cell communication structure.
Methods: Creation of *Nostoc* strains with truncated AmiC2 variants. Fluorescence microscopy to localize AmiC2 and derivatives by GFP tags and immune fluorescence. Overexpression of GST- tagged proteins and purification. Lytic activity measurement by dye release assay using stained PG. Structure of the catalytic domain by crystal structure analysis.
Results: Like AmiC from *E. coli*, AmiC2 localizes preferentially to the cell septa. In contrast to *E. coli*, AmiC2 has two AMIN domains at its N-terminus. For the localization of AmiC2, one out of two AMIN domains seems to be sufficient. The crystal structure of the C-terminal catalytic domain (CD) of AmiC2 could be resolved (1.3 Å). The structure showed interesting differences to structures of amidases from other bacteria. The CD of AmiC2 lacks a regulatory α-helix, which is prominent in other amidases suggesting a different way of regulation. This corresponds well to the distinct function of AmiC2 in multicellular cyanobacteria. The purified CD of AmiC2 showed high hydrolytic activity against *E. coli* PG. A truncated AmiC2 version lacking the 1st AMIN domain was less active.
Conclusion: AmiC2 from *N. punctiforme* is an unusual amidase with a different mode of activation/inhibition and novel function.

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CCP29

Nanopores and cell communication channels in the septal cell wall of the multicellular cyanobacterium *Anabaena* sp. PCC 7120

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Introduction: *Anabaena* sp. PCC 7120 cells are connected by the continuous outer membrane and the peptidoglycan (PG) layer. The septal PG layer is perforated by an array of nanopores. A mutant in the *amiC1* gene (encoding an N-acetylmuramyl-L-alanine amidase) does not form the nanopore array, indicating that AmiC1 activity is required for PG perforation. Furthermore, the mutant is unable to differentiate heterocysts, cells specialized for N_2 fixation, and to perform intercellular molecular exchange, showing that the nanopore array is required for this process [1, 3]. Recent studies show that the *sepJ*, *fraC* and *fraD* genes encode septum localized membrane proteins. Mutants of these genes show filament fragmentation and cannot perform intercellular molecular exchange [2, 4, 5]. Therefore, they are candidates for cell-cell joining communication structures and may traverse the septum through the nanopores formed by AmiC1.
Objectives: We want to find out how AmiC1 forms the nanopore array in the septal PG as a structural basis for communication and how this protein is recruited and regulated.
Methods: The *amiC1* gene was mutated in *sepJ* and *fra* mutants. To study the relationship between the septal proteins (SepJ, FraC & FraD) and AmiC1 and the nanopore array, we isolated the septal PG from the mutants and analyzed it by electron microscopy.
Results: All septal proteins seem to have an influence on nanopore array formation, since their mutants have a reduced amount of nanopores of their septal discs. Furthermore, a deletion of *amiC1* in the strains with the mutant background weakens the fragmentation phenotype, indicating that AmiC1 is involved in filament fragmentation.
Conclusion: We assume that a regulatory interaction between AmiC1 and the Fra-proteins takes place and plan to analyze possible protein interactions and to perform co-localization studies.

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CCP30

Cell biology of microbial co-cultures involved in the anaerobic oxidation of methane

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Introduction: Methanotrophs metabolize methane as their main carbon and energy source (6) and are ecologically important as methane sinks (1). Oxidation of methane, a well-known greenhouse gas (5), can be performed via aerobic or anaerobic pathways (3). In anaerobic oxidation of methane (AOM), different strategies have evolved. One example is the recently discovered '*Candidatus* Methylomirabilis oxyfera'. *M. oxyfera* performs methane oxidation coupled to nitrite reduction via an intra-aerobic pathway (2). Alternatively, AOM-associated archaea (AAA) oxidize methane with nitrate via a reverse methanogenesis pathway (4).
Objectives: Here we investigated the cell biology and microbial interactions of *M. oxyfera*/AAA in co-cultures.
Methods: To study this we used advanced electron microscopy techniques, molecular tools and metagenomics.
Results and Conclusion: The cell plan of *M. oxyfera* had some peculiar features. Unlike most other methanotrophs, these bacteria seemed to lack intracellular membranes (ICMs), showed a remarkably unusual polygonal cell shape and had a putative surface protein (S-) layer (7). Currently, we

are investigating the identity of the shape-determining structure and putative S-layer of *M. oxyfera* and the unexplored cell biology of AAA. In addition we are looking at the microbial interactions in the *M. oxyfera*-AAA co-cultures.

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CCP31

Analysis of the *Staphylococcus aureus* capsule biosynthesis pathway in vitro and the role of the CapAB kinase complex in pathway regulation

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Staphylococcus aureus is an opportunistic bacterial pathogen responsible for a diverse spectrum of human diseases, like wound infections, sepsis and other invasive diseases.
Most microorganisms that cause invasive diseases produce extracellular capsular polysaccharides, which protect the pathogen from opsonophagocytosis and thereby enhance virulence.[1]
Despite its importance for pathogenicity, staphylococcal capsule biosynthesis is not fully understood on the molecular level; especially the membrane-associated biosynthetic reactions and the posttranslational regulation of CP production, mediated by the tyrosine kinase complex CapAB, remain largely ambiguous.[2]
In vitro synthesis of the lipid-bound capsule precursors lipid Icap and/or lipid IIcap was only achieved in the presence of the tyrosine kinase complex CapAB.
Phosphorylation assays identified several proteins involved in capsule biosynthesis as protein substrates of the tyrosine kinase CapB. The impact of tyrosine phosphorylation on the individual enzymatic reactions has been analysed and specific phosphorylation sites have been determined.

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CCP32

Characterization of *Thermococcus kodakarensis* chemoreceptors

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Introduction: The hyperthermophilic Archaeon *Thermococcus kodakarensis* was isolated from a solfatara in Kagoshima, Japan. It is an obligate heterotrophic organism which grows on complex organic compounds with elemental sulfur and an optimal growth temperature of 85 °C [1]. Earlier investigations using different growth media showed that *T. kodakarensis* possesses polar flagella and is a fast swimmer [2].
Objectives: The influence of different media and growth phases on the expression of chemoreceptors should be determined using specific antibodies for Western Blot and electron microscope analysis.
Methods: Chemoreceptors were analyzed bioinformatically and recombinantly expressed in *E. coli* to generate polyclonal antibodies. *T. kodakarensis* was grown in various media to different growth phases and expression of chemoreceptors was determined with Western Blots and immuno-labeling of ultrathin sections. For electron microscopy different preparation techniques such as high pressure freezing/freeze substitution or negative staining were used.
Results: Five chemoreceptors, one cytoplasmic and four transmembrane receptors, were identified in the genome of *T. kodakarensis*. The C-terminus of the receptors is highly conserved, whereas the N-terminus which is possibly required for the binding of ligands varies. Four receptors were successfully cloned, expressed in *E. coli* and two polyclonal antibodies (including the cytoplasmic receptor) were generated. Furthermore, an antibody against the C-terminal part was generated and used for localization studies of *T. kodakarensis* chemoreceptors. Western Blot and electron microscopic analyses will show the influence of different growth conditions on flagellation and expression of chemoreceptors in *T. kodakarensis*.
Conclusion: The influence of different media and growth phases on the expression of chemoreceptors in *T. kodakarensis* could be shown. Hence, we suggest *T. kodakarensis* as a model organism to study chemoreceptors and flagellation in Archaea to better understand the link between sensing and movement.

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CCP33

Autolysis processes during cannibalistic growth of *B. subtilis*

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To survive nutrient limitation, *Bacillus subtilis* has developed a strategy, named cannibalism, that allows to feed on nutrients which are released by lysed sibling cells.[1] Cannibalism is initiated by the global regulator Spo0A, which delays energy intensive spore formation and ensures resistance in *spo0A*-expressing cells.[2] One of the cannibalism toxins, SDP, is produced by *spo0A*-expressing cells and causes rapid depolymerization of the membrane of *spo0A*-non-expressing cells, resulting in a collapse of its proton motive force (PMF). Changes in PMF can alter the degree of D-alanylation of the wall teichoic acids in the cell wall, which are normally creating a buffer zone between the peptidoglycan near the cytoplasmic membrane and external space. With the collapse of the proton motive force the buffer zone is not present any more and murein hydrolases/autolysins, which are usually suppressed by the acidic pH, get activated and the cell lyses.[3] We wanted to identify autolysins that are involved in cell lysis process and a recovery pathway that allows *B. subtilis* to feed on peptidoglycan and also wall teichoic acid fragments of the bacterial cell wall during cannibalism. We constructed double mutants of *spo0A* and autolysins that might be involved in cannibalism. These double mutants where then tested with spot assays. To test whether *B. subtilis* can feed on wall teichoic acid fragments we isolated and purified the peptidoglycan-teichoic acid-linker of *B. subtilis*. We show that this disaccharide can sustain growth of *B. subtilis* on minimal media. Our results suggest that distinct autolysins are involved in cannibalism (LytD, LytE and LytF) and *B. subtilis* is able to reuse peptidoglycan-teichoic acid-linker of his cell wall. Using proteomics we are currently identifying proteins involved in the recovery of the linker unit.

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CCP34

UDP-MurNAc biosynthetic pathway in the oral pathogen *Tannerella forsythia*

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T. forsythia is a Gram-negative bacterium that is associated with periodontitis, an oral disease affecting the tooth supporting tissue that leads to tooth loss. Intriguingly growth of *T. forsythia* strictly depends on exogenous *N*-acetylmuramic acid (MurNAc), a unique sugar of the bacterial peptidoglycan. *T. forsythia* lacks genes of cell wall synthesis (*murA* and *murB*) that are essential among other bacteria. Recently our group identified an alternative pathway for cell wall synthesis in Gram-negative bacteria which can bypass the MurAB de novo biosynthesis pathway via recovery of cell wall sugars by a sugar kinase (AmgK) and an uridylyl transferase (MurU) (Gisin et al., 2013). Orthologs of *amgK* (TF2530) and *murU* (TF2529) were found on the genome of *T. forsythia.* In this study we functionally characterized these two enzymes. His-tagged TF2529 and TF2530 proteins were expressed in *E. coli* using the pET expression system and the purified proteins were tested for activity in enzymatic tests using LC-MS and radioactive assays. The TF2529 protein exhibited uridylyl transferase activity and was specific for MurNAc-1-phosphate, generating UDP-MurNAc. Furthermore TF2530 showed kinase activity, phosphorylating both MurNAc and GlcNAc. From these results we could confirm that TF2529 and TF2530 are functional identical to the AmgK kinase and MurU uridylyl transferase of *P. putida.* We assume however that the AmgK/MurU pathway is the only pathway for cell wall synthesis in *T. forsythia.*

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CCP35

Maturation of the [NiFe]-Hydrogenases: A Useful Tool to Dissect the Function of the *isc* Operon Gene Products in Iron-Sulphur Cluster Biosynthesis

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Formate dehydrogenases and [NiFe]-hydrogenases (Hyd) are modular redox enzymes used by anaerobically growing *Escherichia coli*, and other microbes, for energy conservation. As well as the catalytic subunit, the electron-transferring, iron sulfur [FeS] cluster-containing small subunit is important for enzyme activity. The biosynthesis of the [FeS] clusters in the small subunit depends on the Isc (iron sulfer cluster) system, which is the main [FeS] insertion machinery in anaerobically growing *E. coli* [1]. The *isc* locus comprises the genes *iscRSUA-hscBA-fdx-iscX* [2] and although the biochemical activities of some of these gene products have been elucidated, much remains to be understood about the precise roles of many of them in [FeS] biosynthesis. Analysis of the biosynthesis and activity of Hyd in *E. coli* has proven to be a particularly useful tool to help identify the functions of these Isc proteins[1]. *E. coli* synthesizes three membrane-bound Hyd enzymes with Hyd-1 and Hyd-2 functioning as hydrogen-oxidizing enzymes, while Hyd-3 forms part of the hydrogen-evolving formate hydrogen lyase complex. To examine the *in vivo* contribution of individual the Isc proteins to the formation of active Hyd enzymes, defined knock-out mutants were used to monitor the respective enzyme activities and immunological methods were employed to analyze the subunit composition. Neither Hyd-1 nor Hyd-2 activity could be detected in a *fdx* mutant, while the hydrogen-evolving Hyd-3 enzyme retained some activity[3]. Lack of enzyme activity proved to due to the absence of the [FeS] cluster-containing small subunit, suggesting that when the [FeS] clusters cannot be inserted the apo-small subunit is degraded. A mutant with a deletion in the *hscB* was also devoid of Hyd-1 and Hyd-2 activity. Thus, the Hyds lend themselves to probing the functions of the components of the Isc machinery, allowing conclusions to be drawn regarding where in the hierarchy of [FeS] biogenesis each protein can be placed.

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CCP36

LIGAND-DEPENDENT INVERSION OF THE THERMOTACTIC RESPONSE IN *ESCHERICHIA COLI*

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Background: An efficient way for bacteria to navigate towards a favorable gradient of nutrients and a physiological optimum of temperature is flagella-mediated motility. The classical unidirectional mode of bacterial chemotaxis relies on temporal comparison of physiological relevant chemical ligand concentrations.
Objectives: Although chemotaxis and thermotaxis in *Escherichia coli* are mediated by the same pathway, the interplay of these two responses is not well understood. It is also not known whether and why cells accumulate at a preferred temperature.
Methods: Here we apply an *in-vivo* FRET assay and microfluidics to monitor pathway activity and to analyze dependence of the thermotactic response on ambient temperature as well as on ligand stimulation. Biochemical analyses were performed to monitor receptor modification states.
Results: We show that in absence of chemoattractants when cells adapt to a higher temperature range the thermophilic (warm-seeking) response decreases, while the adaptive methylation of chemoreceptors increases. We further investigated the influence of chemoattractants on the thermotactic response and found that it reduces or inverts the response to be cryophilic (cold-seeking) in a dose-dependent manner. Interestingly, when cells were adapted to an intermediate concentration of ligands, we observed an inverted bidirectional thermotactic response, being thermophilic at low temperatures but cryophilic at high temperatures. Additionally, by changing the Tar to Tsr receptor ratio the accumulation temperature was shifted.
Conclusions: A model is proposed where the response to thermotactic and chemotactic stimuli is coordinated by receptor methylation of the two major chemoreceptors, Tar and Tsr, in an additive fashion. We further demonstrate that the interplay of Tar and Tsr enables bacteria to accumulate at a preferred temperature gradient.

CCP37

Regulation of Flagellar Number in Dual Flagellar Systems

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Question: Flagella are organelles of locomotion, which differ in number and arrangement in different bacterial species. Together with the signal recognition particle (SRP)-GTPase FlhF, the ATPase FlhG seems to regulate flagellation patterns in polar as well as in peritrichously flagellated bacteria. Flagella number drastically increases when FlhG is depleted, whereas cells lacking FlhF show no or mislocalized flagella. However, the molecular mechanisms how FlhG establishes these patterns are widely unknown.
Methods: *Shewanella putrefaciens* CN-32 possesses two distinct flagellar systems which are encoded in two different gene clusters. *FlhG* is only present in the cluster for the polar flagellar system, but is absent in the lateral flagellar gene cluster. Using physiological and fluorescent microscopy approaches on various mutants, we explored the role of FLHG in the dual flagellar system of *S. putrefaciens*.
Results: We demonstrated that, in *S. putrefaciens*, FlhG exclusively activates the expression of the polar flagellar system and shows apparently no effect on expression and assembly of the 1 - 2 lateral flagella. Furthermore, we found evidence for interaction of FlhG with a complex of the polar flagellar basal body proteins $FliM_1$ and $FliN_1$. Mechanistically, FlhG appears to interact with FliM/FliN by interaction with the N-terminal EIDAL motif of FliM. This domain is also known to interact with the chemotaxis response regulator CheY. Since $FliM_2$ of the secondary flagellar system lacks this motif, discrimination between the two flagellar systems is likely due to FlhG-FliM interaction.
Conclusions: These findings enable new insight into the control of flagellar number and assembly and show the specificity of regulatory elements in dual flagellar systems.

CCP38

Extracellular *Streptomyces* vesicles

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Introduction: Streptomycetes have a complex life cycle, are highly abundant in soils and some other habitats, produce many secondary metabolites and enzymes being relevant for ecology, biotechnology and/or medical applications.
Objectives: We designed to deepen our knowledge on the extracellular vesicles that we discovered newly in streptomycetes.
Methods: We performed a range of physiological studies, enriched metabolites and protein, analyzed them by LC-MS, and performed investigations by fluorescence- and electron-microscopy.
Results: We determined the composition of vesicles. These comprise specific metabolites as well as proteins that include different enzyme-types, and components for signal transduction cascades. In addition, we explored the biogenesis of vesicles, their interaction to large assemblies, and their role in killing microbes including selected fungal and bacterial strains.
Conclusion: The findings provide novel insights to the composition, biogenesis, and antimicrobial activity of vescicles, and deepen our understanding of the extracellular and environmental biology of streptomycetes.

CCP39

The impact of structural variations of the cell wall precursor lipid II on the enzymatic activity of Fem peptidyltransferases in *S. aureus*

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In *S. aureus* the cell wall precursor lipid II is characteristically modified by a pentaglycine crossbridge attached to the L-Lys of the stem peptide, which is catalyzed by FemXAB peptidyltransferases. In VRE and vancomycin-resistant *S. aureus*, the mechanism of resistance results from alteration of the molecular target, i.e. the replacement of the lipid II D-Ala-D-Ala terminus by D-Ala-D-Lac. Here we provide first biochemical evidence, that the Fem-catalyzed addition of glycine residues to lipid II-D-Lac is strongly hampered. Therefore, we synthesized modified lipid II containing D-Ala-D-Lac in large scale for the first time. Compared to the control reaction using lipid II-D-Ala, the addition of the first glycine catalyzed by FemX is strongly decreased when lipid II-D-Lac was used as a substrate. This effect was even more pronounced in a coupled FemXA synthesis assay, providing biochemical evidence that the depsipeptide precursor is a poor substrate for Fem enzymes of *S. aureus*. Most likely, the modification of the stem peptide terminus interferes with substrate recognition. Until now the *vanA*-mediated mechanism of vancomycin resistance is not very widespread among MRSA strains. Although characterized by an abnormal cell morphology, reduced cell wall turnover, and retarded cell separation, cells expressing at least monoglycine cross-bridges are generally viable, but hypersusceptible to oxacillin. It has been shown that the simultaneous expression of chromosomally located *mecA* and plasmid-borne *vanA* resistance determinants is mutually antagonistic and it was concluded that this phenomenon is based on the inefficient recognition of depsipeptide precursors by the alternative penicillin binding protein PBP2a. Since pentaglycine bridge formation is severely hampered in VRSA, a reduction in FemX activity results in a synthetically lethal phenotype in the presence of oxacillin, because PBP2a is unable to crosslink monoglycyl containing precursors.

CCP40

Investigations on Daptomycin Mode of Action and Mechanisms of Resistance

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The first-in-class lipopeptide antibiotic daptomycin (DAP) is one of the last-resort agents with potent antimicrobial activity against Gram-positive pathogens including methicillin-resistant *Staphylococcus aureus* (MRSA),vancomycin-resistant *S. aureus* (VISA/ VRSA) and enterococci (VRE). In spite of its enormous success on the market as a resistance breaking antibiotic drug, the mode of action of DAP and the mechanism(s) of resistance are not fully understood. The emergence of DAP resistance has been encountered both *in vitro* and *in vivo* after prolonged DAP exposure and appears to be multifaceted, involving alterations in membrane composition and charge, as well as changes in cell wall homeostasis.
Here we compared two *S. aureus* strains with different genetic background, to examine the diverse mechanisms that contribute to DAP resistance. Characterization of morphological, phenotypic and biochemical attributes of DAP resistant mutants, corroborated by comprehensive analyses of the underlying genetic, transcriptomic and proteomic changes revealed that both strains have taken distinct routes to a common non-susceptibility level. In addition, detailed investigations on the impact of DAP on single cells and on individual cell wall biosynthesis reactions *in vitro* gave valuable hints towards (a) putative target structure (s) and DAP mode of action.

CCP41

Structural characterization of the alkaline shock protein 23 (Asp23) in *Staphylococcus aureus*

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The alkaline shock protein 23 (Asp23) of *S. aureus* is the eponymous member of the Asp23 (DUF322) protein family. Proteins with Asp23 domains are highly conserved in Gram-positive bacteria but their exact function remains unknown so far.
Recently, we showed that Asp23 which is one of the most abundant cytoplasmic proteins in *S. aureus* is localized next to the cell membrane. It is linked to the membrane by the integral membrane protein AmaP which also contains a cytoplasmic Asp23 domain. Transcriptome analysis of an *asp23* mutant revealed a small set of genes to be induced in the mutant. Most of the induced genes were connected to cell wall stress, suggesting a cell surface related function of Asp23 in *S. aureus.*
The 19 kDa Asp23 protein consists of a central Asp23 domain, an N-terminally predicted coiled coil domain and a C-terminus with clustered glutamine and asparagine residues, a typical feature of amyloid proteins.
Here we show, that Asp23 of *S. aureus* forms corkscrew-like filaments *in vitro*. Using a combination of BACTH experiments, fluorescence and electron microscopy, we investigated which parts of the Asp23 protein are important for the ability to form filaments *in vitro* and the interaction with its membrane anchor AmaP. To this end, single amino acid exchanges in Asp23 and constructs representing the individual Asp23 domains (Asp23 domain, N- and C-terminus) or combinations thereof were created.
We show, that Asp23 had to consist of at least the Asp23 core domain linked to the C-terminus, to exhibit a clear self-interaction. Moreover, we identified single amino acid exchanges which led to an impairment of filament formation *in vitro*. A particularly clear effect was observed for the Asp23 K51A amino acid substitution. This amino acid exchange also had an impact on the localization of Asp23 in *S. aureus* cells as shown by fluorescence microscopy providing a first hint, that filaments may also form *in vivo*.

CCP42

Molecular basis for SMC rod formation and its dissolution upon DNA binding

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SMC condensin complexes are central modulators of chromosome superstructure in all branches of life. Their SMC subunits comprise a long intramolecular coiled coil, which connects a constitutive “hinge” dimerization domain with an ATP regulated “head” dimerization module. Here, we address the structural arrangement of the long coiled coils in SMC complexes. We unequivocally show that prokaryotic Smc-ScpAB forms rod-like structures with its coiled coils being closely juxtaposed and accurately anchored to the hinge. Upon ATP-induced binding of DNA to the hinge, however, *Bacillus subtilis* Smc switches to a more open configuration. Our data suggest that a long-distance structural transition is transmitted from the Smc head domains to regulate Smc-ScpAB’s association with DNA.

CCP43

Applying data-independent mass spectrometry to comprehensive characterization of the *Staphylococcus aureus* proteome under iron-restricted conditions

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Introduction: *Staphylococcus aureus* is a pathogen that plays a major role in community-acquired and nosocomial infections. Treatment of these infections becomes more complicated due to the spread of antibiotic resistance [1]. Development of new strategies for infection control relies on the comprehensive investigation of infection mechanisms.
With new methods like data-independent acquisition (DIA) mass spectrometry (MS) [2] a new level of data quality becomes available for analysis of so far unresolved questions in host-pathogen studies.
Objectives: The comparison of the traditional shotgun MS (data-dependent acquisition (DDA)) with a DIA approach was performed using samples from *S. aureus* grown under iron-deficient conditions. These conditions mimic the nutritional state of a typical host environment and were further compared with standard conditions in a defined medium.
Methods: *S. aureus* HG001 was cultivated in pMEM with or without Bipyridyl. Cytoplasmic and secreted proteins were analyzed with DDA and DIA on a Q Exactive™. The DIA workflow can further be applied to bacterial cell samples from an infection experiment.
Results: The combined analysis of secreted and cytoplasmic proteins revealed about 1,400 identifications using the DDA approach and these data were used to generate a spectral library for DIA. A sample by sample comparison showed about 200 more proteins and significant higher peptide coverage in the DIA analyses.
The DIA analysis revealed that 5 proteins of the isd-family, the sirA and the fhuC protein from the staphyloferrin uptake system were upregulated in iron-restricted conditions. Furthermore, many proteins belonging to virulence (e. g. splF or sspB) or host-pathogen interactions (clfB, SCIN or fib) were specifically regulated.
Conclusion: In this study we have generated an iron limitation specific *S. aureus* DIA library which allows proteome analysis of *S. aureus* under *in vivo* simulating conditions with a very high accuracy. With these data we can now monitor the metabolic behavior of this pathogen more comprehensively in order to obtain a holistic view of its adaptation to conditions mimicking natural settings.

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CCP44

Resistance against antibiotics in the "living drug" bacteria *Bdellovibrio bacteriovorus*

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Introduction: *Bdellovibrio bacteriovorus* is a small gram-negative bacterium preying upon other gram-negative bacteria by invading and growing in the periplasm of its prey. The ability of killing gram-negative bacteria, including known human pathogens, makes *B. bacteriovorus* an attractive option as a pro- or prebiotic agent with applications in agriculture, industry and clinics.
Methods: Development of sensitivity drug tests in *B. bacteriovorus*. Complementation studies in *E. coli*. Other methods for the determination of enzymatic activity.
Objectives: Whilst resistance of bacteria against conventional antibiotics is an ever-rising problem, *B. bacteriovorus* might offer a new path to fight drug-resistant gram-negative pathogens. One of the options is to use *B. bacteriovorus* in combination with conventional antibiotics to potentiate the effectiveness against highly drug-resistant gram-negative pathogens. In order to make this approach feasible more has to be known on the ability of *B. bacteriovorus* to resist antibiotics itself.
Results: We tested the susceptibility of *B. bacteriovorus* towards several antibiotics from different classes and compared it to the antibiotic susceptibility pattern of the *Escherichia coli* prey. In this way we identified the antibiotics against which *B. bacteriovorus* express high resistance.
Conclusions: We developed two novel assays, performed either in liquid or solid media, which allow to test *B. bacteriovorus* sensitivity against antibiotics. The underlying molecular mechanisms for the observed high resistance of *B. bacteriovorus* against some of the antibiotics is currently investigated.

CCP45

A tightly-controlled, tetracycline-inducible expression system for integration into the genome of *Streptococcus pneumoniae*

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Streptococcus pneumoniae is an important human pathogen, which is able to cause severe diseases such as pneumonia, septicemia, and meningitis. To understand processes implicated in virulence and pathogenesis, it is important to be able to analyze regulatory events and networks in this organisms. For that purpose controlled gene expression is quite helpful to modulate expression levels for phenotypic analysis. Despite its importance, relatively few gene control systems have been described for *S. pneumoniae*. Therefore, we tested the tetracycline repressor TetR based expression system from *Staphylococcus aureus* for its suitability in *S. pneumoniae*. The *tetR* gene and the tetracycline-inducible $P_{xyl/tet}$ promoter were moved from plasmid pRAB11 to an integrative plasmid pSW1 resulting in pTEX2. This plasmid, which can be propagated in *Escherichia coli*, integrates into to the genome of *S. pneumoniae* in the intergenic region downstream of the ß-galactosidase gene. Measuring $P_{xyl/tet}$ promoter activity as well as expression of several *S. pneumoniae* genes cloned behind $P_{xyl/tet}$ demonstrated tight control by TetR. The expression platform showed a very low background expression in the absence of tetracycline and a very sensitive response to anhydrotetracycline, which less antibiotically active than tetracycline. In conclusion, an integrative expression system for *S. pneumoniae* is available, which allows to adjust gene expression to different levels in response to anhydrotetracycline.

CCP46

The accessory protein CpxP promotes *Salmonella enterica* serovar Typhimurium biofilm formation

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Salmonella spp. is an important foodborne pathogen, which causes a variety of human diseases ranging from self-limiting gastroenteritis to serious systemic infections like typhoid fever. Biofilm formations of *Salmonella* spp. seem to develop a stress response, resulting in an increased resistance to antibacterial agents [1]. A major influence on the biofilm genesis and stress response regulation is given by two-component systems (TCS), the main mechanisms by which bacteria sense and respond to environmental stimuli [2].
Several studies connect the Cpx envelope stress TCS, composed of the membrane-bound sensor kinase CpxA, the cytosolic response regulator CpxR and the accessory protein CpxP, with biofilm formation [3]. However, for *E. coli*, the function of the Cpx-TCS is ambiguous. Activated CpxR inhibits the expression of the major biofilm regulator *csgD* and the motility genes *motABcheAW* [4, 5]. In contrast, motility is under positive CpxR control in *S. enterica* serovar Typhimurium. Since *cpxP* is the most induced gene during early phases of biofilm formation [6] we specifically studied the role of CpxP for *S.* Typhimurium biofilm formation. Biofilm formation was investigated by macrocolony morphology, cell aggregation, pellicle formation, confocal fluorescence microscopy and atomic force microscopy. We observed distinct differences between wild type *Salmonella*, *cpxP* deletion and *cpxP* overexpression strain. In particular, biofilm formation was faster and biofilms themselves were thicker when *cpxP* was overepxressed. Thus, our results assign CpxP an important function during *Salmonella* biofilm formation and lead a deeper insight how the Cpx TCS is integrated into biofilm cascade.

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CCP47
Computational and Genetic Reduction of a Cell Cycle to Its Simplest, Primordial Components
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Question: What are the minimal requirements to sustain an asymmetric cell cycle?
Methods: We use mathematical modelling and forward genetics to reduce an asymmetric cell cycle to its simplest, primordial components.
Results: In the Alphaproteobacterium Caulobacter crescentus, cell cycle progression is believed to be controlled by a cyclical genetic circuit comprising four essential master regulators. Unexpectedly, our in silico modelling predicted that one of these regulators, GcrA, is in fact dispensable. We confirmed this experimentally, finding that ΔgcrA cells are viable, but slow-growing and elongated, with the latter mostly due to an insufficiency of a key cell division protein. Furthermore, suppressor analysis showed that another cell cycle regulator, the methyltransferase CcrM, is similarly dispensable with simultaneous gcrA/ccrM disruption ameliorating the cytokinetic and growth defect of DgcrA cells.
Conclusions: Within the Alphaproteobacteria, gcrA and ccrM are consistently present or absent together, rather than either gene being present alone, suggesting that gcrA/ccrM constitutes an independent, dispensable genetic module. Together our approaches unveil the essential elements of a primordial asymmetric cell cycle that should help illuminate more complex cell cycles.

CCP48
Choreography of prokaryotic multipartite genome replication
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Faithful maintenance of genome content is crucial to ensure prokaryotic proliferation. The initiation of genome replication is the fundamental decision every single cell has to take, and many bacterial species including N_2-fixing symbionts and pathogens keep more than one replicon, consistently copying these only-once per cell cycle.
How is this multiple replication initiation coordinated in the endosymbiont *Sinorhizobium meliloti* harboring three replicons?
We applied a combination of methods in cell biology and genomics to gain insights into the *S. meliloti* 2011 replication strategy involving the chromosome, megaplasmid pSymA and chromid pSymB. FROS (fluorescent repressor operator system) tagging of DNA loci and fluorescent tagging of proteins involved in replication initiation or the replisome revealed details of the spatiotemporal dynamics of replication and cell cycle progression. FACS and CGH in different genetic backgrounds furthermore provided data on the importance of DnaA activity within this only-once per cell cycle replication initiation of multiple replicons.
This work enhanced our understanding of how secondary replicons are integrated into the conserved asymmetric core cell cycle of an alpha-proteobacterium.

CCP49
Coping with stress - filamentation of *Caulobacter crescentus* as a response to high salinity
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Introduction: Free-living bacteria have to deal with various stresses in the environment, including heat, changes in osmolarity or nutrient depletion. To survive under such adverse conditions, cells must induce pathways that alleviate cellular damages, but also adjust their cell cycle to guarantee cellular integrity. During various stress conditions different bacteria block cell division and form long filaments. The molecular causes of cell filamentation and the benefit that cells gain from altering their morphology remain unclear.
Objectives: In this project I want to investigate the response of the model organism *Caulobacter crescentus* to salt stress. By using a combination of genetics and cell biology techniques I address how elevated salt concentrations affect the cell cycle in this organism and whether the observed phenotypes contribute to the survival of *C. crescentus* in this condition.
Results: We observed that upon exposure to increased salinity *C. crescentus* cells stop to divide while growth and the initiation of DNA replication continue, leading to filamentous cells. This response occurred in sublethal NaCl concentrations between 70 and 100 µM. Time-lapse microscopy shows that filamentous cells are able to reenter cell divisions and to recover after stress release, demonstrating that filamentation is a reversible process. Our data show that induction of the SOS-response is not responsible for the cell division block. Instead, a rapid decrease in the levels of the major transcriptional regulator CtrA is observed upon salt exposure. The drop in CtrA levels is accompanied by a downregulation of CtrA-regulated genes important for cell division and morphogenesis. Our data show that the decrease in CtrA steady-state levels is mainly caused by faster proteolysis by the protease ClpXP.
Conclusions: Here, we demonstrate that external salt concentrations can alter the abundance of a major cell cycle regulator and thereby affect cell division and cell morphology. This work provides a better understanding of how external conditions affect the morphology and proliferation of bacteria.

CVP01
A mass balance of somatic coliphages in an activated sludge system
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Question: To decrease viral pollution in receiving waters at the most probable source, municipal wastewater treatment plants (WWTPs), the elimination mechanisms of viruses had to be understood. Therefore a mass balance of somatic coliphages (SCP, indicator for enteroviruses) was determined in a municipal WWTP. Additionally some batch tests were performed to verify the influence of temperature and MLSS content onto adsorption and inactivation of viruses in activated sludge (AS).
Methods: The samples were taken at several points of a municipal WWTP in summer and winter season (temperature influence). The concentrations of SCPs within the WWTP and the batch tests were determined according to ISO 10705-2:2000 (2001). Some samples were analysed by qPCR to detect adenoviruses (AV) as representative of a pathogenic viruses.
Results: The results of the mass balance showed, that SCPs were mainly inactivated (85%) in the AS e.g. by predation of bacteria and protozoa [1]. In the primary treatment an elimination of SCPs was not detected. Only 1% of the viruses entering the WWTP were released in the environment. The concentration of phages in the liquid phase of the AS tank was equal to that in the secondary clarifier. Additionally, the reduction of SCPs was comparable with that of AV.
The batch tests showed that inactivation is a very slow process and temperature dependent (after 42 d at RT: decrease of 2.7 log, at 12°C 1.4 log). This is in agreement with the results of the WWTP, where inactivation was significantly higher in summer (95%, winter: 85%). Furthermore the adsorption tests showed that MLSS contents between 2-5 g/l had no significant effect on the adsorption of SCPs.
Conclusion: This study showed that the main elimination mechanism of viruses in a municipal WWTP was adsorption at solids in the AS tank. An increase of the MLSS content between 2-5 g/l does not optimize the virus elimination in municipal WWTPs. Inactivation of viruses in the activated sludge is slow process, but can be positively influenced by higher temperatures.

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CVP02
Enhanced disinfection and regular closure of wet markets reduced the risk of avian influenza A virus transmission
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Introduction: Recently, avian influenza virus A (H7N9) emerged inChina. As of October 2014, a total of 453 laboratory-confirmed cases of human infection with avian influenza A (H7N9) virus, including 175 deaths, have been reported to WHO [http://www.who.int/influenza/human_animal_interface/influenza_h7n9/riskassessment_h7n9_2Oct14.pdf?ua=1].
Objective: Since most H7N9-infected human cases have reported contact with poultry or live-poultry markets (LPMs), we carried out surveillance for avian influenza virus (AIV) in 144 LPMs inGuangzhou.
Methods: From 1 April to 30 June 2013, we sampled a total of 4,598 environmental and animal swab specimens in 137 retail LPMs and 7

wholesale LPMs for the detection of AIV by RT-PCR for H5, H7 and H9, respectively.
Results: AIV was continually detected in both environmental and animal samples with a positive rate of 4.22% (194/4,598), while H9 and H5 subtypes were identified for 1.65% (76/4,598) and 0.54% (25/4,598), respectively. However, H7 virus was not found from these samples. AIV positive rate in the retail LPMs was 4.56% (177/3,884), which was significantly higher than that (2.38% 17/714) in the wholesale LPMs（x^2=7.07, P<0.01), suggesting that the retail LPMs were favorable microenvironment for AIV transmission between poultry. On 3 May 2013, Guangzhou government issued a new guideline for enhanced disinfection of LPMs, including daily cleaning and weekly one-day closure of the markets for complete disinfection. The positive rate of AIV decreased significantly from 6.95% (before the enhanced measures) to 5.97% (1st month after the enhanced measures) and 1.74% (2nd months after the enhanced measures), respectively (trend x^2=14.01, P<0.01). Notable, the positive rate of H9 reduced significantly from 3.48% to 2.88% and 0% during this period (trend x^2=12.92, P<0.01). However, positive rates of AIV in animal specimens maintained at similar levels.
Conclusion: These results indicated that enhanced disinfection measures may reduce the risk of environment-to-human transmission of AIV in LPMs.

CVP03
Changes of the enzyme system in the body of white mice with experimental flu under the influence of Piler light

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Question. The aim of this study was to investigate the effect of polarized, polychromatic, incoherent (Piler) light on the changes of proteinase, infectious, hemagglutinating activity and total protein in the body of white mice at experimental influenza.
Methods. There were 60 white mice of "Balb/c" line, weight 13-14 g., influenza virus A/PR/8/34 (H1N1), Piler light device. The mice were infected with influenza A virus intranasally under Rausch-anesthesia. The animals were divided into 6 groups, each of 10 pcs. The first group was the control one for animals. The second group of animals was exposed to Pilar light (control). The third group of the animals was infected with a lethal dose of influenza virus A. The fourth group also was infected with a lethal dose of influenza A virus and exposed to Pilar light (treatment). The fifth and sixth groups of animals were infected with influenza virus therapeutic dose (10^{-2} LD 50/0.1 ml), when 50% of the animals die. The sixth group underwent a course of phototherapy (treatment). Each mouse in experimental groups received 11sessions of Pilar light. Fence of lungs and blood sampling was conducted under deep ether anesthesia. In the supernatants of lungs and blood serum proteinase, infectious, hemagglutinating, inhibitory activity and total protein were determined.
Results. Under the action of a lethal dose of influenza A 100% mortality of animals on the sixth day after infection took place. In the groups of animals previously infected with a lethal dose of influenza virus and underwent 11 sessions of light therapy, 20% of the animals survived. Under the influence of therapeutic sublethal doses 80% of animals survived and remained alive for the whole study period. The animals which underwent light therapy on the 15th day after infection, there was an increase of inhibitory activity independent on the dose of infection.
Conclusions. Phototherapy with Piler light of the animals infected with a lethal dose of influenza A virus activity delayed replication of the virus. Infectious and hemagglutinating activity was determined in small quantities i.e. under the influence of Pilar light influenza A virus did not die, but its reproduction was decelerated. During this period restoration of inhibitory protecting activity took place and the experimental animals survived.

CVP04
Viral production in the subsurface of tidal-flat sediments

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Marine sediments harbor huge amounts of viruses that affect microbial community structures and nutrient availability within the subsurface [1]. The number of viruses is balanced by production and decay, both of them rarely studied in subseafloor sediments. Here, we report on virus production rates performed on tidal-flat sediments from 2 to 375 centimeters. Tidal flats are characterized by high microbial activity down to several meters due to high input of organic matter caused by tidal pumping. Viral production rates were determined with the dilution method, i.e., sediment was diluted in anoxic artificial seawater [3]. The increase of virus like particles (VLP) was followed over up to 24 hours via epifluorescence microscopy. Numbers of bacteria and viruses (up to 8.4 x 10^9 cells cm^{-3} and 4.5 x 10^{10} VLP cm^{-3}) as well as viral production rates in surface sediments (up to 1.4 x 10^{10} VLP cm^{-3} d^{-1}) were amongst the highest ever reported for marine sediments. As an alternative method for viral production measurements, we tested ^{3}H-thymidine incorporation into viral DNA in incubations of several days [2]. Using the theoretical conversion factor to relate radiolabel incorporation to viral numbers, the viral production rates were three to four orders of magnitude lower than with the dilution method. We conclude that these results represent an underestimation, probably because the conversion factor does not apply for our anoxic sediment incubations or because of the rather long incubation time. In conclusion, the high production rates of the dilution method indicate a major impact of viruses on the microbial community and biogeochemical processes within the highly dynamic system of tidal-flat sediments.

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CVP05
Investigation of the protein complex at the heart of the immune defence system

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The prokaryotic immune system CRISPR-Cas protects prokaryotic cells against foreign genetic elements such as viruses or plasmids. The defence reaction progresses in three stages: adaptation, where new spacers are acquired; expression, where mature CRISPR-RNAs are generated; and interference, where the invader-DNA is degraded. Different versions of the CRISPR-Cas system exist, that show variations of the molecular mechanisms in the three stages. These different CRISPR-Cas systems have been grouped into three major classes (I-III) and currently 14 subclasses (IA-IIID).
The subtype I-B system in *Haloferax volcanii* consists of three CRISPR loci and eight Cas proteins (Cas1-Cas8b) (1, 2). We could show that the Cascade complex of this I-B system contains the Cas5, Cas6 and Cas7 proteins as well as the crRNA (3). To investigate the interaction in the Cascade complex in more detail we generated Cas5 and Cas7 variants and analysed their activity *in vivo*. For the Cas7 protein 18 variants of the *cas7* gene were generated and the activity of the Cas7 protein variants concerning crRNA maintenance and interference was analysed. Mutation of several conserved residues of the Cas7 protein resulted in reduced amounts of crRNA as well as an impaired defence reaction. A similar approach was made with the Cas5 protein. Here, 15 gene variants were generated and analysed in a Δ*cas5* strain in respect to crRNA amounts and interference activity, revealing several amino acids that are essential for the functionality of the protein.

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CVP06
CRISPR-Cas with a grain of salt - Insights into the CRISPR-Cas system of *Haloferax volcanii*

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KeyWords: CRISPR; Cas proteins; *Haloferax volcanii;* PAM; Seed; interference
Defense against foreign genetic elements is key to the survival of prokaryotic organisms. The CRISPR (clustered regularely interspaced short palindromic repeats)-Cas (CRISPR associated) system is one of the most elaborate answers to this need in it being adaptive, hereditary and highly sequence specific. CRISPR-Cas systems comprise clusters of

repetitive sequences interspaced by short sequences derived from invaders previously encountered by the cell. These CRISPR loci are accompanied by a set of *cas* genes encoding the protein machinery required for the reaction.
Although present in more than 90 % of the archaea and app. 40 % of bacteria, so far only a few organisms have been studied in detail. Here we present insights into the type I-B defense system of *Haloferax volcanii*.
We could show that the *H. volcanii* system is active in triggering a defense reaction (1). Using a plasmid mimicking an invader, we could define prerequisites for successful identification and elimination of the intruder (1). A short sequence called PAM (protospacer adjacent motif) encoded on the invader DNA must be given as one of six motifs (1). And additionally a systematic analysis revealed the requirement for a ten nucleotide long non contiguous seed sequence (2).

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CVP07
A neglected field - Bacteriophages of solventogenic clostridia
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Introduction: In November 2014, the European Nucleotide Archive of the European Bioinformatics Institute listed 26 different sequenced bacteriophages obtained from clostridia. All of their hosts belong to pathogens, but none to clostridia which have industrial importance such as the solvent producers *Clostridium ljungdahlii*, *C. beijerinckii*, or *C. acetobutylicum*. The bacteria are frequently used in fermentation processes to convert substrates such as waste gas or *lignocellulosic* biomass to valuable chemicals such as acetate, acetone, butanol, or ethanol.
First reports of abnormal fermentation processes of clostridia due to bacteriophage infections stem from the early twenties of the 20th century. Unfortunately, companies showed more interest in removing these bacteriophages than to study the infecting agents in detail [1]. Therefore, up to the present day, there are only few publications about phages infecting solventogenic clostridia. None of these studies put great emphasis on the molecular level of these phages.
Objectives: Identify potential molecular tools within the genomes of bacteriophages of solventogenic clostridia using next-generation sequencing.
Methods: To characterize both host and phage genomes, DNA of both origins were isolated and sequenced by illumina sequencing.
Results: By sequencing of the two host strains *C. saccharoperbutylacetonicum* N1-4 (HMT) and *C. beijerinckii* 479, it was possible to gain information of phage-host interaction at the molecular level.
Conclusion: To improve and extend the spectrum of molecular tools for clostridia, it is inevitable to have a closer look upon their bacteriophages at the molecular level, leading to a tremendous potential in successful construction and further development of new biocatalytic strains of solventogenic clostridia.

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CVP08
The Cas8 protein is essential for the immune defense reaction
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About half of the bacteria and most of the archaea feature CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeat) systems to fend off invading genetic elements like viruses or plasmids. The CRISPR-Cas systems exist in different versions, that have been grouped into three major classes (I-III) and currently 14 subtypes. All versions carry out the same function, namely the defense against invaders. They differ from another since they have a different set of Cas proteins and slightly different modes of carrying out the defense reaction.
In type I-B systems, the interference reaction is catalysed by the Cascade (CRISPR-associated complex for antiviral defense) complex consisting of Cas5, Cas6, Cas7 and Cas8. The latter has previously been shown to be a nuclease with ATPase activity. To identify the role of the Cas8 protein, two Cas8 homologues from the archaea *Haloferax volcanii* (Hvo) and *Methanothermobacter thermoautotrophicus* (Mth) were investigated genetically and biochemically for their functions. A *Haloferax cas8* deletion strain (Δ*cas8*) was not active in interference anymore showing the indispensable role of Cas8 for interference. Mutational analysis revealed several amino acids as essential for the reaction. *In vitro* analyses of Mth Cas8 demonstrated binding to specific DNA substrates like R-loops. Interestingly, binding activity was even higher when a potential Mth PAM (ProtospacerAdjacentMotif) was present. Nuclease assays confirmed cleavage of ssDNA flaps or flayed duplexes and nuclease activity was highest with ssRNA flaps.

CVP09
Molecular insights into the archaeal DNA interference machinery of type I-A CRISPR immunity
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Introduction: Viral DNA is identified in the adaptive CRISPR-Cas immune systems of prokaryotes via small RNA molecules. These CRISPR RNAs (crRNAs) guide a ribonucleoprotein surveillance complex for DNA interference. In type I CRISPR-Cas systems the targeting and degradation of DNA is mediated by the multi-protein Cascade complex.
Objectives: We aimed to analyze the diversification of CRISPR systems in Bacteria as well as Archaea and characterized the assembly and DNA interference of the archaeal type I-A Cascade from *Thermoproteus tenax*.
Methods: Cascade assembly and DNA interference activity could be established *in vitro* via the cooperative refolding of the six recombinant subunits and synthetic designed crRNAs.
Results: The archaeal I-A Cascade complex consists of the small and large subunits Csa5 and Cas8a2 and the crRNA-binding proteins Cas5 and Cas7. In contrast to Bacteria, the Cas3 helicase (Cas3') and nuclease (Cas3") subunits are an integral part of the complex, while the Cas6 endonuclease is absent (1). All stages for implementing DNA interference including crRNA binding, R-loop formation with the target and DNA degradation could be shown *in vitro*. Additionally, short target motifs (PAM) were identified that influence efficient DNA degradation (2). Finally, we investigated the role of the small and large subunits Csa5 and Cas8a2 in DNA targeting (3).
Conclusion: The established assembly of the type I-A Cascade shows for the first time interference activity in Archaea *in vitro* and provides information about similarities and differences in the evolution of bacterial and archaeal DNA interference machineries in CRISPR immunity.

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CVP10
Two CRISPR-Cas systems in *Methanosarcina mazei* strain Gö1
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Objective: The CRISPR system is an RNA-based immune system in bacteria and archaea. This defense mechanism against exogenous nucleic acids is highly adaptive and heritable. The CRISPR-array is flanked by different *cas*-genes, encoding the crucial proteins for the defense. Our aim was to analyze the two CRISPR-Cas systems of *Methanosarcina mazei* strain Gö1 (I-B and III-C) and their regulation mechanism.
Methods: The expression and maturation of CRISPR-derived RNAs (crRNAs) were studied *in vitro* and *in vivo* via Northern blot analysis and by RNA sequencing under different growth conditions and stresses. Further, qRT-PCR, electrophoresis mobility shift assays (EMSA) and Western Blots were used to analyze potential regulatory mechanism.
Results: In *M. mazei* strain Gö1 two different subtypes (I-B and III-C) of a CRISPR-Cas system were observed. Northern blots and differential RNAseq analyses demonstrated generally very low expression of both systems. In the presence of high NaCl concentrations, a significant increase in expression levels of the crRNA was observed. The analysis

further revealed a 5'-hydroxy and 3'-phosphate termini architecture of small crRNAs due to cleavage of Cas6 endonucleases. Each purified protein showed significant activity and was able to cleave both repeat RNAs (type I-B and type III-C) at the identical processing site.
We recently isolated a spherical virus (MSV) from anaerobic sewage sludge sampled from a waste water treatment plant, which is able to specifically target *M. mazei*. Sequence analysis of the archaeal virus genome now allows to screen for the occurrence of viral DNA in CRISPR-arrays e.g. of several newly isolated *M. mazei* strains in parallel to virus challenging.
Conclusion: The finding of overall very low activities of the CRISPR systems under optimal growth conditions argues for a strong repression of the two CRISPR-loci, and let us conclude that the CRISPR-Cas system in *M. mazei* is evolutionary old and evolves very slowly. Potential regulatory mechanisms will be presented and discussed.

CVP11
Characterization of a minimal type-I CRISPR-Cas system found in *Shewanella putrefaciens* CN-32

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Introduction: *Shewanella putrefaciens* CN-32 is a mesophilic gamma-proteobacterium that has a single minimal type-I CRISPR-Cas system, which does not fit into standard classification schemes. The CRISPR locus contains 81 spacers and is accompanied by 5 *cas* genes. Three of these genes encode Cas1 (integrase), Cas6f (CRISPR RNA endonuclease) and Cas3 (DNA helicase/nuclease). The two genes *Sputcn32_1821* and *Sputcn32_1822* cannot be classified into known *cas* gene families.
Objectives: We aimed to characterize the CRISPR-Cas-mediated targeting of plasmid DNA *in vivo* to understand the functionality of a minimal DNA interference system.
Methods: RNA-Seq methodology was used to analyze CRISPR RNA transcription. Plasmid conjugation assays of *cas* gene mutants were applied to study the *in vivo* activity of this CRISPR-Cas system.
Results: The CRISPR-Cas system of *S. putrefaciens* CN-32 was found to be active and displayed characteristics similar to other type-I systems. Four of the five Cas proteins were found to be essential for DNA interference activity. The efficiency of DNA targeting correlated with CRISPR RNA abundance. The presence of a short sequence motif (the protospacer-adjacent motif (PAM), 5′-GG-3′) was found to be required for DNA target recognition.
Conclusion: A minimal CRISPR-Cas system is active in *S. putrefaciens* CN-32 and DNA selectivity could be shown. Known proteins that could recognize the PAM sequence are absent which suggests that a novel DNA recognition mechanism is present.

CVP12
A small coding RNA regulates glutathione homeostasis upon singlet oxygen stress in *Rhodobacter sphaeroides*

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The photosynthetic model bacterium *Rhodobacter sphaeroides* faces photooxidative stress due to the bacteriochlorophyll-mediated generation of singlet oxygen ($^1O_2$) in the light. Our aim is to investigate the underlying response mechanisms, focusing in particular on the involvement of small RNAs.
On top of the $^1O_2$-dependent regulation one can find the alternative sigma factor RpoE, which induces among other genes, the 219 nt long sRNA RSs0019 (1). RSs0019 contains a small ORF (150 nt), which is translated at low levels under aerobic growth and is further induced upon $^1O_2$ stress. Over-expression of RSs0019 negatively affected mRNA levels for several genes involved in sulfur metabolism as well as for the hypothetical protein RSP_0557, as shown by microarray analysis. To distinguish between peptide- versus sRNA-driven effects, several RSs0019 mutant variants were over-expressed and compared to the genuine sRNA by real time RT-PCR. These experiments suggested RSs0019 to be a potential bifunctional RNA, as loss of peptide expression did not affect gene regulation by RSs0019. We used a *lacZ*-based *in vivo* reporter system to further uncover the effect of RSs0019 ORF expression on the potential main target RSP_0557. These studies also suggested RSs0019 to be Hfq-dependent and binding of RSs0019 to Hfq was shown by co-immunoprecipitation.
Physiological experiments, such as measurement of glutathione and zone inhibition assays, identified the likely main target of RSs0019, the hypothetical protein RSP_0557, as regulator of glutathione (GSH) biosynthesis. We provide a model, in which RSs0019 regulates sulfur metabolism genes and RSP_0557 to maintain GSH homeostasis under conditions of $^1O_2$ as well as peroxide stress.

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CVP13
CopraRNA based sRNA target prediction boosts the analysis of two major regulators of photosynthesis in cyanobacteria.

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Small RNAs (sRNAs) constitute a large heterogeneous class of bacterial gene expression regulators. These sRNAs typically target multiple mRNAs, thereby acting as global post-transcriptional regulators. However, although high-throughput sequencing of transcriptomes is producing an ever-increasing number of sRNAs, the identification of their targets has remained tedious. We present a strategy that integrates phylogenetic information to predict sRNA targets at the genomic scale and reconstructs regulatory networks upon functional enrichment [1]. CopraRNA has a superior performance compared to currently available bioinformatic approaches and rivals the results of experimental target prediction by microarrays. The approach proved to be sound for various bacterial phylae [1,2,3] and an easy-to-use web interface has been implemented [1].We give a short overview of CopraRNA and exemplary show its power by uncovering the regulatory networks of two widely conserved cyanobacterial sRNAs, PsrR1 and IsaR1. Those are the first known sRNAs involved in the regulation of oxygenic photosynthesis. PsrR1 is up-regulated upon high-light and helps to adapt the photosynthesis apparatus to changed light conditions. PsrR1 regulates photosystem components, phycobili proteins, and chlorophyll biogenesis proteins by e.g. translation inhibition and RNase E recruitment. Photosynthesis involves many iron-containing proteins not found in other bacteria, e.g. in the electron transport chain or in photosystem I. IsaR1 is up-regulated upon iron limitation and a major regulator of iron homeostasis. It has an extended target set and directly and indirectly controls photosynthesis.

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CVP14
Bacillus subtilis* 6S-2 RNA serves as a template for pRNA transcription *in vivo

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Introduction: 6S RNA is abundant in a broad range of bacteria where it acts as a global transcriptional regulator by binding to the active site of housekeeping RNA polymerase (RNAP) holoenzymes to inhibit transcription from DNA promoters [1]. The transcriptional block is released upon a structural rearrangement of 6S RNA, induced by so-called product RNAs (pRNAs) transcribed from 6S RNA itself as a template. While most bacteria express a single 6S RNA, some harbor a second 6S RNA homolog (termed 6S-2 RNA in *Bacillus subtilis*) [2,3].
Objectives: Clarification whether *B. subtilis* RNAP synthesizes pRNAs on 6S-2 RNA to release RNAP from sequestration by 6S-2 RNA.
Methods: In this work, we analyzed if *B. subtilis* 6S-2 pRNAs are synthesized *in vivo*, applying (i) RNA-seq of cellular RNA preparations enriched for small RNAs, (ii) a special Northern blot procedure and (iii) a newly developed primer extension assay that utilizes pRNAs as primers for revers transcription.
Results: While *B. subtilis* 6S-2 RNA was recently shown to exhibit essentially all hallmark features of a *bona fide* 6S RNA *in vitro* [4],

evidence for the synthesis of 6S-2 RNA derived pRNAs *in vivo* has been lacking so far, which raised doubts about 6S-2 RNA acting as a genuine 6S RNA. Here we demonstrate, for the first time and by application of three independent approaches, that 6S-2 RNA is able to serve as a template for pRNA synthesis *in vivo*, thereby answering the question how RNAP is released from its sequestration by 6S-2 RNA *in vivo*.
Conclusion: The second 6S RNA homolog of *B. subtilis*, 6S-2 RNA, is a genuine 6S RNA that serves as a template for pRNA synthesis *in vivo* to release RNAP from its sequestration by 6S-2 RNA.

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CVP15

Double-comparative transcriptomics suggests a high level of variability within the non-coding share of the transcriptome and the existence of actuatons, a new type of genetic element in bacteria

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Question: In eukaryotic organisms, differences in the regulation of gene expression and the composition of the transcriptome have been recognized as important elements for physiological and developmental differences between closely related species. Accordingly, a substantial share of the transcriptome consists of non-coding and antisense RNAs, many of which have regulatory impact. In a striking analogy, up to two thirds of all transcription start sites (TSSs) in bacteria produce non-coding transcripts. However, the extent at which this pervasive transcription is functional is only partly understood.
Methods: We present a double-comparative approach in which we have compared the primary transcriptomes of the cyanobacterium *Synechocystis* sp. PCC 6714 under 10 different conditions with those of *Synechocystis* sp. PCC 6803.
Results: Both strains are closely related to each other, as is indicated by 2854 shared protein-coding genes and a 16S rRNA identity of 99.4%. Based on genome-wide maps of transcriptional start sites (TSSs), operon structures, and the classification of different types of transcripts, non-coding transcripts were identified as the most dynamic component of the transcriptome. We identified a class of genes that lack a specific TSS but whose mRNAs instead originate from the transcription of a small RNA that accumulates as a discrete and abundant transcript while also serving as the 5' UTR of the adjacent protein-coding gene. Such an sRNA/mRNA structure, which we name 'actuaton', appears as a means by which bacteria remodel their transcriptional network.
Conclusions: Our findings support the hypothesis that fluctuations in the non-coding transcriptome constitute a major evolutionary element of inter-strain divergence and the capability for physiological adaptation among bacteria.

CVP16

Hfq and YbeY - impact on *Agrobacterium tumefaciens* riboregulation

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In the past years small noncoding RNAs (sRNAs) have received enormous attention as a new class of gene expression regulators. The largest and most extensively studied set of sRNAs act through base pairing with target RNAs, usually modulating the translation and stability of mRNAs.
The RNA chaperone Hfq directly influences the stability of RNA molecules and mediates the interaction between regulatory sRNAs and their mRNA targets [1]. Hfq affects bacterial physiology including growth, motility and resistance towards environmental stresses and plays an important role in microbe-host interactions as shown for a number of pathogenic and symbiotic bacteria. The ribonuclease YbeY was recently identified in *Escherichia coli* and potentially influences riboregulation in several proteobacteria. YbeY has been shown to participate in sRNA regulation and the processing of all three types of rRNA thus influencing ribosome maturation [2].
Here, we demonstrate the significance of Hfq and the YbeY homolog Atu0358 for physiology of the plant pathogen *Agrobacterium tumefaciens* [3, 4]. Construction of *hfq* and *ybeY* deletion strains and their phenotypical characterization as well as detailed comparison of WT vs mutant proteomes indicate a crucial role of both proteins in nutrient acquisition, general metabolism and virulence. RNA-immunoprecipitation sequencing (RIP-seq) with Hfq^{3xFLAG} bound RNAs revealed binding of numerous cellular RNAs by Hfq. The majority of Hfq-bound regulatory RNAs in *A. tumefaciens* are encoded antisense to protein-coding genes, indicating or role for Hfq in antisense RNA-mediated gene regulation.

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CVP17

RSs0827 - A small non-coding RNA which is strongly induced during iron limitation in *Rhodobacter sphaeroides*

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The phototrophic model bacterium *Rhodobacter sphaeroides* expresses several small non-coding RNAs (sRNAs) upon various stress conditions. In an RNA-seq approach under iron limitation we identified the strongly induced trans-encoded sRNA RSs0827 (Remes *et al*., 2014). A role of sRNAs in regulation of iron metabolism is known in other bacteria for instance in *E. coli* RyhB regulates the expression of the *fur* gene (Vecerek *et al*., 2007) and is responsible for differential degradation of the polycistronic *iscRSUA* mRNA (Desnoyers *et al*., 2009). A global stress screening showed that RSs0827 is further induced in response to heat, $^1O_2$, $CdCl_2$ or peroxide stress in stationary phase. In response to these stress conditions alternative sigma factors are activated. Northern blot analysis revealed that RSs0827 is transcribed from an $RpoH_I/RpoH_{II}$-dependent promoter (Nuss *et al*., 2010).
To elucidate the impact of RSs0827, we constructed an RSs0827 deletion strain and compared mRNA levels in the mutant and wildtyp by applying a high-density oligonucleotide microarray comparing the mutant to its parental wild type strain. Combining our transcriptome data and a bioinformatic prediction (IntaRNA) we identified several putative targets. To test the putative interaction of RSs0827 with the predicted mRNAs we used an established *in vivo* reporter system for *R. sphaeroides* (Mank *et al*., 2012) in the wildtyp, the RSs0827 deletion and RSs0827 overexpression strain. In this two-plasmid system the mRNAs of the putative target genes are translationally fused to the *lacZ* gene on plasmid pPHU235 under the control of the constitutive 16S rRNA promoter, respectively.

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CVP18

Comparative study of tRNA processing in *Aquifex aeolicus* and *Bacillus subtilis*

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Transfer RNAs (tRNAs) are adaptor molecules that link the genetic code to specific amino acids to be delivered to the ribosome. In almost all organisms, tRNAs are transcribed as precursor tRNAs (pre-tRNAs) that require processing to produce functional tRNA molecules. One of these processing enzymes is RNase P which endonucleolytically removes the 5'-leader of the tRNA. In bacteria, archaea, many eukaryotic nuclei and organelles, RNase P is composed of a catalytically RNA subunit and a varying number of protein subunits, one in bacteria and upto ten in eukarya. A few years ago, a new type of RNase P was found in human mitochondria and subsequently discovered in land plants as well as kinetoplastida. This form lacks the RNA subunit and consists of one to three protein subunits. It is called proteinaceous RNase P (PRORP) [1-3]. In the hyperthermophilic bacterium *Aquifex aeolicus*, neither a gene for the RNA nor the protein component of bacterial RNase P could be identified in its sequenced genome [4-5]. However, RNase P activity can be detected in *A. aeolicus* cell lysates [6] and we were able to enrich the

RNase P activity of *A. aeolicus* by several chromatography steps. With this study we want to address the mechanism of the tRNA 5´-end processing in *A. aeolicus*.
Starting from a conformationally stable class I tRNAas reference substrate we designed different tRNA variants that were incubated with partially purified *A. aeolicus* cell lysate and compared to reactions catalysed by the *Bacillus subtilis* RNase P holoenzyme.
A.eolicus RNase P is able to process different tRNA variants which sometimes differs from *B. subtilis* RNase P processing.

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CVP19
Functional network of tRNA wobble base and anticodon loop modifications
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Post-transcriptional modifications of tRNA occur at different positions, including the anticodon but individual and potentially collaborative contributions to tRNA function are not well understood. Synthetic genetic array data from the model organism *Saccharomyces cerevisiae* indicated the existence of a negative genetic interaction network between wobble uridine modifications and modified bases at the 3' side of the anticodon. We confirm such networks by demonstrating that combined loss of modifications results in synergistic growth defects and stress sensitivity, indicating the importance of simultaneous presence of base modifications for tRNA function. We predicted affected tRNA species based on a combination of primary tRNA sequence and known identity of modification targets and verified them by phenotypic suppression induced by overexpression of individual tRNAs. These efforts identify specific tRNAs that become functionally impaired in the combined absence of wobble uridine and anticodon loop modifications, suggesting a functional cross talk of base modifications occurring within the anticodon itself and neighboring positions in two specific tRNAs.
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CVP20
The bifunctional Urm1-Uba4 system is conserved in eukaryotes
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Urm1 (ubiquitin related modifier 1) from the budding yeast *Saccharomyces cerevisiae* has dual roles as a ubiquitin-like modifier in a protein conjugation pathway termed urmylation and as a sulfur donor in a thiolation pathway that modifies tRNA anticodons. To study whether both of these functions are conserved among lower and higher eukaryal models, we substituted yeast Urm1 and its E1-like activator, Uba4, for their respective human counterparts, hURM1 and hUBA4 (also known as MOCS3). Here, we demonstrate that either alone or in combination, hURM1 and hUBA4 are able to functionally replace their yeast orthologs. Thus, they operate in tRNA anticodon thiolation and mediate urmylation of Ahp1, a peroxiredoxin from yeast known to be targeted for conjugation by Urm1. In addition, we find that similar to previous reports on urmylation of hUba4, yeast Uba4 in itself qualifies as a target for Urm1 conjugation in *S. cerevisiae*, suggesting that urmylation targets are conserved among yeast and human cells. Taken together, our study shows that dual roles of Urm1 and the Urm1-Uba4 system in protein urmylation and tRNA thiolation are conserved and hence, functionally exchangeable between eukaryal cells.

CVP21
Identification of the small RNA interactome of *Sulfolobus acidocaldarius*
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Introduction: Small non-coding RNAs (sRNAs) are key elements for the regulation of cellular processes. C/D box sRNAs guide the site-specific methylation of ribosomal RNA in archaea and eukarya.
Objectives: We aim to characterize the sRNA interactome of *Sulfolobus acidocaldarius* in order to identify C/D box sRNAs involved in ribosomal RNA methylation process and to experimentally verify the methylation sites. Furthermore, we aim to identify sRNA interactions with the archaeal LSm proteins and mRNA targets.
Methods: Three small RNA-seq data sets are produced in this study: i) sRNome; small RNAs are isolated from *S. acidocaldarius* and deep-sequencing is performed via Illumina technology. ii) sRNA-protein interactome; the C/D box sRNA binding protein L7Ae and the LSm proteins are genomically tagged for subsequent co-immunoprecipitation of the interacting sRNAs. iii) rRNA-methylome; ribosomal methylation sites are analyzed combining the previously developed tool RTL-P with RNA-seq [1].
Results: The sRNome profile revealed a high abundance of C/D box sRNAs in *S. acidocaldarius*. The Flag-HA tagging of the sRNA binding proteins L7Ae and LSm 1-3 resulted in significant growth deficiency for tagged LSm1 and LSm2 strains, presumably due to hindrance of the LSm ring formation caused by the tag. In contrast, His-tagging of the three LSm proteins exhibited normal growth. L7Ae and the LSm proteins were purified via immunoprecipitation, revealing the co-isolation of small RNAs. The L7Ae purification showed co-immunoprecipitation of proteins matching to the size of fibrillarin and Nop5, which are part of the methylation complex formed by C/D box sRNAs and L7Ae.
Conclusion: These first results provide a basis for the analysis of rRNA methylation events guided by the C/D box sRNAs. Sequencing of the co-isolated sRNAs of the LSm proteins should allow the discovery of novel targets for trans-acting sRNAs.

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CVP22
The sRNA SorY confers resistance during photooxidative stress by affecting a metabolite transporter in *Rhodobacter sphaeroides*
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Exposure to oxygen and light generates photooxidative stress by the bacteriochlorophyll *a* mediated formation of singlet oxygen ($^1O_2$) in the facultative photosynthetic bacterium *Rhodobacter sphaeroides* (1). We have identified SorY as an Hfq dependent sRNA, which is induced under several stress conditions and confers increased resistance against $^1O_2$. SorY by direct interaction decreases the levels of *takP* mRNA, encoding a TRAP-T transporter. A *takP* mutant shows higher resistance to $^1O_2$ than the wild type, which is no longer affected by SorY. We present a model in which SorY reduces the metabolite flux into the TCA cycle by reducing malate import through TakP. It was previously shown that oxidative stress in bacteria leads to a switch from glycolysis to the pentose phosphate pathway and to reduced activity of the tricaboxylic acid cycle (2). As a consequence the production of the prooxidant NADH is reduced and production of the protective NADPH is enhanced. In *R. sphaeroides* enzymes for glycolysis, pentose phosphate pathway, Entner-Doudoroff pathway and gluconeogenesis are induced in response to $^1O_2$ by the alternative sigma factor RpoHII. The same is true for the sRNA SorY. By limiting malate import SorY thus contributes to the balance of the metabolic fluxes under photooxidative stress conditions. This assigns a so far unknown function to an sRNA in oxidative stress response.

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CVP23

***Streptococcus pneumoniae* infection of bronchial epithelial cells induces specific changes in microRNA profile**

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Streptococcus pneumoniae is a Gram-positive lactobacillales that usually colonizes the human nasopharynx but is also an important pathogen causing fatal infections like pneumonia. Within the past years it was found that microRNAs (miRNAs) play a crucial role as important transcriptional regulators of gene expression and inflammatory host response in infectious diseases. To date, there are no data addressing miRNA expression after pneumococci infection. For this purpose, we performed a global miRNA expression analysis of human bronchial epithelial cells (Beas-2B) infected with two different multiplicities of infection (MOIs) of *S. pneumoniae* (strain D39) compared to mock-infected and LTA-stimulated cells by Taqman Low Density Arrays (TLDA). Out of 759 examined miRNAs we found 356 to be expressed. In total one miRNAs was detected in all infected and stimulated cells compared to mock infection. This miRNA, the inflammation-associated miRNA-146a, was validated in qPCR as significantly regulated. With regard to the MOIs we identified 30 and 17 significantly deregulated miRNAs after *S. pneumoniae* infection in contrast to mock infection. In LTA-stimulated Beas-2B compared to mock infection we determined 18 significantly deregulated miRNAs. To identify miRNAs specific for *S. pneumoniae* infection we compared the results with LTA-stimulated cells. There, only three overlapping miRNAs to *S. pneumoniae* infection were detected. The results indicate that the miRNA profile is specifically altered in *S. pneumoniae* infection compared to LTA stimulation. These miRNAs might play an important role in human host response to *Streptococcus pneumoniae*. Further analysis and greater knowledge of the effect of this deregulated miRNAs within the infection could reveal potential therapeutic targets.

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CVP24

Target identification of sRNAs 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 30 sRNA genes, the biological roles of a variety of sRNAs could be unraveled (3). Apart from two examples in *Methanosarcina mazei*, target mRNAs of archaeal sRNAs and the interaction sites are unknown. To identify putative targets, we selected several sRNA genes and compared the transcriptomes of the respective deletion mutants with that of the wildtype. This led to the identification of several putative target mRNAs.

For $sRNA_{132}$, a potential target operon encoding an ABC-transporter with phosphate as potential substrate was identified. Using Northern Blot analysis, the transcript levels were determined in the wildtype and the deletion mutant, and the phosphate-dependency was quantified. A potential binding site in the 3´-UTR of the operon mRNA could be predicted. Fusion of the 3´-UTR of the potential target mRNA with a reporter gene confirmed the influence of the $sRNA_{132}$ on the 3´-UTR of the regulated gene.

Another example is $sRNA_{63,}$ for which the flagellin transcript was identified as a potential target. In this case, the predicted interaction site lies within the ORF. Interestingly, the transcript level of the flagellin gene is higher in the $sRNA_{63}$ deletion mutant. In congruence with this observation, the mutant has a higher swarming motility than the wildtype. Complementation of $sRNA_{63}$ nearly restored the amount of wildtype flagellin transcript level and will be further investigated. Mutations in the $sRNA_{63}$ sequence will be used to characterize the interaction between $sRNA_{63}$ and *fla*-mRNA.

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CVP25

Role of Kti12 in the tRNA modification function of yeast Elongator complex

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Elongator is a six subunit complex (Elp1-6) required for efficient tRNA anticodon modifications. Recent studies showed that Elongator function depends on antagonistic regulation by casein kinase Hrr25 and protein phosphatase Sit4 (1). Although Hrr25 kinase copurifies with Elongator complex, there is no evidence that Sit4 interacts directly with Elongator. Here we show that, Kti12, a protein that always copurifies with Elongator complex, also interacts with Sit4. Kti12 interactions with Elongator complex or Sit4 depends on a conserved P-loop and a putative calmodulin binding motif in the Kti12 amino terminal domain.

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CVP26

Regulation of the RNA polymerase by 6S RNAs in *Bacillus subtilis*: role of the delta subunit

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Introduction: 6S RNA (~200 nt) is a global regulator of RNA polymerase (RNAP) in bacteria. Unlike most other bacteria, the *Bacillus subtilis* genome harbors two 6S RNA homologs, termed 6S-1 (*bsrA*) and 6S-2 RNA (*bsrB*) [1]. After binding to the active site of RNAP, 6S RNA itself serves as a template for the transcription of short "product RNAs", called pRNAs [2].

The *B. subtilis* housekeeping RNAP holoenzyme is a complex of several subunits ($\alpha_2\beta\beta'$) forming the core enzyme which displays minimal catalytic activity. The core enzyme must bind to σ^A, the most abundant sigma factor in *B. subtilis,* for specific recognition of promoter regions [3]. Furthermore, $\alpha_2\beta\beta'$ is associated with two small accessory subunits (ω_1 and ω_2) of unknown function and a 20.4 kDa delta subunit (δ) encoded by the *rpoE* gene [4]. Depending on growth conditions and template, delta can diminish or enhance the transcription rate [5].

Objectives: In this work, we investigated the role of delta on the 6S-1 RNA-templated pRNA synthesis rate and the pRNAs length distribution.

Methods: pRNA transcription and electrophoretic mobility shift assays were performed to characterize the effect of delta *in vitro*. 6S-1 RNA levels *in vivo* were analyzed by Northern blotting.

Results: We could show that increasing concentrations of delta favour shorter 6S-1 pRNA transcripts, possibly due to lower affinity of 6S-1 RNA to RNAP. Furthermore, Northern blot experiments revealed an increase in the levels of mature 6S-1 RNA and its 5'-precursor in delta knockout strains, indicating that the average dwell time of 6S-1 RNA on RNAP is longer in the absence of delta, resulting in enhanced overall protection of the RNA from decay.

Conclusion: We show that the delta subunit affects the cellular levels of 6S-1 RNA as well as the 6S-1 pRNA length distribution, thus revealing the interdigitation of two regulatory factors of RNAP activity.

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EMP01

A Genomic Approach for Probing a Possible Soil-Borne Life Cycle of *Bacillus anthracis*

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Introduction: *Bacillus anthracis*, the causative agent of anthrax-disease, uses its host for massive cell proliferation. After demise of the animal the pathogen is disseminated from the carcass as endospores that can survive in the environment for several decades. However we still know very little about the ability of the bacteria to replicate in the environment without the host-animal. In experimental settings *B. anthracis* is able to multiply in soil-dwelling amoebae and even survive as a saprophyte. Strains lacking one or both virulence plasmids can frequently be isolated from soil. This loss might be an indication of a soil-borne cycle of the pathogen.
Objectives: To test the hypothesis of a soil-borne lifecycle we investigated two burial sites where two bovines were buried during an anthrax epidemic in Pollino Natural Park (Italy) in 2004. If there is *B. anthracis*-proliferation then genotypes of strains isolated from near the surface (5-cm) of contaminated soil should be on a different evolutionary trajectory from those residing at 100-cm depth near the carcass. It was the expectation that the surface population would yield a higher genetic diversity. The aim of this project was testing such microevolution using genomic tools.
Methods: The genetic diversity of randomly picked *B. anthracis*-isolates was compared by 31-Loci MLVA, 4-loci SNR analysis and whole genome sequencing.
Results: MLVA-31 analysis of 93 isolates only revealed three differences, none of which in near-surface isolates. SNR 4-loci-screening of 93 isolates revealed eight differences (in loci HM1 or HM2, respectively) three of which in 5-cm isolates. Except of one repeat count reduction by 4 bp in a surface-isolate, which was a novel allele, the other SNR-alleles had been observed before in genotyping analysis of the 2004 outbreak isolates. Genome sequencing of nine 5-cm- and 100-cm-isolates, respectively, revealed five isolate-specific SNPs, four of which only found in different isolates from 5-cm. Notably, one of these randomly-picked surface-isolates lacked plasmid pXO1.
Conclusion: Our findings do not yet provide a clear-cut answer on the extent of a soil-borne cycle of *B. anthracis*. However, loss of a virulence plasmid and a higher number of isolate-specific SNPs in 5-cm-compared to 100-cm-isolates suggest possible proliferation in soil.

EMP02

Detection of allelic variants of hexokinases and the hexose transporter Hxt3p in strains of *Saccharomyces cerevisiae* and interspecies hybrids with different fermentation performances

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Introduction: The ability of most yeast strains to utilize preferentially glucose in comparison to fructose is a major cause of sluggish and stuck grape wine fermentation, in particular in combination with nutritional imbalance and high ethanol concentrations in the must. Variations in the molecular structure of hexose transporters and kinases may have an impact on the ability of *Saccharomyces* wine strains to finish fructose fermentation even under stressful environmental conditions.
Objectives: It has been demonstrated that a complex interaction of hexose transporters and hexokinases determines the fermentation efficiency of yeast strains and their preference for glucose or fructose. However, most of the studies have been performed with laboratory strains of *S. cerevisiae* and mutants thereof. The identification and characterization of hexose transporters and hexokinases gene variants is a tool to select strains with high fermentation performance despite environmental stress factors. For a better understanding and control of grape must fermentations we studied the distribution of allelic variations of *HXT3, HXK1* and *HXK2* in a number of laboratory, industrial and natural occurring *Saccharomyces* strains and interspecies hybrids.
Materials & Methods: We sequenced and compared genes encoding Hxt3p and the kinases Hxk1p/Hxk2p of *Saccharomyces* strains and interspecies hybrids with different industrial usage and regional background.
Results: The Hxt3p primary structure varied in a small set of amino acids, which characterized robust yeast strains used for production of sparkling wine or to restart stuck fermentations. In addition, interspecies hybrid strains, previously isolated at the end of spontaneous fermentations, revealed a common amino acids signature. In contrast, both hexokinase genes were rather conserved in different *Saccharomyces* strains and hybrids.
Conclusion: Molecular variants of the hexose carrier Hxt3p but not of kinases correlate with different fermentation performances of yeast.

EMP03

Poking around in the 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- and single cell genomics powerful tools to probe biological "dark matter"[1].
Objectives: In order to maximize the gain of sequence information from environmental samples we combined meta- and single cell genomic approaches. We also evaluated different bioinformatics tools and their effect on the interpretation of phylogenetic and functional diversity, using Marburg forest soil as an example.
Methods: DNA extracts and single cells from Marburg forest soil were sequenced on Illumina® HiSeq and MiSeq platforms, respectively. 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.
Results: We show that the choice of assembly algorithm and analysis tools affects the observed phylogenetic and functional composition of metagenome datasets and therefore their utilization for complementing single cell genomes. In addition, single cell assemblies can be greatly optimized in terms of genome completeness by tweaking the corresponding assembly pipelines.
Conclusion: Metagenomic and single cell sequencing approaches are highly complementary for the analyses of yet uncultivated microorganisms. Metagenomic data can enhance single cell sequencing approaches, not only by producing highly specific screening primers, but also by supplementing single cell assemblies to produce more complete pan genomes. However, fine-tuning the applied bioinformatics pipelines for individual dataset is crucial for reconstructing genomes of single community members from metagenomes as well as from single cells.

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EMP04

Genome sequence of *Clostridium sporogenes* DSM 795^T, an amino acid-degrading, nontoxic surrogate of neurotoxin-producing *Clostridium botulinum*

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Introduction: *Clostridium sporogenes* DSM 795^T is a Gram-positive, rod-shaped, anaerobic bacterium isolated from human faeces [1] but can also be found in soil and marine or fresh water sediments [2]. This species belongs to the proteolytic branch of clostridia and is known as nontoxic surrogate of the food-borne and neurotoxin-producing pathogen *C. botulinum*. Analysis of the 16S rDNA revealed a 99.7 % sequence similarity to proteolytic *C. botulinum* strains of serotypes A, B and F. *C. sporogenes* attracts special interest because of its potential use in a bacterial therapy for certain cancer types.
Objectives: The genome of *C. sporogenes* was sequenced to get information about the metabolic potential of this organism, but also to

show how closely related it is to the neurotoxin-producing pathogen *C. botulinum*.
Methods: Whole genome sequencing was done with the 454 GS-FLX Titanium XL, the Genome Analyzer II and the MiSeq system**.** The hybrid *de novo* assembly was performed with the Mira 3.4 and the Newbler 2.8 software. For scaffolding, we used the Mauve Genome Alignment Software, the Gene Ortholog Neighborhood tool [3] and the genomes of *C. sporogenes* ATCC15579 and *C. botulinum* ATCC3502 as references.
Results: The genome of *C. sporogenes* consists of a circular chromosome of 4.1 Mb with an overall GC content of 27.81 mol% harboring 3744 protein-coding genes, and 80 RNAs. Genome sequencing and annotation revealed several gene clusters coding for proteins involved in anaerobic degradation of amino acids, such as glycine and betaine, via Stickland reaction. Organisation of these clusters is very similar to those identified in *Eubacterium acidaminophilum* al-2 DSM 3953 and *Sporomusa ovata* H1 DSM 2662. Genome comparison showed that *C. sporogenes* is closely related to *C. botulinum serotypes* A, B and F.
Conclusion: *C. sporogenes* is a member of the proteolytic branch of clostridia and shows high sequence homology to *C. botulinum,* but is due to the lack of the neurotoxin cluster the nontoxic counterpart of this pathogenic organism**.**

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EMP05
Evolutionary changes in bacterial genomes on the level of speciation: possibilities for genetic barcoding of closely related species and their biological activities
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Availability of complete genome sequences of micro-organisms belonging to the same species and/or subspecies made it possible to study micro-evolutionary processes on the level of individual genes. Only from recently the scientists have become able to have a closer look at genetic peculiarities of bacteria, which having no differences in phenotype might show significant differences in the severity of virulence manifestations or in their biotechnological activities. Firstly the attention was paid to the presence or absence of several accessory genes, mostly horizontally transferred ones. However, the focus of this work is on adaptive changes in the core genome of micro-organisms. It was found out that the adaptation to a new habitat causes a fast accumulation of positively selected nucleotide and amino acid substitutions in a part of the core genome, while other genes experience only random neutral substitutions. In this work a group of recently sequenced *Bacillus* strains was studied, all belonging to closely related species of *B. subtilis* group, which included active plant growth promoting bacteria from rhizosphere and inactive soil dwelling strains. In total 150 core genes were found, which experienced the positive Darwinian selection when plant associated and soil dwelling strains were compared. Among proteins encoded by these genes there were amino acid, carbohydrate and nitrogen metabolism enzymes; membrane transporters and proteins of several other categories. Strain specific genetic barcodes were developed based on DNA sequences of these genes. These barcodes then were tested on several publically available metagenomic datasets by using an in-house Python script based on a local BLASTN mapping followed by a statistical processing of alignment scores. Remarkably dissimilar patterns of abundance of different representatives of this group were obtained despite of the fact that all these bacteria hardly were distinguishable neither by phenotype nor by 16S rRNA sequences. A Python based program for visualization and selection of the genes involved in bacterial speciation is proposed.

EMP06
Anaerobic Degradation of BP Oil Spills in the Coastal Sediments of Louisiana, USA
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The significant challenges presented by the April 20, 2010 explosion, sinking, and subsequent oil spill of the Deep water Horizon drilling platform in Canyon Block 252 about 52 miles southeast of Venice, Louisiana, USA greatly impacted Louisiana's coastal ecosystem including the sea food industry, recreational fishing, and tourism. The short term and long term impact of this oil spill are significant and the Deep water Horizon spill is potentially both an economic and an ecological disaster. Microbes present in the water column and sediments have the potential to degrade the oil. Oil degradation could be enhanced by bio-stimulation method.
The conventional approach to bioremediation of petroleum hydrocarbon is based on aerobic processes. Anaerobic bioremediation has been tested only in a very few cases and is still considered experimental. The currently practiced conventional in-situ biorestoration of petroleum-contaminated soils, and ground water relies on the supply of oxygen to the sub-surface to enhance natural aerobic processes to remediate the contaminants. However, anaerobic microbial processes can be significant in oxygen-depleted sub-surface environments and sediments that are contaminated with petroleum-based compounds such as oil-impacted marshes in Louisiana. The goal of this work was to identify the right conditions for the indigenous anaerobic bacteria present in the contaminated sites to enhance degradation of petroleum hydrocarbons. We evaluated the ability of microorganisms under a variety of electron acceptor conditions to degrade petroleum hydrocarbons. Researched microbial systems include sulfate-, nitrate-reducing bacteria, and fermenting bacteria. The results indicated that anaerobic bacteria are viable candidates for bioremediation. Enhanced biodegradation was attained under mixed electron acceptor conditions, where various electron-accepting anaerobes co-existed and aided in degrading complex petroleum hydrocarbon components of marsh sediments in the coastal Louisiana. Significant degradation of oil also occurred under sulfate reducing and nitrate reducing conditions.

EMP07
A metaproteomic approach for analysis of microbial community structure and function for improvement of biogas plant performance
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Introduction: To avoid downtime in operation and to further optimize operation of biogas plant (BGP) profound understanding of the composition and function of microbial community converting the biomass to methane is required.
Objectives: Our main goal is to understand how process conditions determine the process performance, the taxonomic and functional composition of the microbial community and vice versa. For such investigations, the quantification of the composition of the active community is essential. In particular, the presence of key enzymes of metabolic pathway correlates well with the community activity. Thus, the present study investigated the metaproteome of 40 industrial-scale BGPs.
Materials & Methods: The applied metaproteome workflow involved protein extraction using liquid phenol, tryptic digestion, peptide separation by liquid chromatography coupled to tandem mass spectrometry (Velos Orbitrap Elite), and data analysis with the *MetaProteomeAnalyzer* software [1].
Results: For each BGP, about 500 proteins, covering the main metabolic pathways of the biogas process, namely hydrolysis, fermentation, acetogenesis and methanogenesis, were identified. The microbial communities detected by metaproteome analysis of the BGPs clustered according process temperature (mesophilic and thermophilic) [2,3], and substrate composition.
Conclusion: Application of metaproteomics allowed the acquisition of profound knowledge about community structure and function of microbial communities and contribute to our understanding of the conversion of biomass into methane. This knowledge could be used to improve monitoring and control of BGP, and to support development of new BGP designs.

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EMP08

Marine hydrocarbonoclastic bacteria and their ability to adapt to their environment and aliphatic substrates

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The marine hydrocarbonoclastic bacterium *Alcanivorax borkumensis* is able to degrade mixtures of n-alkanes as they occur in marine oil spills. Growth behaviour and physiology of these bacteria cultivated with n-alkanes of different chain lengths (C6-C30) as substrates was investigated. Growth rates increased with increasing alkane chain length up to a maximum between C12 and C19, with no evident difference between even and odd numbered chain lengths, before decreasing with chain length greater than C19. Surface hydrophobicity of alkane-grown cells, measured as water contact angles, showed a similar pattern with maximum values associated with growth rates on alkanes with chain lengths between C11 and C19, and was significantly lower for cells grown on pyruvate. *A. borkumensis* was found to incorporate and modify the fatty acid intermediates generated by the corresponding n-alkane degradation pathway. Cell grown on distinct n-alkanes proved the capability to not only incorporate but also modify fatty intermediates derived from the alkane degradation pathway. Comparing cells grown on pyruvate with those cultivated on hexadecane showed similar tolerances towards toxic concentrations of chlorophenols, whereas tolerance to different n-alkanols was significantly increased when hexadecane was used as carbon source. These findings could be verified by a detailed transcriptomic comparison between cultures grown on hexadecane and pyruvate including solvent stress caused by addition of 1-octanol as the most toxic intermediate of n-alkane degradation.

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EMP09

Identification and characterization of a novel diamine monooxygenase from *Gordonia* sp. CWB2

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Introduction: The microbial diamine monooxygenases are explored as drug targets and part of siderophore production machinery [1]. But, they are still little characterized to date. Usually these enzymes hydroxylate an amino group of a diamine as putrescine or cadaverine and some even act on amino acids as leucine and ornithine.
Objectives: Attempt was made here to identify, clone, overexpress and characterize monooxygenases from the *Gordonia* sp. CWB2 as well as from an enrichment culture which was made possible through the corresponding Genome-Projects (unpublished).
Materials & Methods: The NCBI and GENDB databases were used for BLAST searches with respect to monooxygenases. Cloning and overexpression of the putative diamine monoxygenases in a suitable vector and expression system was done according to conventional procedures [2]. Purification of His_{10}Tag-protein was achieved by Ni chelate chromatography on a 1-ml HisTrap FF column, using an ÄKTA fast-performance liquid chromatographer. Production of the novel diamine monooxygenase was confirmed by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis and activity assays [1,2].
Results: Two novel diamine monooxygenases from *Gordonia* sp. CWB2 and enrichment culture were predicted as belonging to the *L*-lysine *N*(6)-hydroxylase/*L*-ornithine *N*(5)-oxygenase family with 438 and 458 amino acid sequence length, respectively. One of these putative enzymes with a molecular weight of approximately 50kDa has been successfully cloned, overexpressed and purified using *Escherichia coli* DH5α and BL21(DE3) as the cloning and expression hosts, respectively, and the plasmid pET16Bp as the vector for expression. However, it converts only smaller diamines and no amino acid like substrates and is restricted to certain buffers. The second enzyme from the enrichment awaits its functional assignment maybe due to improper substrates applied so far.
Conclusion: A further analysis on the activity of these enzymes is necessary to elucidate more on their functions and to draw conclusions on their metabolic role.

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EMP10

The German Federation for the Curation of Biological Data (GFBio)

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Introduction: Environmental and biological data are constantly increasing in scale and complexity. This adds new challenges to data handling, comparison, integration, publication and long-term archiving. The GFBio project, funded by the German Science Foundation (DFG), was established in 2013 to develop practical solutions for individual scientists and research projects.
Objectives: The main goal of GFBio is to provide a sustainable, service-oriented, national infrastructure to significantly improve the management, sharing, and reusability of biological data. The services provided by GFBio cover the full life cycle of research data from acquisition, through analysis, to long-term archiving and publication [1]. Researchers will be able to search, retrieve, visualize, analyze, and archive integrated biological and environmental data through the GFBio web portal.
Methods: The GFBio consortium brings together 19 national partners from all fields of biodiversity research, representing data producers, consumers and archives. It combines the extensive expertise of all partners to design, develop, and operate the infrastructure and its services.
Results: GFBio was recently launched and has already made considerable progress in harmonization of terminologies, standards and formats. A major effort to mobilize data from all relevant data sources like natural history collections and molecular databases is underway. New tools for integrated data submission, discovery, visualization and aggregation are being developed. All services will be accessible through a unified portal (www.gfbio.org).
Conclusion: GFBio will soon become the single point of entry for archiving, searching and retrieving integrated biological and environmental data in Germany.

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EMP11

Growth control by extrachromosomal elements in *Phaeobacter inhibens* DSM 17395

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The alphaproteobacterial *Roseobacter* group is one of the most abundant and ecophysiologically relevant marine bacterioplankton groups. With their diverse metabolic capabilities, roseobacters have a global impact on marine carbon and sulfur cycles. Sequencing of many *Roseobacter* genomes revealed an extraordinary wealth in extrachromosomal replication systems (plasmids, chromids) of variable size and number (up to twelve), which encode essential lifestyle determinants (e.g., photoheterotrophy). The role of extrachromosomal replicons for growth, survival and habitat success of roseobacters is largely unknown.
The chemoheterotrophic bacterium *Phaeobacter inhibens* DSM 17395 is a nutritionally versatile member of the *Roseobacter* group, harboring a chromosome (4.7 Mb) and three chromids with 65, 78 and 262 kb. Successive curing of chromids from wild type *P. inhibens* DSM 17395 resulted in seven mutants lacking one (Δ65, Δ78, Δ262), two (Δ65Δ78, Δ65Δ262, Δ78Δ262) or all three (Δ65Δ78Δ262) chromids. The impact of chromid loss on growth physiology and stoichiometry was studied in process-controlled bioreactors with casamino acids as carbon and energy source. Absence of chromids resulted in dramatic changes in growth rates

and growth yields that were specific (non-additive) for each genotype. Compared to the wild type, most chromid mutants displayed strongly elevated growth rates and yields (up to 3.7-fold). This unexpected growth benefit was accompanied by an increased consumption of casamino acids, including also those amino acids that were not utilized by the wild type. The results implicate that chromids negatively control the growth of wild type *P. inhibens* DSM 17395 (e.g., reduced substrate consumption, higher energy dissipation). The mechanistic basis and (eco-)physiological role of this chromid-mediated metabolic "brake" remains to be elucidated.

EMP12

6-thioguanine biosynthesis by pathogenic and non-pathogenic *Erwinia* species

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Introduction: The fire blight pathogen *Erwinia amylovora* secretes a compound with an absorbance maximum at 340 nm that forms a yellow colored complex with copper-ions. This compound could be identified as 6-thioguanine (6TG), a guanine analogue with medical applications used in chemotherapy. It is incorporated in DNA and competes with phosphorylation of guanosine. Synthesis of 6TG is common to pathogenic and non-pathogenic *Erwinia* species.
Objective: The influence of 6TG production on *E. amylovora* pathogenicity and on competitiveness of *Erwinia* species in their environment was evaluated.
Methods: Natural *E. amylovora* strains deficient in 6TG synthesis were compared to positive strains for virulence and knock-out mutants for 6TG production were created. 6TG synthesis in *Escherichia coli* was achieved and its influence on other bacterial species was compared with synthetic 6TG.
Results: Expression of four *Erwina tasmaniensis* genes (*tgsA-D*) was sufficient for heterologous production of 6TG in *E. coli*. Bacterial and synthetic 6TG had a strong growth inhibitory effect on many species, such as *E. coli* or several *Pantoea agglomerans* strains. Heterologous expression of the *tgs* operon did not increase tolerance against 6TG for susceptible isolates. In contrast to recent other publications, neither natural deficient isolates nor a *tgsA* deletion mutant of *E. amylovora* showed any link between 6-thioguanine production and pathogenicity. Symptom formation on apple and pear shoots was identical for 6TG positive and 6TG negative *E. amylovora* strains.
Conclusions: Synthesis of 6TG is highly conserved among pathogenic and non-pathogenic *Erwinia* species, although natural mutants occur with low frequency. Mutant analysis could not confirm a link between pathogenicity and 6TG production for *Erwinia amylovora*. The strong growth inhibitory effect of 6TG to several common epiphytic bacteria might provide a growth advantage also in the presence of other microorganisms. An additional regulatory function of 6TG cannot be excluded.

EMP13

Evaluation of the biodegradation products of Amlodipine Orotate by *Phanerochaete chrysosporium*

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Pharmaceuticals have long ago been used to treat diseases of humans and they are excreted to the environment sometimes in their original form or as byproducts of human metabolism. These pharmaceutical metabolites have been proven by studies to be harmful to aquatic life and may be persistent in different water systems. In this regard, there is a growing need to eliminate these compounds as well as their harmful metabolites in water. Biodegradation using white-rot fungi is a promising technology for the removal of recalcitrant compounds; however, products of fungal biodegradation can also be detrimental. Meanwhile, antihypertensive drugs are being used worldwide to treat high blood pressure and prevent complications such as stroke, myocardial infarction, etc. The extensive use of these drugs led to its detection in water matrices. In this study, we evaluated the extracellular metabolites of *Phanerochaete chrysosporium* in the presence of the anti-hypertensive drug Amlodipine after the degradation set-up of 120 hours. 92 significant metabolites (q value≤0.05) were found after FDR adjustment of *p* values of different m/z from q-TOF. Pyridine derivatives of amlodipine were still seen at 120 hours.
Objectives of the study: To degrade amlodipine and to evaluate the metabolites produced in the process with the aide of metabolomics and their possible ecotoxicity.
Methods: Biodegradation->q-TOF->Metabolomics->Metlin->KEGG
Results: Significant metabolites from differentially expressed features at a significance threshold of 0.05 were collected and m/z values were analyzed in Metlin online database for metabolites for the identification of the degradation products of Amlodipine. Among the many detected metabolites, a group of pyridine-containing compounds became the basis of our proposed pathway for amlodipine degradation by white-rot fungi. Despite the harmful effects of pyridine, this compound is readily degraded by bacteria to ammonia and carbon dioxide (Sims, G.K. and O'Loughlin, E.J., 1989).
Conclusion: In this study, we have shown the possibility of the antihypertensive drug amlodipine to be degraded by *Phanerochaete chrysosporium* based on the extracellular metabolites produced by the organism in the presence of the pharmaceutical.

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EMP14

Effects of Oxytetracycline on the Nitrogen Conversion in a Recirculating Aquaculture System

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Common aquaculture practices include the use of certain pharmaceutical products such as antibiotics in avoiding diseases and promoting healthier growth of the culture. The aim of this study to look into the effect of oxytetracycline, which are widely used in aquaculture practice, on the nitrogen transformation and its correlation to a selected nitrifier, *Nitrosomonas europaea,* and the possible microbial diversity shifts with respect to different levels of oxytetracycline present in the system. Results showed that 1 ppm and 2ppm of oxytetracycline could already entail responses from the system. The persistence of very low concentration of antibiotic corresponded to reduced performance in ammonia oxidation. Initial ammonia levels had a range of 0.16-0.32mg/L as compared to Phase 3 values which ranged from 0.4-0.57mg/L. The oxytetracycline was also found to inhibit the production of nitrous oxide.This inhibition corresponds to an observable increase in nitrite levels which is considered to be a key compound in the denitrification by nitrifying bacteria and a toxic compound in aquaculture. The oxytetracycline presence also significantly shifts the microbial diversity (pvalue= 0.039) which may also further explain the earlier result and may even entail other implications to the system. Oxytetracycline presence therefore reduces or even inhibits the release of nitrous oxide at the cost of reducing water quality of the system. Comparison between the pure culture experiment and the aquaculture experiment shows a clearer role of the ammonia oxidizers in the nitrogen conversion of a recirculating aquaculture system. The effect of oxytetracycline on the ammonia oxidation can be attributed solely to the ammonia oxidizers. Nitrous oxide in the system can be said to be mainly produced by denitrifiers so with the sharp decrease in N_2O production shows that the ability of denitrifiers were highly affected by the antibiotic. Therefore, it can be said that it might be because of the distruption in the denitrification causing an elevated flux in nitrate production. This could then be attributed to nitrite oxidizers and or denitrifiers.

FAO. The State of World Fisheries and Aquaculture. Food and Agriculture Organization of United Nations, Rome, Italy, 2012

EMP15

Latex clearing protein of *Streptomyces* sp. K30 is a *b*-type cytochrome

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Introduction: Two types of enzymes for oxidative cleavage of poly(*cis*-1,4-isoprene) (rubber) are known. One is rubber oxygenase A (RoxA) that is secreted by Gram-negative *Xanthomonas* sp. 35Y during growth on rubber. The RoxA structure shows a relationship to bacterial cytochrome *c* peroxidases (1). The other enzyme is latex-clearing-protein (Lcp) that is secreted by rubber degrading actinomycetes (2). Controversial reports of the metal content of Lcp were published: Hiessl *et al.* claimed that Lcp of *Gordonia polyisoprenivorans* VH2 is a copper containing protein (3) but our group did not detect copper in a related Lcp protein of *Streptomyces* sp. K30 (4).
Objectives: The aim of our research was to increase the yield of Lcp_{K30} by improvement of the expression system and subsequent purification via Strep-tag. Furthermore, the presence of metal ions in Lcp was investigated and a biochemical characterization of Lcp was conducted.
Methods: Overexpression in *E.coli*, Strep-tag purification, UV-vis spectroscopy, HPLC-MS.
Results: Two repetitions of metal analysis using high concentrations of purified Lcp_{K30} showed that it contained Fe in a 1 to 1 stoichiometry. In addition, sub-stoichiometric amounts of Ni (0.05 mol/mol Lcp) but no copper (≤0.008 mol/mol) were detected. We used the pyridine hemochrome assay (5) to show that Lcp is a *b*-type cytochrome with a non-covalently bound heme. The specific activities of purified Lcp and RoxA were in the same order of magnitude but Lcp degraded polyisoprene via endo-cleavage to tetra-(C_{20}) and higher oligo-isoprenoids with aldehyde and keto end groups whereas RoxA employed an exo-cleavage type mechanism to yield the main C_{15} product ODTD.
Conclusions: In conclusion, RoxA and Lcp represent two different extracellular enzymes catalyzing the oxidative cleavage of the hydrocarbon polyisoprene. We improved the expression system and purification significantly, established an activity assay and proved the presence of an iron containing, non-covalently bound *b*-type heme in Lcp compared to the *c*-type heme in RoxA.

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EMP16

Quorum sensing restrains growth and activates desiccation resistance in *Sinorhizobium meliloti*

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Microbial cooperative behaviors, such as quorum sensing (QS), improve survival but typically require costly contributions from limited resources. Inevitably, cooperation is vulnerable to damaging mutations which results in mutants that are relieved of the burden of contributing but nonetheless benefit from the contributions of their parent. Unless somehow prevented, such mutants may outcompete and replace the parent. This raises the question of how cooperation is stabilized. The bacterium *Sinorhizobium meliloti* uses QS to activate the production of copious levels of exopolysaccharide (EPS). Domestication of this bacterium is typified by the appearance of spontaneous mutants incapable of EPS production. We found that these mutants took advantage of EPS production by the parent and out-competed the parent. These were found to be QS mutants, demonstrating that QS was unstable under laboratory conditions and that the loss of QS capacity represents domestication of this bacterium. We traced this instability to several QS-regulated processes. Paradoxically, the major contribution is because QS restrains growth, providing the mutant with a significant growth advantage. In search of conditions where QS might be advantageous and more stable, we found that QS strongly enhances survival of desiccation, independently of EPS. A model is proposed whereby QS restrains population growth to prevent over-crowding and prepares the individual for the survival of desiccation and other severe conditions. These are novel roles for QS that can explain both the loss of EPS production during domestication and the advantage and stability of cooperation in natural microbial populations.

EMP17

The genomics of *Bacillus amyloliquefaciens* LFB112 and its regulations on intestinal microbiota and meat quality in broilers

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Introduction: *Bacillus amyloliquefaciens* LFB112 (LFB112) was isolated from Chinese herbs and the secretary substances of this strain show inhibitory activity against a wide range of pathogens [1, 2]. However, our knowledge of how LFB112 affects these processes is mostly unknown.
Objectives: The aim of the present study was to investigate the complete genome sequence of the probiotic strain LFB112 and its regulations on intestinal microbiota at the metagenomic level and meat quality of broilers.
Methods or Materials & Methods: The genome sequence of LFB112 was sequenced with Illumina Hiseq 2000 and gaps were closed by PCR and subsequent Sanger sequencing. Then the changes in the structure and abundance of broiler intestinal microbiota using Miseq sequencing technology after being fed with LFB112 were studied to clarify the mechanism of action of probiotics and the relationship between the structure changes of intestinal microbiota and meat quality.
Results: The genome sequence was deposited into the NCBI (Accession no. CP006952). The complete genome sequence of the strain is characterized by a circular chromosome of 3,942,754 bp with a 46.7% G + C content without plasmids. A total of 3961 coding DNA sequences, 94 tRNA genes and 10 RNA operons were predicted. RAST server based annotation of the whole genome showed the presence of 459 subsystems [2]. Genome analysis showed that LFB112 contains many gene clusters of secondary metabolite synthesis, especially the antibacterial substance gene clusters, confirming its excellent antibacterial properties. The bacterial diversity of the LFB112 group did not increase, but the structure stability of ileal microbiota and the abundance of the probiotic dominant bacteria, especially *Lactobacillus*, were strengthened, producing more fatty acids and improving growth performance and meat quality.
Conclusion: In conclusion, the *B. amyloliquefaciens* LFB112 genome information is consistent with antibacterial activity, whose effects on optimization of the structure of intestinal microbiota with *Lactobacillus* being the advantage bacteria may be the fundamental mechanism of regulating and optimizing meat quality in broilers.

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EMP18

Carbon flows during the anaerobic digestion in biogas plants

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Introduction: So far, more than 13.000 biogas plants all over Europa (Germany: > 7.800) were connected to the electricity network. Beyond many technical progresses, the complex microbiological degradation process of polymeric organic compounds and the carbon fluxes to CH_4 and CO_2 are not fully understood yet.
Objectives: With focus on the methanogenesis we investigated the impact of each methanogenic pathway (hydrogenotrophic, acetoclastic or methylotrophic) and the involved methanogenic species under defined process conditions.
Methods: To draw conclusions about the present methanogenic pathways and the correspondent microbiota, we used a combination of microbiological methods and stable isotope techniques. For this purpose, we selected and cultured organisms of four different methanogenic orders that are commonly found in biogas plants [1]. To study the pathway-dependent carbon-isotope fractionation in the evolving CH_4 and CO_2 we inoculated growth media including specific carbon sources with pure and mixed cultures. Additionally, we applied a combination of ^{2}H- and ^{13}C-labelled substrates to trace the specific metabolic pathways and typical carbon flows [2]. In addition, we performed real-time PCR analyses of weekly withdrawn reactor samples during a period of 3 months to get a quantitative overview about the concomitant microbiota.
Results: Batch- and pure culture experiments evidenced that the isotopic signature depends on the available carbon. These analyses also showed

some new results about the impact of the methylotrophic pathway which so far has been presumed to be negligible in biogas plants. Furthermore, the usage of ^{1-13}C and ^{2-13}C labelled acetate revealed novel informations about the usage and fluxes of the methyl and carboxyl group during acetate degradation.

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EMP19

Shifts in fungal and bacterial community structure and composition in the interface of vineyard and fruit-growing soils and preserved wood

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Copper-based preservatives are routinely used for both wood and plant protection, which finally leads to an enrichment of copper tolerant microbial communities in respective soil environments. Such communities are overall able to decompose copper-based preserved wood over a long time period and thus leading to major damages in wooden stakes. To investigate the effect of wood preservatives on fungal and bacterial community structure and composition, five different vineyard and fruit-growing soil environments were evaluated over time. In total, 440 soil samples (5 soil environments, 4 incubation times, 5 preservative treatments, 4 replicates, + 40 virulence controls) were collected across Germany and southern Europe and incubated in accelerated soil incubation studies. To test the efficacy of wood preservatives, wooden specimens were impregnated with water (A as reference) or different biocide-based preservation treatments (B=containing copper, triazoles and benzalkonium chloride; C=containing triazoles and benzalkonium chloride, encapsulated; D=containing triazoles and benzalkonium chloride, non-encapsulated; and E=containing copper). Samples were selected for next-generation sequencing and quantitative PCR by 16S rRNA and ITS gene region, respectively, based on mass loss and bending elasticity results. For all dominant taxa, the composition and diversity of fungal and bacterial communities were significantly environment specific and remained less affected by the wood preservative treatment and incubation time. Surprisingly, about 80 % and 30 % of the genera of the bacterial and fungal community, respectively, were phylogenetically similar but uneven distributed within the samples. The Shannon diversity index (*H'*) over time was even distributed in the bacterial community and was not influenced by preservative treatments. In contrast, the corresponding *H'* of the fungal community shifted towards high abundances of *Ascomycota Talaromyces* in treatment E of northern and central Germany as well as in southern France. Members of the genus *Talaromyces* are known cellulose-degrading organisms with potentially high tolerance towards copper. In conclusion, a decreasing fungal community composition over time indicates that few fungi were functionally superior in the main wood decay process.

EMP20

Hungry and freezing? Insights into microbial life in seafloor sediments north of Svalbard

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Introduction: Low nutrient input caused by varying temperatures and changing ice coverage is one of the key challenges of microbial life in the arctic regions. To get more knowledge of the indigenous microorganisms and their habitat, sediment samples were taken on a scientific cruise to the areas north of Svalbard in September 2013.
Objectives: Our aim is to study the subseafloor geochemistry in the Arctic, and to quantify and identify the prokaryotes, which may drive seafloor ecosystems in the Northern Barents Sea. Another objective is the investigation of the oil biodegradation potential of the indigenous microbiota under in situ conditions.
Methods: The samples from the seafloor were taken with a gravity corer from shallow (200m) areas on the Svalbard shelf as well as deep sea areas on the eastern Yermak Plateau (3200m water depths). The Bacteria and Archaea as well as important functional genes have been quantified via qPCR. This will be combined with a detailed diversity study using pyrosequencing and physiological analyses of the microbiota under in situ conditions.
Results: Shelf sediments showed the highest organic carbon content which decreased with increasing sediment depth. Also, potential sulfate reduction, carbon dioxide and methane production rates were highest in these sediments. Possible electron acceptors, like sulfate, iron and manganese were present in substantial amounts throughout the cores. On average the gene copy numbers per g sediment of the archaeal community (10^6) are one magnitude lower than the bacterial community (10^7). Numbers of methanogens determined via the functional gene for methanogenesis (mcrA) were low and the number of sulfate reducing prokaryotes varied strongly between 10^7 to 10^3 gene copy numbers per g sediment.
Conclusion: These first results indicate that these Arctic sediments are a suitable habitat for microorganisms despite cold temperatures and a low nutrient input. This study will provide information regarding global distributions of activities and communities of subsurface microbes.

EMP21

Dispersal of degrading bacteria impedes outgassing of organic contaminants

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Introduction: Contaminants are only hazardous if they become bioavailable. Therefore, the most effective remediation technique should lead to an optimal coverage with degrading microorganisms in order to prevent a release of contaminants sorbed in biogeochemical interfaces (BGI) to the environment. Simultaneously it should reduce the contaminant concentration at its source.
Objectives: Here, we investigated the impact of bacterial dispersal on BGI on the outgassing of phenanthrene (PHE). Our study challenged the hypothesis that the presence of dispersal networks of bacteria leads to: (i) bacterial distribution along the transport network, (ii) efficient bacterial distribution on the surface, and (iii) an increased biomass production fostering the degradation of PHE, that are released from the system.
Methods: We designed a laboratory microcosm that mimicked a continuous PHE release from a PHE hotspot to a model surface (agar surface) in the presence and absence of model dispersal networks that facilitated the transport of the poorly motile PHE-degrading *Pseudomonas fluorescens* on agar surfaces.
Results: In our experiments, we observed that the presence of the glass fibres, which imitate the widespread soil fungal networks, resulted in (i) an increased spatio-temporal spreading of bacteria, (ii) an increased bacterial coverage and growth on the agar surface, and (iii) a subsequent effective degradation of outgassing PHE and effective reduction of PHE contamination beyond the PHE hotspot.
Conclusion: Our data suggest that effective dispersal networks such as fungal mycelia may promote the formation of an adapted microbial population that will degrade hazardous molecules that desorb from the contaminant source. Potentially, such an activity produce no emission of contaminants to the pore and groundwater, and hence, to higher organisms.

EMP22

The µAqua-Chip – a new tool to monitor water quality

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Introduction: The µAqua-Chip represents an RNA-based microrarry systems allowing for an efficient, sensitive, robust, rapid and inexpensive monitoring of various aspects of microbiological water quality. It was developed by Scienion and several European partners in the course of a project funded by the 7th Framework Programme of the European Commission since 2011. The present chip prototype allows for simultaneous testing of more than 50 organisms. These organisms include pathogens that are considered to be potentially most dangerous for human

health and represent the standard pathogens whose presence is tested by all national water authorities in Europe.
Objectives, Materials & Methods: The µAqua-Chip system was used to analyze the microbial community composition in water samples taken in the course of the "Rheines Wasser" project of Furtwangen University (http://www.rheines-wasser.eu). In August 2014, the entire German Rhine river was swum longitudinally (1231 km) by A. Fath. Water samples for microbiological analyses (2 x 50 l) were taken at 12 of the 25 stages, i.e ~ every 100 km and immediately processed following a protocol developed by Scienion and partners. The water samples were exposed to a multistep filtration procedure and total RNA of the microorganisms of each individual sample was extracted.
Results & Discussion: The RNA targets were tested for existence of selected microorganisms using the µAqua-Chip technology. Preliminary results will be presented and discussed in the context of water quality.

EMP23
Microbial populations in iron-rich sediments of Lake Towuti at varying oxygenation levels in bottom waters
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Introduction: Lake Towuti is a deep tectonic basin surrounded by ophiolitic rocks and lateritic soils. The catchment exerts strong control on the trophic state of the lake due to massive iron (hydr)oxide inflows, leading to strong phosphorus adsorption. As Lake Towuti mixes at least occasionally, its bottom waters face different degrees of oxygenation, making the sediment and its metalliferous substrates a peculiar environment for microbial communities. These settings possibly result in enhanced preservation of organic matter (OM) in the lacustrine record.
Objectives: The present multiproxy study aims to establish the relationship between biogeochemistry and microbial communities in iron-rich anoxic sediments.
Methods: Short sediment cores were retrieved from three different sites representing oxic, suboxic and anoxic conditions at the water/sediment interface. Sampling was carried on site for pore water, cell counts, sulfate reduction rates and genetic analyses. 16S rRNA fingerprinting of microbial populations was performed on separate intra- and extracellular DNA fractions.
Results: Nutrient sorption varied in accordance to bottom waters oxygenation, resulting in differential primary production and preservation of OM. Microbial cell densities were highest at the oxic site and were related to the availability of labile OM and sulfate in pore water. At the suboxic site, sporadic mixing of the water column was recorded as variations of the organic content while significantly affecting the development of archaeal populations. At the anoxic site, the highly refractory OM coupled to the prior depletion of e- acceptors in the water column resulted in lower microbial cell densities.
Conclusion: SO_4^{2-} reducing bacteria were shown to be present and active at the oxic site, whereas they only appeared viable at the suboxic and anoxic sites, due to different OM types and e^- acceptor availabilities. In the absence of phosphate and nitrate/nitrite in the pore water, microbes turned to alternative sources of nutrients and used extracellular DNA as such, leading to its rapid turnover in uppermost sediments. Despite iron-rich and ultra-oligotrophic conditions of the sediment, microbial populations were, nevertheless, shown to be dense and active throughout the sedimentary records of the three sites.

EMP24
Microbial sulfur transformations in novel laboratory-scale constructed wetlands
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Constructed wetlands are near-natural wastewater treatment systems. There, pollutant transformations are either direct components of or interlinked with the redox cycles of major chemical elements, such as sulfur. The aim of the present study is to generate an enhanced view of microbial transformations of sulfide and elemental sulfur in constructed wetlands. To this end, we newly designed and built two laboratory-scale horizontal subsurface-flow constructed wetland models (CW1, CW2) in which the hydraulic characteristics limit the physicochemical heterogeneity rectangular to the flow direction. Each model has six separate compartments filled with gravel and is fed with artificial wastewater containing 300 mg/L of sulfate. In CW1, all of the six compartments were planted with the rush, *Juncus effusus*, whereas only the two middle compartments of CW2 were planted. Samples for chemical measurements as well as molecular analyses of sulfur and sulfide oxidizing bacteria (SOB) and the general microbial community were collected from the individual compartments at three different depths along the flow path. As phylogenetic markers, the 16S rRNA gene and the genes encoding sulfide:quinone oxidoreductase (*sqr*), adenylsulfate reductase (*aprA*), and *soxB* of the Sox multi-enzyme complex were used. The results highlight the dynamics of sulfur transformations in constructed wetlands. Better efficiency in sulfide removal and higher concentrations of elemental sulfur produced was observed in CW1 as compared to that in CW2. The formation of elemental sulfur indicated the reoxidation of reduced sulfur compounds. The molecular analyses of the functional gene markers suggested the activity of specific SOBs in the different compartments of both CWs. In conclusion, our findings enhance the understanding of sulfur and sulfide oxidation in constructed wetlands.

EMP25
Spontaneous release of fluoride during the oxidative cleavage of 5-fluorosalicylate by the salicylate 1,2-dioxygenase from *Pseudaminobacter salicylatoxidans* BN12
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Introduction: The α-Proteobacterium *Pseudaminobacter salicylatoxidans* BN12 forms a peculiar gentisate 1,2-dioxygenase (SDO) which oxidatively cleaves salicylate, gentisate (2,5-dihydroxybenzoate), and additionally 1-hydroxy-2-naphthoate and various amino-, chloro-, fluoro-, hydroxy-, and methylsalicylates [1,2].
Objectives: The products formed during the ring fission of 5-fluorosalicylate by the SDO were analyzed.
Methods: The SDO was produced in recombinant *E. coli* cells and purified as previously described [2]. In some experiments a whole cell system with recombinant *E. coli* cells was used in order to produce larger amounts of the reaction products. Analysis of reaction products was performed by means of HPLC, NMR, ion chromatography and UV/Vis-spectroscopy.
Results: The conversion of 5-fluorosalicylate by the purified enzyme was analyzed at pH 8.0 spectrophotometrically and it was found that the reaction resulted in the formation of a new absorbance maximum at λ=292 nm. The reaction products were unstable and different decomposition reactions were observed when either Tris/HCl- or Na-phosphate buffers were used. The analysis of the enzymatic reaction in Na-phosphate buffer by HPLC showed that two main products with absorbance maxima at λ=292-296 nm were formed from 5-fluorosalicylate. The same two products (although in different relative proportions) were also formed when the SDO converted 5-chlorosalicylate or a purified 5-nitrosalicylate 1,2-dioxygenase from *Bradyrhizobium* sp. JS329 [3] 5-nitrosalicylate. It was demonstrated with the whole cell system by ion-chromatography and ^{19}F-NMR that fluoride was released in quantitative amounts in the course of the enzymatic reaction.
Conclusion: Our results demonstrate that the oxidative 1,2-cleavage of 5-fluorosalicylate results in an unexpected fluoride release from the 5-position probably via a spontaneous lactonization reaction.

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EMP26
Eco-friendly textile dye degradation coupled to bioelectricity generation using Microbial Fuel Cell (MFC) technology
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Background: Reactive azo dyes are considered as one of the most detrimental pollutants from industrial effluents and therefore their biodegradation is receiving constant scientific consideration. Textile effluent treatment has been a challenge since long, hitherto, no sustainable technology has yet been developed. Very recently the microbial fuel cell (MFC) technology has emerged as one of the promising technology for treatment of azo dye degradation concomitantly generating electricity.

Methods and Results: In the present study, a bacterial culture designated as HHGP was isolated which was able to decolorize textile industrial dye - Reactive Brown as evaluated by spectroscopic analysis. Cultural parameter optimizations was also performed to enhance the biodegradation ability of the culture. The degradation of Reactive Brown was evaluated by various analytical techniques (FTIR, UV-Visible spectroscopy and GC-MS analysis) and consequently a pathway of degradation of reactive brown was also elucidated. The toxicity profile of degraded metabolites was assessed by phyto-toxicity assay. The bacterium HHGP was also evaluated for bioelectricity generation via an MFC set up. The maximum voltage output of 700mV and current generation of 1.03mA was observed with dual chamber system connected by agar salt bridge with copper plates as electrodes. Thus, the microbe HHGP demonstrates ability to degrade completely the textile dye - Reactive Brown alongwith loss of its toxicity.
Conclusion: The culture HHGP identifies itself as a potential candidate for its application in textile effluent bioremediation as well as bioelectricity generation via MFC technology. To the best of our knowledge, this is the first study focusing on biodegradation of reactive brown dye from textile effluent using microbial isolate and concomitant generation of bioelectricity in the process.

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EMP27

Energetically depleted *Dinoroseobacter shibae* maintains strong membrane potential

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One of the most abundant groups of marine bacteria world-wide is the *Roseobacter* clade. *Dinoroseobacter shibae*, a prominent member of this clade, is capable of anoxygenic photosynthesis under oxic conditions and uses light as additional energy source, which supports survival during long-term starvation (1). The ATP level of *D. shibae* is drastically reduced during 2 hours of anoxia but quickly regenerates after 5 minutes of aeration and light exposure (2). It could recently be shown, via permeabilisation of the cell membranes with butanol (3), that the intracellular pH (pHi) of *D. shibae* lies around 7.3 and is therefore slightly more acidic then the medium. Consequently, the ΔpH remains close to zero and does not contribute to the proton-motive force, leaving the membrane potential (ΔΨ) as the main force for fast ATP regeneration. Our hypothesis is that ΔΨ has major impact on the quick ATP regeneration of *D. shibae* after anoxia-induced ATP depletion. First experiments with the ΔΨ-indicating dye $DiOC_2$(3) revealed that the membrane potential of *D. shibae* is not reduced during the time of anoxia, but rather slightly increased. This was evaluated and documented via epifluorescence microscopy with a carefully established staining protocol that includes short exposure times for excitation and photography. The benefit of this method is the visualization of ΔΨ-driven dye accumulates within the stained cells in different intensities which reveal heterogeneity of membrane potential within the same culture. A drawback compared to flow cytometric analysis is a higher detection minimum of dye uptake. Therefore the ultimate quantification of the membrane potential needs flow cytometric experiments eventually. Since 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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EMP28

Identification of a phenylpropanoid degradation pathway in *Corynebacterium glutamicum*

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Introduction: The Gram-positive soil bacterium *Corynebacterium glutamicum* is able to utilize a broad range of compounds as carbon and energy sources. Amongst these compounds are also plant-derived phenylpropanoids, which serve as building blocks for lignin and secondary metabolites in plants. To this date, the catabolic pathway(s) for these substances in *C. glutamicum* is unknown.
Objectives: Identification of the pathway responsible for phenylpropanoid catabolism in *C. glutamicum* and investigation of growth on phenylpropanoids.
Methods: DNA microarray experiments for comparative transcriptome analyses, growth experiments with phenylpropanoids as sole carbon and energy source, and construction and characterization of *C. glutamicum* gene deletion mutants.
Results: Comparison of the transcriptomes of *C. glutamicum* wild type cells and wild type cells pulsed with 5 mM cinnamic acid, *p*-coumaric acid, caffeic acid or ferulic acid revealed up-regulation of a gene cluster in presence of these phenylpropanoids. Detailed sequence analyses revealed, that the genes cg0340-cg0347 might encode for enzymes involved in a β-oxidation of phenylpropanoids to yield 4-hydroxybenzoate, which can be easily converted to TCA cycle intermediates. Deletion of cg0344-47 already abolished growth on *p*-coumaric acid, caffeic acid and ferulic acid. The gene cg0343 is part of the cluster and shows pronounced homologies to MarR-type transcriptional regulators. Transcriptome analysis of a constructed *C. glutamicum* Δcg0343 deletion mutant (compared to wild type *C. glutamicum*) was performed to uncover a role of this putative transcription factor in the regulation of the gene cluster potentially involved in phenylpropanoid degradation. Indeed, cg0343 controls expression of two operons cg0341-40 and cg0344-47 by repression of gene expression in absence of all tested phenylpropanoids.
Conclusions: The obtained results revealed that the gene cluster cg0340-47 of *C. glutamicum* encodes for an hitherto unknown CoA-dependent β-oxidative phenylpropanoid side-chain shortening pathway. Future experiments will focus on the individual enzymatic steps during the utilization of phenylpropanoids in this organism.

EMP29

Analysis of microbial community diversity and function responsible for transformation of micropollutants in managed aquifer recharge systems using metatranscriptomics

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Microorganisms play an important role in the biotransformation of dissolved organic carbon (DOC) and trace organic chemicals (TOrC) during managed aquifer recharge (MAR) like riverbank filtration, soil aquifer treatment and artificial recharge and recovery[1,2]. Regarding transformation of TOrC, co-metabolism is the most likely mechanism for degradation[3]. Recent research using high-throughput pyrosequencing revealed that the diversity of the microbiome in the infiltration layer and vadose zone of MAR systems is directly influenced by the concentration and composition of the DOC[1]. These investigations have resulted in the establishment of a sequential managed aquifer recharge technology (SMART) that is utilizing a sequence of recharge basins to establish oxic and oligotrophic conditions to enhance TOrC transformation.
The objective of this study was to utilize next-generation microbiome pyrosequencing (metagenomics and metatranscriptomis) in combination with target analytical methods (LC-MS/MS) to identify composition and function of microbial communities responsible for biodegradation of trace organic compounds. Two sequential laboratory-scale systems were established that employ an in between aeration step to provide favorable aerobic, carbon limited infiltration in the second system in order to simulate the innovative SMART concept. The project focuses on the optimization of bank filtration and artificial recharge systems in Berlin for a better attenuation of TOrC such as household chemicals, pesticides and disinfection byproducts by adopting the SMART approach.
Results revealed that in particular oxic and oligotrophic conditions resulted not only in an upregulation of enzymes including P450

cytrochrome, but also in an enhanced removal of micropollutants. These results suggest that biofiltration systems like groundwater recharge can be engineered by establishing more favorable operating conditions to further improve the attenuation of TOrC.

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EMP30
Tackling the microbiome of the International space station - the ARBEX project

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Introduction: ARBEX - ARchaeal and Bacterial EXtremophiles onboard the international space station ISS - is part of the European Programme for Life and Physical Sciences in Space. The ISS represents a special living area for humans and accompanying microorganisms under extreme conditions, almost completely sealed off from the outside world. In such an environment regular monitoring of the microbial population is mandatory to assess eventual risks for the crew´s health or for the integrity of the spacecraft itself and to enable appropriate countermeasures if needed.
Objectives: The ARBEX project is designed to look beyond the confinements of the regular, standardized, microbial monitoring and aims for microorganisms yet uncultured onboard the ISS. The main focus lies on the detection of hardy, extremophilic bacteria and archaea via different cultivation assays and state-of-the-art molecular analyses. The obtained results will be compared to the cultivable and uncultivable microbiome of ground controls (i.e. spacecraft assembly clean rooms). Isolates will be analyzed with regard to their physiology and resistances against e.g. (UV- and γ-) radiaton, desiccation and antibiotics.
Results: Besides introducing the ARBEX project in detail, we will also present results of the first ground control, namely the S5C clean room in Kourou, French Guiana. More than 50 different microbial isolates were obtained under different conditions, such as e.g. high and low pH values and oligotrophic medium, including two putative new species.
Conclusion: This assortment of microbes will be the baseline for comparative analyses with the ISS microbiome. The launch for the ARBEX project is scheduled for 2015 from Baikonur, Russia, and the first ground control results presented by this poster show that the used methods cover the already known and expected range of microorganisms in clean rooms [1] and onboard the ISS and are also well suited to discover organisms yet unknown to live in these environments.

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EMP31
Hydroquinone-driven Fenton Reactions for Pollutant Degradation by White-Rot Fungi

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White-rot fungi have been established as highly interesting organisms for the oxidative degradation of a wide range of xenobiotics [1]. They achieve these feats mainly through utilization of their ligninolytic enzymes, which include laccase and various peroxidases. In the past, there have also been reports that white-rot fungi were able to initiate Fenton chemistry with the help of hydroquinones, which would allow them to extend their degradation spectrum [2,3]
A selection of nine white-rot fungal strains was employed to test whether supplementation of a hydroquinone would enable them to depolymerize polystyrene sulfonate (PSS), a polymer normally inert to the ligninolytic system. Samples were analyzed with size exclusion chromatography. An active strain of *Trametes hirsuta* was also chosen in order to investigate whether hydroquinones would improve degradation of three recalcitrant dyes, monitored by photometric decolourization.
Out of the nine strains, four were capable of causing strong PSS depolymerization when supplemented with hydroquinones and reduced PSS molecular mass by approx. 90 % within 20 days. The effects were independent of ligninolytic enzyme activities. Decolourization of the dyes Acid Red 299 and Reactive Black 5 by *T. hirsuta* was also improved in the presence of hydroquinones, while there was no effect for Azure B.
Although not a common ability of all white-rot fungi, quinone-mediated Fenton reactions appear to be able to improve degradative capabilities of certain strains. Future work will elucidate the extended degradation spectrum more in detail.

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EMP32
Characterization of a hydrocarbon contaminated aquifer by comparative enrichment of denitrifying BTEX-degrading microorganisms from different zones of a plume

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Introduction: Monoaromatic BTEX compounds (benzene, toluene, ethylbenzene, *o*-, *m*-, *p*-xylene) are often found in the aquifer of industrial sites, where refined petroleum products and coal are used. Contaminated aquifers suffer from oxygen depletion and anaerobic microorganisms usually dominate the naturally occurring attenuation processes [1]. Bioremediation with nitrate as an alternative electron acceptor is a promising alternative to classical aeration strategies: nitrate can be added in high concentrations to the groundwater and supports the already established anaerobic community.
Objectives: Polluted aquifers are characterized by different redox zones and potentially toxic concentrations of contaminants. The aim of this study was to identify areas within the plume where denitrifying BTEX degraders are present, and to compare degradation rates for the single BTEX compounds. The information may help to decide, where bioremediation can be applied.
Methods: Groundwater of a former gasworks site, which was previously characterized by its geological and chemical properties, was sampled from three different zones ($\sum_{BTEX} \approx$ 116, 19, or 2 µM) and incubated in anaerobic batch-cultures with additional nitrate and BTEX. Community structures were characterized by 16S rRNA-PCR and DGGE.
Results: BTEX degrading microorganisms were preferentially enriched in groundwater originating from the vicinity of the contamination source: highest degradation rates were found for ethylbenzene and toluene, followed by *p*-xylene und *m*-xylene, whereas *o*-xylene and benzene were not degraded. Further downstream of the contamination, no xylene-degraders could be enriched. Degradation was accompanied by the stoichiometric consumption of nitrate. Transcripts of genes associated with anaerobic BTEX-degradation were detected. Different strains of *Azoarcus* sp. were identified and isolation attempts were conducted.
Conclusions: High concentrations of hydrocarbon pollutants ($\sum_{BTEX} \approx$ 116 µM) favor the presence of BTEX-degraders, whereas concentrations below a threshold may be insufficient for them to establish. Considerable degradation rates for *p*-xylene are noteworthy, as to date no nitrate-reducing pure culture exists.

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EMP33
Overseen, but everywhere – Archaea the hidden players in the human microbiome?

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Introduction: Although, at first sight, resembling Bacteria, Archaea are totally different in cellular organization, molecular processes and general behavior. For instance, most medically used antibiotics do not affect Archaea in their growth. Due to their distinctiveness from Bacteria, Archaea are often overlooked when molecular methods, e.g. 16S rRNA gene-based amplicon sequencing, are applied. Interestingly, at least to the recent state of research there is no known archaeal pathogen. Although probably not directly pathogenic, there are some striking correlations with for instance obesity[1], atherosclerosis[2], oral infections[3] and probably skin pH regulation[4].
Objectives: Due to the fact that human associated Archaea were investigated only in a low number of studies (compared to the human bacterial microbiome), we are currently particularly screening for Archaea within the human microbiome.Of certain interest are the changes in

diversity and abundance of these organisms comparing healthy and diseased persons.
Materials & Methods: For the detection of Archaea different primers targeting the 16S rRNA gene will be tested and applied. The samples for our screenings are obtained from healthy/ diseased patients of the hospital Graz, whose medical history is well described hereby delivering important additional informations for a comprehensive insight in the archaeal microbiome.
Results: We will map the diversity and abundance of Archaea in several samples (digestive tract, lung, skin and stool) compared to Bacteria and Fungi.
Conclusion: The presented results will deliver better insights in where to search for certain Archaea and maybe give hints on their association with certain disseases.

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EMP34
Microbial life in continental salt pan sediments in south-western Africa
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Introduction: Terrestrial climate archives will be used to get a better understanding of climate evolution and environmental condition in south-western Africa (Namibia and South Africa) in the framework of the project "Signals of climate and landscape change preserved in southern African GeoArchives" which belongs to the BMBF SPACES program. Continental salt pans represent sediments from terrestrial sources with the potential to preserve climate signals during phases of deposition. To understand the climate impacts on the salt pan microbial ecosystem the composition and diversity of indigenous microbial communities related to different soil parameters are investigated. Special emphasis is placed on the characterization of halophilic microorganisms. For microbiological analyses outcrops or short cores (0-100 cm) were drilled at four different salt pans (Aminuis, Koes and Witpan region) having rather different geochemical properties.
Objectives: This work focused on changes within the microbial communities due to the impact of long-term climatic variation and the associated environmental changes.
Methods: For a quantitative characterization of microbial communities molecular techniques such as polymerase chain reaction (PCR) and real-time quantitative PCR (qPCR) based on the 16S rRNA genes are used. Moreover, 454 sequencing technique is utilized to describe the diversity and abundance of microorganisms in detail. Soil parameters are described by standard soil scientific methods.
Results: The distribution of bacteria and archaea in salt pan sediments is strongly correlated to the abundance of total organic carbon (TOC), which varied between 0.2 and 1.5%. Gene copy numbers of bacteria and archaea decrease with depth. In the upper 10 cm of the different salt pan sediments 10^4 to 10^6 copies g^{-1} soil are quantified, while gene copy numbers decrease with depth down to 10^3 copies g^{-1} soil. In general gene copy numbers of bacteria are higher than those of archaea.
Conclusion: Quantitative analyses of bacterial and archaeal copy numbers show a similar pattern in different salt pan sediments. TOC values increase due to higher terrestrial input and the increase coincides with a shift within the microbial community. Furthermore, microbial lipid biomarker analyses are planned to characterize living and past microbial biomass in relation to climate change.

EMP35
Exploring the plastic microbiome in the marine system
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While data on the widespread distribution of microplastic in marine waters are increasing, we still do not understand the impact of this pollution on the marine foodweb. In particular, the role of microbial biofilms on microplastic particles represents a major research gap. Many microorganisms prefer to grow in biofilms, as this lifestyle offers efficient nutrient utilization, protection and dispersal. Microplastic represents an abundant and durable hence attractive surface for colonizing microorganisms in marine waters, and the understanding of plastic biofilms and their role within the marine foodweb is essential. We carried out exposure experiments and field sampling of microplastic in European waters, comparing biofilms at different stations, seasons and different polymers. Biofilm composition was analyzed using scanning-electron-microscopy and high-throughput sequencing. Results suggest an important role within plastic biofilms of photosynthetically active colonizers, as well as members of the *Bacteriodetes*. Distinct differences to reference communities from seawater were observed, while taxa overlaps with communities from non-plastic surfaces hint at a generic biofilm community. Strong spatial and seasonal influence on the plastic communities were evident. Currently, we tackle the question whether marine plastic serves as vector for potential pathogens, considering different sources.

EMP36
Isolation and characterization of *Longimicrobium salmoneum* gen. nov., sp. nov., an oligotrophic bacterium belonging to a new class of the underrepresented phylum *Gemmatimonadetes* isolated with a Diffusion Sandwich System.
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Introduction: The phylum *Gemmatimonadetes* was proposed by Zhang et al. in 2003 and so far only harbors the species *Gemmatimonas aurantiaca* [1]. DeBruyn et al. (2013) proposed the second genus *Gemmatirosa* harboring also the nonvalidated species *Gemmatirosa kalamazoonesis* [2] (http://ijs.sgmjournals.org/). Despite the cosmopolitan distribution of this genus on the basis of metagenomic data, the vast majority of bacteria belonging to these taxa are yet to be cultured laboratory conditions.
Objectives: The aim of this study was to isolate and characterize polyphasically new previously uncultured bacteria using a Diffusion Sandwich System from soils collected from the Tejeda, Almijara and Alhama Natural Park, Granada, Spain.
Methods or Materials & Methods: The strain CB286315^T was isolated using a Diffusion Sandwich System and gradually adapted to cultivation on oligotrophic solid media in laboratory conditions. The strain was characterized following standard polyphasic procedures [1, 2]. Whole genome sequencing using the Illumina sequencing technology has been applied to obtain a first draft of the bacterial genome.
Results: The strain is a Gram negative, aerobic bacterium with non-motile long and irregular rod-shaped cells with G+C content of its genomic DNA is 67.0 mol%. The strain is catalase and oxidase-positive, and grows between 4 and 30 ºC (optimum 20 ºC), at pH 6.0 and 7.0, and at saline concentration lower than 1.5% NaCl (w/v). It does not tolerate rich culture media. Major chemotaxonomic characters include the presence meso-diaminopimelic acid, PG, PE, PC as major polar lipids, a high content of the fatty acid ISO 17:1 w9c (45%) and MK-8 as major menaquinone. The strain CB-286315 shares 84.36% and 83.57% 16S rRNA gene similarity with *G. kalamazoonesis* and *G. aurantiaca* respectively.
Conclusion: The strain CB286315^T (=DSM 29007^T =CECT 8659^T) represents a new genus belonging to a new class, *Longimicrobiae* classis. nov., of the phylum *Gemmatimonadetes*, for which we propose the name *Longimicrobium salmoneun* gen. nov., sp. nov.

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EMP37
Kinetics and toxicity tests of the dymethylphenol degrading bacteria *Delftia acidovorans*
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Dimethylphenols (DMP, xylenols) are toxic compounds with high environmental mobility in water and one of the main constituents of coal pyrolysis industry effluents. In order understand the DMP degradative metabolic pathways of activity of these compounds, as a first step, it is necessary to isolate and characterize potential DMPs bacterial degraders.
Outflow water from a horizontal sub-surface Constructed Wetland fed with contaminated groundwater containing benzene, phenols and *m*-cresols, was collected. Water samples were enriched in liquid minimum media with 70 mg/L of a equimolar ratio of 2,6-; 3,4- and 3,5-DMP as sole carbon and energy source and isolates were obtained by spread-plating on minimum medium agar with a mix of DMPs. Kinetics, toxicity test and

FAME analysis were also performed. Finally, selected bacteria were taxonomically identified by sequencing of 16s rDNA.
From isolates, the β-proteobacteria *Delftia acidovorans* was the strain able to completely degrade o-xylenols 3,4-DMP within 14 hours and 2,3-DMP within 80 hours, showing in both cases the accumulation of 2-hydroxymuconic semialdehyde, a compound formed by the meta-cleavage of catechol by the catechol 2,3-dioxygenase. Furthermore, toxicity of the different DMP-isomers represented by the logarithm of its partition coefficient in *n*-octanol and water (log Pow), exhibited that 3,5-DMP is the less toxic compound for bacteria, presenting the highest Log Pow value (2.55), followed by 2,4- and 2,3- with 2.49 and 2.48 Log Pow values respectively. Meanwhile 3,4-; 2,5- and 2,6-DMP are the most toxic isomers with the lowest Log Pow (2.23, 2.33 and 2.36 respectively).
Additionally, the high exposition of *D. acidovorans* to the different DMPs-isomers did not showed a short term response of lipids membrane modification by bacteria, non isomerization of *cis*-into *trans*-unsaturated fatty acids were evidenced. Other possible long term mechanisms, as alteration of phospholipids head-groups composition, modification of LPS of outer membrane or active excretion by energy-consuming transport systems, among others mechanisms could lead the solvent tolerance in *D. acidovorans*.
D. acidovorans showed to be an optimal bacterium model for studying the mechanisms of degradation and tolerance of DMPs, and a suitable candidate for further studies for the reconstruction of the metabolic DMPs pathway by genomic approaches.

EMP38
Assessment of toluene degradation by an isolated strain of *Magnetospirillum* and its detection in a Planted Fixed-Bed 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, under microaerophilic and anaerobic conditions. Numerous studies on this genus are focused on their magnetic properties, however almost no studies have assessed the extent at which these bacteria can metabolize toluene under denitrifying anaerobic conditions. Furthermore a few studies have identified and analyze 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. An isolated strain was taxonomically and physiologically characterized, and defined genes related to general toluene degradation pathways for anaerobic conditions were detected and specific primers were designed. Finally, their expressions were assessed in the reactors using quantitative polymerase chain reaction (qPCR). Through *16s rDNA*, our results showed that the strain were closely related to *Magnetospirillum* TS-6, a strain able to degrade phenol, previously describe by Shinoda et al. (2000). Furthermore, *Magnetospirillum* was able to degrade toluene up to a concentration of 35-50 mg/L in liquid cultures and other aromatic compounds, with nitrate as the electron acceptor under anaerobic conditions. Genes were analyzed through BLAST and related to toluene degradation, specifically *bssA* (benzylsuccinate synthase) and *bcrC* (benzoyl-CoA reductase). With qPCR the abundance of *Magnetospirillum* sp. were possible to quantify in two different PFR systems, 9,17E+01 and 2,96E+03 (copy numbers/µl) respectively.

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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.

EMP39
A microbe from arctic waters catabolizes pectic fiber from citrus fruits
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Introduction: Microbes adapt to new glycan resources by acquiring polysaccharide utilization loci (PULs) encoded on mobile genetic elements. We recently identified a pectinolytic phenotype in the psychrophilic, marine bacterium *Pseudoalteromonas haloplanktis* ANT/505 [1]; a result which was intriguing because pectin, a glycan fiber of methanol esterified galacturonate, is abundant in fruits from terrestrial plants but not in marine habitats.
Objectives: The ability to use pectin as a carbon and energy source has been rarely described in marine bacteria.
Results: Here we show that the arctic, marine bacterium *P. haloplanktis* ANT/505 gained a mobile element carrying a gene cluster for the digestion of pectin. The genetic island encodes PelA, a multimodular enzyme of >200 kDa size with fused methylesterase (CE8) and pectate lyase (PL1) catalytic domains, which process consecutive steps in the degradation of pectin. The methylesterase CE8 domain cleaves the methyl ester bonds such that the pectate lyase PL1 domain can cleave the non-decorated pectate chain. Our proteomic analysis revealed that genes of the island are induced by pectin and that *P. haloplanktis* ANT/505 secretes PelA and its second pectate lyase PelB into the environment. A phylogenetic analysis showed the ancestors of PelA were two independent enzymes suggesting that their assembly into one secreted protein was an adaptation to the marine environment - i.e. to the high diffusion rate in seawater.
Conclusion: Our results show that marine gammaproteobacteria use pectin as a carbon and energy resource and our results exemplified that glycan degrading pathways can adapt to new environments.

1. Truong LV, Tuyen H, Helmke E, Binh LT, Schweder T., Extremophiles. (2001), 5(1):35-44.

EMP40
Effect of electron acceptor fluctuations on the anaerobic toluene degrader community in a toluene contaminated model aquifer
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Introduction: Groundwater microbes play an important part in hydrocarbon pollutant removal. An emerging perspective is that groundwater systems can be dynamic habitats due to recharge-connected hydraulic fluctuations which may strongly influence also microbial degrader community composition and degradation activity, also by changing local redox conditions. A novel *in situ* remediation technology (1) is aimed to enhance natural attenuation by providing easily accessible electron acceptors (Fe^{3+}) to the microbes via the injection of nanoscale iron-oxide particles. Test of this technology is conducted in a toluene contaminated indoor aquifer (2).
Objectives: From an ecological point of view, nanoparticles injection represents a profound change in electron acceptor availability. We hypothesize that it will restructure the anaerobic toluene degrader community. Here, we aim to follow the response of an established, anaerobic toluene degrader community in the aquifer sediments to the injection of iron-oxide nanoparticles.
Methods: Changes in the anaerobic toluene degrader community are assessed via 16S rRNA genes and the functional marker gene benzylsuccinate synthase (bssA) using T-RFLP and pyrosequencing approaches. We link changes in degrader diversity to degradation rates by monitoring toluene and electron acceptor concentrations.
Results: Before the injection distinct toluene degrader communities were found along the plume length. Dominating degraders were affiliated with known denitrifying (*Thauera, Azoarcus spp.*) and iron reducing (*Georgfuchsia spp.)* toluene degraders. Elevated toluene degradation rates and high Fe^{2+} concentrations one month after the injection indicate that the nanoparticles stimulated toluene biodegradation.
Conclusion: These primary results indicate that iron-reducing toluene degraders might indeed have been stimulated within the community by

iron-oxide injection. Therefore, functional redundant degraders sustained degradation activity during a drastic change in electron acceptor availability.

(1) Braunschweig, J., Bosch, J., & Meckenstock, R. U. (2013). Iron oxide nanoparticles in geomicrobiology: from biogeochemistry to bioremediation. New biotechnology, 30(6), 793-802.
(2) http://www.iws.uni-stuttgart.de/institut/index_lehrstuhl.en.php?Abteilung=7

EMP41
Evaluation of 16S rDNA primer sets for monitoring archaeal and bacterial community structure – a comparative study estimating method-based bias
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Introduction: In the still rising field of microbial community analysis, metagenomics and amplicon sequencing play an important role in monitoring and comparing large numbers of samples in terms of their microbial composition. These datasets allow detailed insight in the diversity and potential ecosystem function.
Objectives: Here we compared the complex microbial community inside an anaerobic biogas reactor using various 16S ribosomal DNA primer sets, targeting different variable regions of archaeal and bacterial 16S rDNA. In addition the community was analyzed using a metagenomic approach.
Materials & Methods: Different 16S rDNA primer sets (3 targeting archaea, 2 bacteria and 1 prokaryotes) were chosen and compared against the curated Silva 16S rDNA database. All primer sets, *in silico* covering more than 60 % of their respective target group, were used to analyze the natural diverse microbial community of a biogas reactor by a 454 pyrosequencing approach applying multiplexing barcodes. Respective metagenomic data were generated by Illumina MiSeq sequencing and assembled using IDBA-UD.
Results: We found strong differences in the observed community structure depending on the choice of the 16S rDNA primer set. An even stronger difference was observed comparing metagenomic data to the 16S rDNA based observation. The respective differences will be presented and discussed.
Conclusion: In conclusion our results show that the choice of the primer set determines the quality and the perspective on the community. Therefore the comparability between communities analyzed with different primer sets might be lower than currently expected. In contrast the metagenomic approach is independent from primer bias but can be distorted resulting from the assembly and annotation process. In our understanding both methods have advantages but also limitations and thus might be applied each for a different purpose.

EMP42
Effects of hydraulic frac fluids and formation waters on groundwater microbial communities
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Introduction: Shale gas is being considered as a complementary energy resource to other fossil fuels. Its exploitation requires using advanced drilling techniques and hydraulic stimulation (fracking). During fracking operations, large amounts of fluids (fresh water, proppants and chemicals) are injected at high pressures into the formations, to create fractures and fissures, and thus to release gas from the source rock into the wellbore. The injected fluid partly remains in the formation, while up to 40% flows back to the surface, together with reservoir waters, sometimes containing dissolved hydrocarbons, high salt concentrations, etc.
Objectives: The aim of our study was to investigate the potential impacts of frac or geogenic chemicals, frac fluid, formation water or flowback on groudnwater microbial communities.
Methods: Laboratory experiments under *in situ* conditions (i.e. at *in situ* temperatures, with high pressure, etc.) were conducted using groundwater samples from three different locations. Series of microcosms (3 of each kind) containing R2 broth medium spiked with either single frac chemicals (including biocides), frac fluids, artificial reservoir water, NaCl, or different mixtures of reservoir water and frac fluid (to simulate flowback) were incubated in the dark. Controls included non-amended and non-inoculated microcosms. Classical microbiological methods and molecular analyses were used to assess changes in the microbial abundance, community structure and function in response to the different treatments. Potential transformations of frac or geogenic chemicals by subsurface microbiota and their lifetime are currently being investigated.
Results and conclusion: 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. 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. Ongoing work will provide information on the biodegradability of frac components by groundwater microbiota.

EMP43
Multicarbon Substrate Spectrum and pH - Drivers of Fungal and Bacterial Methylotroph Diversity in a Forest Soil
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Methylotrophs in temperate soils are an important sink in the global methanol cycle. Parameters determining their diversity, distribution, and activity have scarcely been understood. Vegetation type and community structure of soil methylotrophs in aerated soils correlate and methylotrophs can symbiotically interact with plants. An own previous study revealed a large diversity of methylotrophs in temperate top soils, although only gene markers of gram negative methylotrophs have been analysed. Most soil-derived methanol-utilizing isolates are polycarbotrophic, i.e. they can utilize multi-carbon compounds, such as mono-, di- and polysaccharides, alcohols, carbonic acids, and aromatic compounds, and belong to Bacteria or yeasts and ascomycota. Thus, it was hypothesized that an important ecological niche defining parameter of methylotrophs in aerated soils is their multi-carbon substrate spectrum. Following this assumption, soil methylotrophs should have divergent preferences to soluble substrates being products of lignin breakdown (vanillic acid), anaerobic organic matter degradation (acetate), or monomers of abundant plant polymers or root exudates (xylose, glucose). Another driver of methylotrophic community structure was soil pH based on an own previous study that was employed a multivariate statistics approach. Methanol-utilizing Bacteria and fungi that assimilated acetate, vanillic acid, glucose, or xylose in presence of unlabeled methanol were identified in a multi-substrate differential DNA stable isotope experiment (dif SIP). Based on the analysis of bacterial 16S rRNA genotypes, genera of *Beijerinckiaceae* and *Hyphomicrobiaceae* were dominant whereas based on *mxaF/xoxF* genotyping also members of the *Methylobacteriacea* were frequent. Analysis of fungal ITS revealed that yeast (*Cryptococcus*) and some ascomycota species assimilated carbon from added methanol suggesting that these soil fungi are important in methanol cycling.

EMP44
Selective enrichment of Fe(III)-reducing bacteria from microaerobic, BTEX-contaminated groundwater
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Introduction: *Rhodoferax* species and other Fe(III)-reducing microbes, like members of the genus *Geobacter* are frequent members of oxygen-limited, BTEX contaminated subsurface environments. *Geobacter* species usually harbor *benzylsuccinate synthase (bssA), the key gene of anaerobic degradation of toluene, while some Rhodoferax species may have a role in the microaerobic degradation of aromatic compounds and harbor subfamily I.2.C-type catechol 2,3-dioxygenase (C23O) genes.*
Objectives: *The main objective of this study was to investigate diversity of bssA genes of the "Siklós" BTEX-contaminated groundwater (Hungary) and to link them to Fe(III)-reducing members of the bacterial community.*
Material & Methods: Acetate enrichment media were inoculated with groundwater samples under anaerobic circumstances. Fe(III)NTA was added to the media to provide Fe(III) as sole electron acceptor. Four different kinds of enrichments were set up: (i) supplemented with 0.05%

(w/v) yeast extract and NH_4Cl; (ii) supplemented solely with 0.05% (w/v) yeast extract; (iii) supplemented solely with NH_4Cl; and finally (iv) omitting both yeast extract and NH_4Cl. A metagenomic approach was used to reveal the bacterial community composition in the initial groundwater, while the methods of T-RFLP and SNuPE were used to follow changes in the community structure during the enrichments.
Results: The diversity of the initial bacterial community considerably decreased during the enrichment culturing, and the lowest diversity was observable in case of enrichment cultures omitting yeast extract. However, selective enrichment of *Geobacter* species was observable also in these latter cultures. Nevertheless, *bssA* genes, even yet unknown genotypes were detectable in every type of enrichment culture.
Conclusion: The selective enrichment of *Geobacter* species was observable in enrichments without yeast extract. By omitting fix nitrogen forms from the enrichment medium the selective enrichment of *Geobacter*-related bacteria became to be more expressed. The enriched *Geobacter*-related bacteria of the „Siklós" BTEX-contaminated groundwater (Hungary) were only distantly related to validly described members of the genus *Geobacter*.

EMP45
Establishing a novel function-based screen for the identification of [NiFe]-hydrogenases in metagenomic libraries
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[NiFe]-hydrogenases catalyze the reversible oxidation of molecular hydrogen to protons and electrons, providing a high energy potential. For the identification and characterisation of novel [NiFe]-hydrogenases from different environments, culture-independent approaches are of great interest in order to avoid growth-limitations. Sequence-based screenings of metagenomic libraries can only detect hydrogenase-sequences showing conserved domains, so that function-based screenings are required. So far no function-based screening method for the identification of [NiFe]-hydrogenases has been reported.
We here present a function-based screening method for the identification of [NiFe]-hydrogenases in metagenomic libraries. The screen is based on the complementation of a [NiFe]-hydrogenase deletion mutant of *Shewanella oneidensis* (*S.o.* Δ*hyaB*) with metagenomic hydrogenase genes. Hydrogenase activity of complemented clones can be detected by a color change of the medium, used for chemolithotrohic growth of *S. oneidensis*. The sensitivity of this screen is currently being evaluated by the complementation with different proteobacterial [NiFe]-hydrogenases.
So far the complementation of *S.o.* Δ*hyaB* with the large subunit of the [NiFe]-hydrogenase from *Sulfurimonas denitrificans* (ε-Proteob.) as well as the measurement of the corresponding hydrogenase activities were successfully performed. Also in a metagenomic scale (1 positive under 47 negative clones) the detection of hydrogenase activity was possible.
After a positive evaluation of the sensitivity of this screen it will serve as a high throughput tool for the screening of metagenomic libraries for [NiFe]-hydrogenases.

EMP46
Effects of blood feeding on water and biological filter associated bacteria in leech rearing systems
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Leeches are blood sucking Annelida of the order *Hirudinis* occurring in brackish water preferably with a low pH. In human medicine leeches are used as natural therapeuticals. Medical leeches can be cultured in water tank systems, but normally, several blood feedings are required before the leeches reach the medical treatment size. A blood feeding event strongly affects the water quality by changing ammonia, nitrate and nitrite concentrations, and also the abundance and community composition of water-associated bacteria, all of which may negatively affect the leech health.
We investigated in two independent experiments the effect of blood feeding events in three different water circulation systems, a system with unaffected neutral pH, but with an additional sand filter beside the biological filter, and two systems with either chemically or biologically decreased water pH values. The aquarium water was analyzed few days before and five days after the blood feeding. Chemical water parameters (ammonia, nitrate, nitrite) were measured and total cell numbers of water associated bacteria were determined after membrane filtration, subsequent SybrGreen I staining, and epifluorescense microscopy. The concentration of culturable water-associated bacteria was determined on lactose peptone medium (DEV-standard medium). Most abundant bacteria were phylogenetically identified by 16S rRNA gene sequencing.
Ammonia concentrations rapidly increased after blood feeding followed by increased nitrate and nitrite concentrations. The total cell numbers and the concentrations of culturable bacteria increased independent from the water systems significantly after blood feeding, whereas the diversity of abundant bacteria decreased after blood feeding. A preconditioning of the biological filter, by feeding the filter-associated bacteria with ammonia before the second experiment started, led to more stable systems. To investigate this finding in more detail,, water and filter-associated bacterial communities are currently analyzed using cultivation independent bacterial community analysis and quantification of ammonium oxidizing bacteria by targeting the *amoA* gene. First results indicate the importance of preconditioned filter systems for leech rearing.

EMP47
A novel bacterial isolate from soil able to degrade the *Pseudomonas* quinolone signal and related alkylhydroxyquinolines
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Introduction: Bacteria coordinate their gene expression in response to population density by a mechanism called quorum sensing (QS). The opportunistic pathogen *P. aeruginosa* employs a complex QS network, which includes *N*-acylhomoserine lactones (AHLs) and 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, to control the production of virulence factors [1]. Interference with QS and thus virulence can be achieved by enzyme-catalyzed modification or degradation of signal molecules that lead to signal inactivation.
Objectives: Isolation and characterization of new AQ degrading bacteria.
Methods: Enrichment cultures with PQS and HHQ were set up, using soil samples, collected in the botanical garden of the University of Münster beneath alkaloid-producing plants, as inocula. Promising strains were tested for AQ degradation in resting cell assays.
Results: Cell suspensions of a new bacterial isolate, which was identified as a *Rhodococcus erythropolis* strain, cometabolically degraded PQS and HHQ to anthranilate. The isolate moreover was able to convert the respiratory electron transport inhibitor 2-heptyl-4-hydroxyquinoline-*N*-oxide, which is also produced by *P. aeruginosa*, to PQS [2]. HHQ transformation by cell extracts was NADH-dependent, whereas no cosubstrates were needed to degrade PQS *in vitro*. Comparison of HHQ and PQS conversion rates by crude extracts from cells grown in the presence and absence of PQS suggested that expression of the genes encoding AQ converting enzymes is inducible by PQS. Remarkably, the isolate also has the potential to disrupt AHL-based signaling.
Conclusion: *R. erythropolis* strain BG43 is the first bacterial isolate identified to degrade the *P. aeruginosa* quinolone signals PQS and HHQ. It opens up new perspectives to characterize new AQ degrading enzymes and to analyze the response of *P. aeruginosa* to interference with its QS systems.

[1] Heeb S, Fletcher MP, Chhabra SR, Diggle SP, Williams P, Cámara M (2011) FEMS Microbiol Rev 35:247-274
[2] Müller C, Birmes FS, Niewerth H, Fetzner S (2014) Appl Environ Microbiol 80:7266-7274

EMP48
Investigation of microbial benzene degradation in a constructed wetland using *in situ* microcosms in combination with stable isotope probing and 16S rRNA sequence analysis
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Constructed wetlands (CWs) are viable ecological remediation approaches for BTEX-contaminated sites. In the present study, we queried for microbes involved in benzene degradation in a horizontal-flow CW that was built next to a former refinery and industrial site. The CW was fed with contaminated groundwater in which benzene was a main organic contaminant. In the pore water of the CW's front section, benzene

constituted up to 50% of Total Organic Carbon. Previously it was shown that almost complete benzene removal in the CW occurred during the warm season, the majority of it was due to microbial transformation. In order to identify the microbes involved in benzene degradation in the CW, we employed *in situ* microcosms (BACTRAPs®) together with ^{13}C stable isotope probing and Illumina sequencing of 16S rRNA fragments. After short-term incubation (6 days) of benzene-loaded BACTRAPs in the CW, no incorporation of ^{13}C into 16S rRNA could be detected. In contrast, RNA isolated from BACTRAPs exposed for 28 days showed a significant incorporation of ^{13}C. Sequencing revealed that the benzene-degrading bacterial community on the BACTRAPs was mostly comprised of members from three genera, namely *Zoogloea*, *Geobacter*, and *Dechloromonas*. While all three genera contain species that have been reported as being associated with benzene degradation, only *Zoogloea* and *Dechloromonas* sequences increased concomitantly with the increase in ^{13}C content of RNA fractions, whereas the abundances of *Geobacter* sequences decreased. The latter was also shown by qPCR measurements. In representative wetland samples that were not exposed to ^{13}C-labelled benzene, sequences affiliated with *Zoogloea* and *Dechloromonas* were present at relative proportions of up to 5%. In conclusion, we identified microbes of likely significance for benzene degradation in a CW used for remediation.

EMP49

Bacterioplankton biogeography and activity in the Southern Ocean with special emphasis on the *Bacteroidetes*

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Marine bacterioplankton communities, including widely distributed and abundant groups such as the *Bacteroidetes* and *Proteobacteria*, play a central role in many ecological processes in the oceans. However, our understanding of how environmental parameters influence bacterial community structure is still limited, especially in remote regions such as the Southern Ocean.

The goals of this study were to identify bacterioplankton community composition including the major active players along a transect of the Southern Ocean from South Africa to the Antarctic Ice Shelf, spanning a wide range of water masses and environmental conditions. A specific focus lies on the *Bacteroidetes* due to their abundance in cold waters and role in central biogeochemical processes. This is achieved using CARD-FISH with probes specific for important subgroups (e.g. NS clades) in combination with probes for other important bacterioplankton components such as the *Roseobacter* clade to obtain a comprehensive view of community structure. This is complemented by MAR-FISH and measurements of bacterial biomass production and activity using radioisotope techniques to obtain insights into active populations. Bacterial diversity and activity will be linked with water mass characteristics using available hydrographic and biogeochemical data (chlorophyll a, nutrients).

The proposed work provides a comprehensive biogeographic picture of *Bacteroidetes* and other taxa in the Southern Ocean. Moreover, setting the obtained results in context to data from other provinces (Gómez-Pereira et al., 2010; Schattenhofer et al., 2009; Wietz et al., 2010) contributes to the understanding of the global biogeography of *Bacteroidetes* and other major bacterioplankton groups.

Gómez-Pereira PR, Fuchs BM, Alonso C, Oliver MJ, van Beusekom JEE, Amann R (2010). Distinct flavobacterial communities in contrasting water masses of the north Atlantic Ocean. ISME J, 4: 472.
Schattenhofer M, Fuchs BM., Amann R, Zubkov MV, Tarran GA, Pernthaler J (2009). Latitudinal distribution of prokaryotic picoplankton populations in the Atlantic Ocean. *Environ Microbiol* 11: 2078.
Wietz M, Gram L, Jørgensen B, Schramm A (2010). Latitudinal patterns in the abundance of major marine bacterioplankton groups. *Aquat Micro Ecol* 61:179.

EMP50

Proposal for the down-stream pathway of anaerobic naphthalene degradation

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Introduction: 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). Also the following metabolic steps are still unknown, but there is strong evidence that β-oxidation like steps are involved (Annweiler *et al.*, 2002).
Objectives: Our aim for this study was the elucidation of the degradation pathway down-stream of the dearomatising reductions with special focus on the two ring-opening reactions and the involved enzymes.
Methods: The conversion of known metabolites and chemical analogues of these substances was tested both with whole cells and with cell-free extracts of N47 and NaphS2. Metabolites were identified via mass-spectrometry. Additionally, selected genes from a gene-cluster co-transcribed with the genes coding for the dearomatising reductase were heterologously expressed in *Escherichia coli* and the activity of the correspondent enzymes was assayed with potential substrate analogues.
Results: The analysis of metabolites occurring from natural substrates or substrate analogues allowed further insights into the degradation pathway. Furthermore, some theoretical enzyme functions deduced from sequence alignments and comparisons with other pathways could be proven for substrate analogues. For example, the activity of a ring-cleaving hydrolase of the crotonase-type could be demonstrated with the substrate analogue 2-oxocyclohexanecarboxyl-CoA.
Conclusion: Combining the results from metabolite analyses and assays with heterologously produced enzymes, we can for the first time make a detailed proposal for the down-stream pathway of anaerobic naphthalene degradation and an attribution of enzymes to the catalysis of the proposed metabolic steps.

EMP51

Identification of iron-cyanide degrading microorganisms in roadside soils

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Introduction: Deicing salt application leads to temporary inputs of anthropogenic ferrocyanide complexes into the road environment, having the potential to release extremely toxic free cyanides (CN^- and HCN). This, on the one hand, could occur via photolysis when contaminated ground water is transferred to the surface. On the other hand, biodegradation of ferrocyanide complexes might occur via specialized microorganisms.
Objectives: The aim of this study was to investigate (1) if ferrocyanide complexes in roadside soils are biodegraded without previous induction via photolysis and (2) to identify the microorganisms involved in their biodegradation.
Methods: A microcosm study was conducted using two roadside soils and one control soil spiked with ^{12}C- or ^{13}C-labelled ferrocyanide (100 mg kg^{-1}), respectively. The microcosms were incubated in the dark. Sampling occurs after 18, 32 and 74 days. Soil cyanide contents were determined spectrophotometrically. The extracted DNA was separated according to its buoyant density using CsCl gradients. Afterwards, the microbial community composition was analyzed using TRFLP fingerprinting and 454 pyrosequencing based on 16S rRNA genes.
Results: Already after 32 days, biodegradation of ferrocyanide complexes was detected, both in roadside soils and the control soil. Although the soils differed in their microbial community composition, microorganisms belonging to *Xanthomonadaceae* and *Flavobacteriaceae* seemed to be involved in ferrocyanide biodegradation in all soils. However, also soil specific biodegraders could be found. More details about the microbial community structure and the microorganisms involved in ferrocyanide biodegradation will be presented on the conference.

EMP52

Biofilm formation of the methanoarchaeal human gut inhabitants *Methanosphaera stadtmanae* and *Methanobrevibacter smithii*

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Introduction: To date, a plethora of microorganisms has been identified as inhabitants of the human intestine. In adaption to this environment, it appears reasonable that the vast majority of the intestinal microbiota adheres to mucosa surfaces by forming biofilms. In particular, *Methanosphaera stadtmanae* and *Methanobrevibacter smithii* are well-known representatives of the archaeal domain reported to be members of the indigenous human microbial flora. Despite this, profound insights into their capability to build biofilm structures as well as into the composition of such are rather scarce.
Objectives: This study aimed to elucidate whether the gut inhabitants *M. stadtmanae* and *M. smithii* are able to grow on surfaces in a biofilm-specific manner. In order to gain substantial knowledge on functional factors regulating the process of methanoarchaeal biofilm formation, the effect of different experimental conditions - e.g. incubation time, media composition, stress - was investigated.
Materials & Methods: *M. stadtmanae* and *M. smithii* were grown on mica in hungate tubes or on plastic µ-dishes^{TM} with 3 mL of minimal medium, respectively [1]. Cells were fixed to mica or µ-dishes^{TM} by 2 % glutaraldehyde. Prior to imaging by means of both confocal laser scanning microscopy and scanning electron microscopy, cells were stained with a PBS buffer solution containing SYTO 9 and propidium iodide (LIVE/DEAD staining).
Results: The two investigated methanoarchaeal strains formed biofilms on both abiotic surfaces provided in this study. Biofilm thickness and surface coverage varied depending on different experimental parameters, for instance the incubation time and the media composition. An increase in biofilm height and density was observed upon cultivation of *M. stadtmanae* in media supplemented with formate and rumen fluid.
Conclusion: The mucosa-associated methanoarchaeal strains *M. stadtmanae* and *M. smithii* are able to form biofilms and are hence potentially present as such in the human intestine. Based upon our findings, we are expecting an enhanced thickness for *M. stadtmanae* environmental biofilms since the human gastrointestinal tract represents an exceeding nutrient-rich habitat.

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EMP53

From acidic stalactites to nanoorganisms

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We studied stalactite-like biofilms in an abandoned pyrite mine in the Harz Mountains (Germany) to analyze a microbial community that is based on autotrophic pyrite oxidizing microorganisms as primary producers.
The analyzed biofilms contain a full iron and sulfur cycle within a micrometer scale. In these snottites the microorganisms are embedded in a matrix of carbohydrates and bio-/geochemical products of pyrite oxidation whereas ferrous-sulfate rich water with a pH of 2.3 drops constantly through them. Since XANES measurements showed jarosite as the major mineral element, oxidative dissolution of pyrite seems to be the primary energy source. $^{14}CO_2$-fixation analyzes indicated carbon fixation mainly in the outer parts of the snottites and *in situ* oxygen-measurements showed rapidly declining oxygen contents from the outer to the inner parts. CARD-FISH-pictures as well as 16S rDNA analyses revealed that the primary producers are lithotrophic *Leptospirillum*- and *Acidithiobacillus* species, which oxidize ferrous to ferric iron in the oxic outer parts. Reduced sulfur species can be oxidized to sulfate by *Acidithiobacillus spp.*. Together with ferric iron sulfate is potentially reduced by *Ferroplasma spp.* and some uncultured members of the *Thermoplasmatales*, which all occur only in the anoxic inner parts.
It remains the main question what role the detected unculturable *Thermoplasmatales* and ARMAN (Archaeal Richmond Mine acidophilic nanoorganisms) play and why it is so far impossible to cultivate them in pure culture.
To answer it, we designed an anoxic medium for the enrichment of the uncultured archaea. It contains $Fe(III)SO_4$ as well as an organic carbon source as necessary compounds. The head space has an H_2/CO_2 atmosphere. CARD-FISH and 16S rDNA analyses show the enrichment of so far uncultured *Thermoplasmatales*. Still the most surprising result is the good development of ARMAN. Against the so far existing results of a strictly aerobic lifestyle, we can postulate a facultatively anaerobic metabolism of these organisms.

FTP01

New insights into the discrimination between an aldolase and a transaldolase

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Introduction: Transaldolases (TAL) catalyse the transfer of a dihydroxyacetone (DHA) moiety from a ketose donor onto an aldose acceptor. This reaction proceeds via a Schiff base intermediate [1]. Whereas, the fructose 6-phosphate (F6P) aldolase (FSA) of *E. coli* catalyses the synthesis of F6P from DHA and glyceraldehyde 3-phosphate [2]. $TalB^{F178Y}$ of *E. coli* was engineered resulting in a catalytic efficiency for the synthesis of F6P similar to FSA but still retains TAL activity [3]. In FSAA, the catalytic water molecule is bound by the side chains of Tyr131, Gln59 and Thr109, whereas in TalB only two residues (Glu96 and Thr156) participate in H-bonding [4,5].
Objectives: We aim to convert a transaldolase completely into an aldolase and a FSA vice versa, and to elucidate the role of the H-bonding pattern of the catalytic water molecule in the reaction mechanism.
Methods: The H-bonding network of the catalytic water molecule was investigated using site-directed mutagenesis. Single and double variants were characterized with respect to FSA and TAL activity, stability, pH-dependence of activity, pK_a-value of the essential lysine residue, as well as 3D structure.
Results: No differences in the structure could be detected via CD/DLS spectroscopy and crystal structures. In FSAA, no TAL activity could be introduced, yet. The exchange of Glu96 to Gln and Phe178 to Tyr in TalB resulted in a lower specific activity for the TAL reaction (0.6 U/mg, 12 % of $TalB^{wt}$) than for the FSA reaction (4.4 U/mg, 40 % of $FSAA^{wt}$). In TalB, the Schiff base forming lysyl residue is positively charged (pK_a 9.4) whereas in FSAA the lysyl residue is neutral (pK_a 5.4).
Conclusion: The observed enzymatic activities of the TalB variants further emphasize the role of Glu96 as general acid/base in TalB. The key player in the in fructose 6-phosphate aldoses is Y131. A new mechanism for the formation of the Schiff base intermediate in FSAA will be proposed.

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FTP02

***trp1*$^+$ vector transformation in auxotrophic *Coprinopsis cinerea* strains results in only half the number of *trp1*$^+$ clones than a co-transformation with a *trp1*$^+$-free plasmid**

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Introduction: Genetic transformation of *Coprinopsis cinerea* makes use of complementation of auxotrophies, such as by defects in gene *trp1* for tryptophan synthetase. DNA integrates in transformation at multiple ectopic sites into the host nucleus, singly or in tandem copies. Multiple integration sites offer the chance of co-transformation of two different plasmids at the same time. With equal amounts of both vectors, equal numbers of nuclear sites should become occupied by the two distinct DNAs in co-transformation.
Objectives: Using the *trp1*$^+$ gene in transformation revealed a surprising phenomenon: Transformation with just a *trp1*$^+$ vector gives only low numbers of clones, whereas co-transformation with a *trp1*$^+$-free plasmid doubles the transformants.

Methods: To explore this phenomenon, different lengths of the *trp1*$^+$ fragment, different vector backbones, different relative plasmid concentrations in co-transformations, the effect of aromatic amino acid and tryptophan precursor supplementation, *pab1*$^+$ (for PABA synthetase) as an alternative selection marker and a Δ*ku70* strain defective in ectopic DNA integration were tested.
Results: Truncations of *C. cinerea* DNA to just the *trp1*$^+$ gene sequence and changing the vector backbone did not alter the phenomenon. Using different relative plasmid concentrations showed that keeping the *trp1*$^+$ vector high caused a sharp decrease in numbers of *trp1*$^+$ transformants. Keeping instead the other plasmid high caused only a minor decrease in numbers. Supplementations in the medium reduced the numbers of *trp1*$^+$ transformants in single and in co-transformations. In contrast, using a *pab1*$^+$ vector for selection always resulted in same numbers of transformants in single and in co-transformations with a *trp1*$^+$ vector. Equal numbers of clones were also observed in single and co-transformation of the Δ*ku70* strain.
Conclusion: The phenomenon of reduction of transformants is specific to *trp1*$^+$. Loss of clones is due to ectopic integration of *trp1*$^+$ at multiple sites. Tryptophan biosynthesis is under feedback inhibition by tryptophan at the first enzyme of the pathway, anthranilate synthase. Integration of more *trp1*$^+$ copies into a nucleus might cause an overexpression with a strong feedback effect up to an irreversible shut-down of the pathway.

FTP03
Archaea on human skin - news and views
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Introduction: Currently, archaea are considered minor, irrelevant components of the human microbiome. Methanogens have been detected in the oral cave as well as in the intestinal tract, Thaumarchaeota have been detected on human skin (1). To date, it is still unclear whether Archaea can be opportunistic pathogens or not, although they seem to be involved in pathogenic processes of the digestive tract.
Objectives: We are currently investigating the archaeal inventory of the human body, with major focus on human skin. Our major goal is to obtain insights into their abundance, distribution and role, interacting with the larges human organ.
Material methods: In a very recent study, we have analysed 50 persons of different age (1-80) with respect to the archaeal diversity associated to their torso skin. The samples were analysed using quantitative PCR and 454 pyrotag-sequencing.
Results: All of the samples analysed revealed detectable amount of Archaea. However, some samples revealed a very low, others very high abundance (up to 10 % in quantitative PCR results). In particular elder people (starting from age of 55) showed significantly increased levels of Thaumarchaeota on their skin.
Conclusion: To date it is unclear how these Archaea are involved in ammonia-turnover on skin or whether they are beneficial, commensals or even (opportunistic) pathogens.
In this talk, we will present the new data obtained from the current study and discuss a potential role.

FTP04
Staphylococcal PSM peptides contribute to influenza infection
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Introduction: Staphylococcal phenol-soluble modulin (PSM) peptides have been shown to be important virulence factors and cytotoxins and to be connected to many infection processes. However, their role in staphylococcal pneumonia has not been elucidated. Secondary bacterial infections are a common risk factor in inducing pneumonia and often follow established Influenza A virus (IAV) infection. Pathogens such as *S. aureus* coinfect the lungs, resulting in an aggravated and occasionally fatal clinical picture. CA-MRSA strains are among the most commonly occurring pathogens in this setting, especially in hospital acquired pneumonia. Their treatment is increasingly complicated due to antibiotic resistance and their virulence repertoire, which is not yet fully understood.
Objectives: We aimed to determine whether staphylococcal phenol-soluble modulin (PSM) peptides influence *S. aureus* coinfection with IAV.
Methods: Both IAV-contaminated and IAV-uncontaminated human lung epithelial cells (A549) were challenged with PSM peptides. Subsequently, cell viability and IL-8 secretion was analyzed. Furthermore, mice were infected with IAV and coinfected with CA-MRSA strain USA300 LAC wild-type or the corresponding PSM knockout mutant. Survival of the mice was determined.
Results: PSMs were cytotoxic for A549 cells and induced the secretion of IL-8. Both effects were boosted by antecedent IAV infection of the eukaryotic cells. Moreover, PSMs significantly exacerbated an existing IAV infection *in vivo*.
Conclusions: With our results we emphasize the cytotoxic versatility of PSMs as well as their influence as proinflammatory agents. In addition, PSMs should also be considered important virulence factors in *S. aureus*-derived pneumonia and consequently important therapy targets in this setting.

FTP05
From flagella to chemoreceptors: Analysing the cell architecture of the hyperthermophilic Archaeon *Methanocaldococcus villosus*
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Introduction: The hyperthermophilic Archaeon *Methanocaldococcus villosus* was isolated from a shallow submarine hydrothermal system at Kolbeinsey Ridge, north of Iceland. Cells possess up to 50 polar flagella which were shown to be multifunctional organelles mediating not only swimming, but also adhesion to abiotic surfaces and formation of cell-cell contacts [1].
Objectives: Using *M. villosus* as a suitable novel model organism, this study was set out to better understand the whole-cell architecture of this organism and Archaea in general with focus on the anchoring of flagella and potential chemoreceptor arrays.
Methods: To analyze the ultrastructure and whole-cell architecture of *M. villosus*, different microscopic techniques were used. Cells were prepared for electron microscopy by high-pressure freezing/freeze substitution or conventional chemical fixation [2]. Specimens were investigated by transmission electron microscopy (TEM) and by (focused ion beam) scanning electron microscopy (FIB-SEM). Antibodies were generated against recombinantly expressed chemoreceptors and were used for immuno-localisation studies.
Results: Electron microscopic analyses of *M. villosus* cells revealed a densely packed cytoplasm surrounded by a cytoplasmic membrane and an S-layer with (pseudo-) sixfold symmetry. A submembraneous layer was identified at that pole of the cell from which flagella protrude suggesting that the layer is related to flagellar anchoring. In some cases, this layer was associated with a complex structure resembling bacterial chemoreceptor arrays. Immuno-labeling studies proved the existence and localisation of chemoreceptors.
Conclusion: The different microscopic techniques used herein extend our current knowledge on the cellular organisation of Archaea. The ultrastructural analyses of this study confirm the suggested connection of the chemotactic system with the flagellar apparatus.

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FTP06

Bioinformatic Analysis and Construction of the Variome of the Virulence Factors and Genetic Regulators in *Streptococcus pneumoniae*

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Introduction: In recent years, the idea of a highly immunogenic protein-based vaccine to combat *Streptococcus pneumoniae* and its severe invasive infectious diseases has gained considerable interest among researchers. However, the target proteins to be included in such a formulation have to accomplish several characteristics in order to ensure its suitability and effectiveness.
Objective: Here, the aim was to get comprehensive insights into the genomic organization, population distribution and genetic conservation of all the pneumococcal surface-exposed proteins, genetic regulators and other virulence factors, whose important function and role in pathogenesis has been demonstrated.
Methods: After retrieving the complete set of DNA and protein sequences reported in databases for all pneumococcal strains whose genomes have been fully sequenced and annotated, a comprehensive bioinformatic analysis and systematic comparison has been performed for each virulence factor, stand-alone regulator and two-component regulatory system encoded in the pan-genome of *S.pneumoniae*.
Results: A total of 25 pneumococcal strains, representing different phylogenetic groups, were considered. A set of 92 different genes and proteins were identified, classified and studied for the construction of a pan-genomic variability map (Variome) for *S. pneumoniae*. Both pneumococcal virulence factor and regulatory genes were well-distributed on the pneumococcal genome and transcriptionally located in a co-oriented way in relation to the region in which the chromosomal replication is initiated. The analysis of the population distribution for each gene and protein showed that 49 of them are belonging to the core-genome, while 43 belong to the flexible-genome. The estimation of the genetic variability allowed to establish that the pneumococcal virulence factors with the highest conservation are the toxin pneumolysin, enolase and Usp45, while the most conserved genetic regulators in the pneumococcus are TCS05, TCS02 and TCS08.
Conclusion: The results obtained here allowed to identify those well-distributed and highly-conserved pneumococcal virulence factors and regulators suitable to be included in a new generation of (protein-based) vaccines to combat pneumococcal infections.

GRP01

Ketone-based janthinobacterial quorum sensing in nature: *Janthinobacterium* and its conversational partners

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Genome analyses of nine isolated, sequenced and characterized purple-pigmented *Janthinobacterium* sp. HH01 [1] and HH100 - HH107, showed an average of 6.7 Mbp, 6,000 ORFs and 63.4% GC content. Interestingly no autoinducer I- and II synthase genes could be identified (i.e. *luxI*- and *luxS* homologs) in any of the sequenced strains. Instead the janthinobacterial quorum sensing system (JQS) [1] is present in all nine strains. The JQS system is homolog to the *Vibrio cholerae* and *Legionella pneumophila* CQS/ LQS systems and consists of an autoinducer synthase (JqsA), a sensor kinase/phosphatase (JqsS) and a response regulator (JqsR) [1,2]. *In silico* analysis and complementation studies indicate the corresponding autoinducer JAI-1 to be 2-aminopentadec-2-en-4-one, deviating in the head group and chain length to the *V. cholerae* CAI-1 and *L. pneumophila* LAI-1 autoinducers. RNA-seq technology of HH01 and the JqsA deficient mutant HH02 [1] showed 34 repressed and 57 JAI-1 induced genes. This includes the polyketide synthase Jab_2c35360, as well as the non-ribosomal peptide synthase Jab_2c35400, two representatives of the high number of NRPS clusters present in HH01. Ongoing work in the laboratory focuses on the role of JAI-1 for the interaction of this microbe with its environment and other microorganisms.

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GRP02

***trp1*$^+$ vector transformation in auxotrophic *Coprinopsis cinerea* strains results in only half the number of *trp1*$^+$ clones than a co-transformation with a *trp1*$^+$-free plasmid**

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Introduction: Genetic transformation of *Coprinopsis cinerea* makes use of complementation of auxotrophies, such as by defects in gene *trp1* for tryptophan synthetase. DNA integrates in transformation at multiple ectopic sites into the host nucleus, singly or in tandem copies. Multiple integration sites offer the chance of co-transformation of two different plasmids at the same time. With equal amounts of both vectors, equal numbers of nuclear sites should become occupied by the two distinct DNAs in co-transformation.
Objectives: Using the *trp1*$^+$ gene in transformation revealed a surprising phenomenon: Transformation with just a *trp1*$^+$ vector gives only low numbers of clones, whereas co-transformation with a *trp1*$^+$-free plasmid doubles the transformants.
Methods: To explore this phenomenon, different lengths of the *trp1*$^+$ fragment, different vector backbones, different relative plasmid concentrations in co-transformations, the effect of aromatic amino acid and tryptophan precursor supplementation, *pab1*$^+$ (for PABA synthetase) as an alternative selection marker and a *Δku70* strain defective in ectopic DNA integration were tested.
Results: Truncations of *C. cinerea* DNA to just the *trp1*$^+$ gene sequence and changing the vector backbone did not alter the phenomenon. Using different relative plasmid concentrations showed that keeping the *trp1*$^+$ vector high caused a sharp decrease in numbers of *trp1*$^+$ transformants. Keeping instead the other plasmid high caused only a minor decrease in numbers. Supplementations in the medium reduced the numbers of *trp1*$^+$ transformants in single and in co-transformations. In contrast, using a *pab1*$^+$ vector for selection always resulted in same numbers of transformants in single and in co-transformations with a *trp1*$^+$ vector. Equal numbers of clones were also observed in single and co-transformation of the *Δku70* strain.
Conclusion: The phenomenon of reduction of transformants is specific to *trp1*$^+$. Loss of clones is due to ectopic integration of *trp1*$^+$ at multiple sites. Tryptophan biosynthesis is under feedback inhibition by tryptophan at the first enzyme of the pathway, anthranilate synthase. Integration of more *trp1*$^+$ copies into a nucleus might cause an overexpression with a strong feedback effect up to an irreversible shut-down of the pathway.

GRP03

Interference of Transcription on H-NS/StpA Repression

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H-NS is a global repressor in *E.coli*. 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. 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[1]. 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 loci (*bgl* and *proU*) the increase in transcription into H-NS binding regions indeed reduces repression by H-NS and StpA. Similar results were obtained for other H-NS repressed loci, namely *ygeH, yahA* and *appY*. Further, we show, using the *bgl* system, that transcription by a mutant RNA polymerase that forms less stable elongating complexes can result in enhanced repression by H-NS.
Taken together, our data suggest that the transcribing RNA polymerase can dislodge H-NS from the DNA. 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. In contrast, at low transcription rates or when an elongation defective RNA polymerase is used H-NS repression is effective.
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GRP04
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 and stress adaptation. Intriguingly, regulation of the H-NS repressed *leuO* gene involves a double-positive feedback loop. LeuO activates expression of *bglJ*, encoded within the H-NS repressed *yjjQ-bglJ* operon [1], and vice versa the *leuO* gene is activated by the BglJ-RcsB heterodimer [2]. Activation of *leuO* by BglJ-RcsB is antagonistically controlled by LeuO [2] suggesting that the double-positive feedback regulation of *leuO* is tightly controlled. Examination of expression of native *leuO* with a fluorescence reporter fusion revealed heterogeneity of *leuO* expression within the population. 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. The antagonistic control of the *leuO* promoter activity by LeuO and BglJ seems to be controlled in a concentration-dependent manner. Furthermore, screening for additional activators of *leuO* revealed a novel regulator which activates a third *leuO* promoter. The obtained data serve as a basis for a theoretical model of the antagonistic regulation of the *leuO* promoter. In a further attempt we try to elucidate the so far unknown inducing conditions of the *leuO* promoter in a natural environment. With these approaches 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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GRP05
IpsA, a novel LacI-type regulator required for cell wall biosynthesis in Corynebacteria and Mycobacteria
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Introduction: The development of new drugs against tuberculosis and diphtheria is focused on disrupting the biogenesis of the cell wall, the unique architecture of which confers resistance against current therapies. The enzymatic pathways involved in cell wall formation in these pathogens are well understood but the underlying regulatory mechanisms are largely unknown.
Objectives: *Corynebacterium glutamicum* lacking the LacI-type regulator IpsA displayed a drastically altered cell morphology hinting towards a function of this protein in the control of cell wall biosynthesis. The aim of this study was the elucidation of the underlying regulatory mechanisms leading to the described phenotype.
Methods: For the analysis of the phenotype, fluorescent microscopy as well as lipid and lipoglycan analysis by TLC we used. DNA Microarrays, EMSAs and promoter studies with fluorescent proteins were applied to identify and verify IpsA target genes.
Results: IpsA was shown to trigger *myo*-inositol formation by activating *ino1* encoding an inositol-phosphate synthase. An *ipsA* deletion mutant of *C. glutamicum* grown on glucose displayed significantly impaired growth, an elongated cell morphology and uneven DNA distribution. Further studies revealed the absence of inositol-derived lipids in the cell wall and a complete loss of mycothiol biosynthesis. The phenotype of the *C. glutamicum ipsA* deletion mutant was partially complemented by homologs of *Corynebacterium diphtheriae* (dip1969) and *Mycobacterium tuberculosis* (rv3575), indicating the conserved function of IpsA in these species. Additional targets of IpsA with putative functions in cell wall biogenesis were identified and IpsA was shown to bind to a conserved palindromic motif within the corresponding promoter regions. *Myo*-inositol was identified as an effector of IpsA, causing the dissociation of the IpsA-DNA complex *in vitro*.
Conclusion: This characterization of the IpsA regulon sheds light on the complex transcriptional control of cell wall biogenesis in the mycolata taxon and generates novel targets for drug development [1]. Based on this study, further factors involved in cell wall biogenesis are currently under investigation.

M. Baumgart, K. Luder, S. Grover, C. Gätgens, G.S. Besra and J. Frunzke, BMC Biol **11** (2013), 122.

GRP06
Gene regulation by molybdenum in *Agrobacterium tumefaciens*
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The vast majority of all bacteria utilizes molybdenum to synthesize the molybdenum cofactor (Moco). Enzymes containing Moco, so-called molybdoenzymes, are key players in the global sulfur, carbon, and nitrogen cycles. To supply molydoenzymes with molybdenum, most bacteria synthesize a high-affinity molybdate transporter (ModABC) [1]. In *Escherichia coli*, ModE functions as a repressor of the *modABC* operon at high molybdate concentrations [2;3;4]. *E. coli* ModE is a one-component regulator sensing the cellular Mo-status by a molybdate-binding domain. In contrast to *E. coli* ModE, *Agrobacterium tumefaciens* ModE lacks a molybdate-binding domain leading us to ask, how *A. tumefaciens* measures the Mo-status. Here we provide evidence that *A. tumefaciens* "Mini-ModE" is essential for repression of *modABC* in response to molybdate. In addition, *modE* transcription and ModE accumulation were determined by reporter gene and western blot analysis.

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GRP07
Finding the happy medium: normalization of RNA sequencing data by applying reference genes
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Introduction: High-throughput RNA sequencing (RNA-seq) techniques have revolutionized the field of transcriptomics. However, standardization of good practice in data evaluation drags behind the capability to generate tremendous amounts of raw data. This is reminiscent of the situation that occurred during the late 1990s when the microarray era was about to revolutionize transcriptomics back then. One possible application for RNA-seq is determination of differentially expressed genes (DEG) and in this regard normalization between data sets represents a critical issue. The most common normalization method is the RPKM (reads per kilo base per million) method. Unfortunately, RPKM values among samples can be unevenly biased by a small number of highly expressed genes [1].
Objectives: Reference genes were applied for normalization of RNA-seq data to find DEG in the DNA damage (SOS) response of *Escherichia coli*.
Methods or Materials & Methods: Total RNA from multiplexed samples was sequenced on an Illumina HiSeq machine (paired-end 100 bp reads) to generate ~10 million reads per sample. Stable expression of selected reference genes was validated by qRT-PCR. Bioinformatics were applied to perform normalization and to analyze DEG.
Results: The selected reference genes differ from each other in terms of expression levels, ranging from low (*cysG*) over medium (*idnT, hcaT, ihfB*) to high (*ssrA, rrsA*), but remain unchanged during the SOS response. Our normalization-by-reference approach will be discussed and compared to other normalization methods (e.g. upper-quartile and DESeq2).
Conclusion: Analysis of the SOS response data significantly improved through appropriate normalization and we therefore hope that our considerations will call attention to a common problem in RNA-seq data analysis.
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GRP08

Rhizobial homologs of fatty acid transporter FadL promote perception of long-chain acyl homoserine lactone quorum sensing signals

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Introduction: Quorum sensing (QS) using N-acyl homoserine lactones (AHLs) as signal molecules is a common strategy used by diverse Gram-negative bacteria. It is employed by bacteria to control functions related to group behavior and enables populations to achieve goals that a single cell could not manage. A widespread mechanism of AHL sensing involves binding of these molecules by cytosolic LuxR-type transcriptional regulators, which requires uptake of external AHLs. The outer membrane is supposed to be an efficient barrier for diffusion of long-chain AHLs.
Objectives: Identify the mechanism of active AHL uptake.
Methods or Materials & Methods: site-directed mutagenesis, promoter-reporter assays, AHL detection by an indicator strain, growth assays.
Results: In *Sinorhizobium meliloti*, sensing of AHLs with acyl chains composed of 14 carbons or more is facilitated by the outer membrane protein $FadL_{Sm}$, a homolog of the *E. coli* FadL long-chain fatty acid transporter. The effect of $fadL_{Sm}$ on AHL sensing was more prominent for longer and more hydrophobic signal molecules. $fadL_{Sm}$ increased sensitivity and accelerated the course of QS. In contrast to $FadL_{Ec}$, $FadL_{Sm}$ did not support uptake of oleic acid, but it contributed to growth on palmitoleic acid. $FadL_{Sm}$ homologs from related symbiotic a-rhizobia and the plant pathogen *Agrobacterium tumefaciens* differed in their ability to facilitate long-chain AHL sensing or to support growth on oleic acid. $FadL_{At}$ was found to be ineffective towards long-chain AHLs. We obtained evidence that a part of the predicted extracellular loop 5 (L5) determines the long-chain AHL-specificity of $FadL_{Sm}$ [1].
Conclusion: An outer membrane protein FadL was identified that facilitated perception of long-chain AHLs by *S. meliloti* and was present in closely related symbiotic a-rhizobia.

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GRP09

The transcriptional repressor NanR controls sialic acid catabolism in *Corynebacterium glutamicum*

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The Gram-positive, non-pathogenic *Corynebacterium glutamicum* is well known for its application in industrial amino acid production. It utilizes various sugars and organic acids as sources of carbon and energy including sialic acid [1]. Sialic acid is metabolized to fructose-6-P by concomitant action of the sialic acid importer $SiaEFGK_2$, *N*-acetylmannosamine kinase NanK, *N*-acetylmannosamine-6-P epimerase NanE, *N*-acetylneuraminic lyase NanA, *N*-acetylglucosamine-6-P deacetylase NagA, and Glucosamine-6-P deaminase NagB. The genes for sialic acid utilization are organized in the three clustered operons namely *nagAB*, *nanAKE*, and *siaEFGK*. Within this cluster of operons a fourth operon is present, which comprises the orf *cg2936* for a putative GntR-type transcriptional regulator.
We here characterize the role of the *cg2936* encoded regulator NanR for the transcriptional control of *nagAB*, *nanAKE*, *siaEFGK* operons and its own expression. In growth experiments we observed consecutive utilization of glucose and sialic acid as well as fructose and sialic acid by *C. glutamicum* WT whereas the *cg2936* deletion mutant *C. glutamicum* ΔnanR simultaneously consumed these carbon sources. In addition no uptake of ^{14}C-labeled sialic acid was detected in cells of *C. glutamicum* WT cultivated on glucose or fructose and high rates of sialic acid uptake were measured for *C. glutamicum* WT cultivated on sialic acid as well as for cells of *C. glutamicum* ΔnanR independent of the substrate used for their cultivation. Microarray studies, reporter gene assays, and qRT-PCR analyses showed the operons *nagAB*, *nanAKE*, and *siaEFGK* as well as *nanR* repressed in *C. glutamicum* WT and highly induced in *C. glutamicum* ΔnanR. Electrophoretic mobility shift assays showed that purified NanR binds specifically to the TANACATNAGACATCANACGT nucleotide motifs located within the *nagA-nanA* and *the nanR-sialA* intergenic regions. Binding of NanR to promoter regions was abolished in the presence of the sialic acid metabolism intermediates GlcNAc-6P and ManNac6-P. Taken together these findings show that sialic acid metabolism is subject to NanR controlled catabolite repression in *C. glutamicum*.

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GRP10

Construction of *E. coli* Reporter Strains for the Study of RpoS as Regulator Protein

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Introduction: The product of the *rpo*S gene is an alternative sigma factor which is essential for cells when they are exposed to various stress conditions (hyperosmolarity, pH downshift, high or low temperature, limitation on C- or N- source). Usually for the study of regulatory proteins reporter strains are being used. These strains contain reporter proteins (such as GFP, GUS, LacZ, YFP or RFP) which are combined with a promoter. The transcription from this promoter should depend on a specific regulator.
Objectives: The reporter system (RpoS dependent promoter) was chromosomally integrated upstream of the *lac*ZYA operon (LJ110 P_{rssA}-*lac*ZYA). As control strains, *E. coli* strains with the deletion of the *rpo*S gene with the same reporter system were constructed (LJ110 Δ*rpo*S P_{rssA}-*lac*ZYA).
Methods: For the construction of the mutant strains, the method of Datsenko and Wanner (1) was used. For measuring the ß-galactosidase activity the method of Miller (2) and Li et. al (3) with modifications was used.
Results: The cells of RpoS-mutants with the reporter system were studied under N- and C- limitation conditions. The activity of ß-galactosidase under N-limitation in the stationary growth phase was nearly two times lower as under excess of N-source. Under glucose limitation in the stationary growth phase, we observed about three times higher activity of ß-galactosidase in comparison to the activity under excess of glucose.
Conclusion: This reporter system can be used for the study of the RpoS regulator. It was shown that the RpoS-mutants under N-limitation overgrow the wild type, but not under C-limitation. The RpoS regulator has a different influence on the same promoter under different limitations.

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GRP11

Classification of phenotypic subpopulations in isogenic bacterial cultures by multiple promoter probing at single cell level

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Question: The Gram negative alphaproteobacterium *Sinorhizobium meliloti* produce exopolysaccharides (EPS) as common goods. These serve multiple roles in enhancing the survival of the population, such as motility, protection against harsh physical conditions, and host interactions during symbiosis. However, EPS production also places a heavy metabolic burden on individuals when nutrients are typically scarce. One way to overcome this challenge might be to restrict EPS production to a subset of the population, i.e., phenotypic heterogeneity. Yet how this could be accomplished in isogenic *S. meliloti* populations is almost a complete mystery and remains to be elucidated.
Method: We constructed a multi-fluorescent reporter gene cassette applicable to simultaneously monitor activities of three promoters, either integrated into the genome or a plasmid vector. Here, we report on the properties of this triple promoter probe construct and demonstrate its performance by characterizing the activity of representative EPS biosynthesis relevant genes in *S. meliloti* populations.
Results: Using the triple reporter construct, detailed information has been collected during time-lapse observations of a single cell growing into a colony of hundreds of individuals. We validated the performance of this reporter cassette and also provided new insights into heterogeneity of EPS production in isogenic *S. meliloti* populations. Further, we developed a novel mathematical method for classification of cell types based on the increase in fluorescence per generation that suggested three subpopulations with regard to EPS biosynthesis: strong-contributors (SC), weak-contributors (WC), and non-contributors (NC).

Conclusions: The new developed triple reporter construct emerged as a powerful tool to discover heterogeneity in EPS production in isogenic populations of *S. meliloti*. To unravel the mechanism(s) which generate heterogeneity in this system, the new developed observation and analysis tools, combined with targeted mutations of relevant genetic components will allow further testing of possible sources of heterogeneity in the EPS production pathway.

GRP12
Regulatory Functions of the Pneumococcal TCS08
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Introduction: *Streptococcus pneumoniae* (pneumococci) is a serious pathogen encoding 14 two-component regulatory systems (TCS). These TCS are crucial for various patho-physiological processes which are indispensable for bacterial competence, fitness and virulence. TCS consist of a sensor, the histidine kinase (HK), and a response regulator (RR). The genes encoding the TCS08 are located (within the core genome) downstream of the gene encoding the adhesin PavB (**P**neumoccal **a**dherence and **v**irulence Factor **B**). PavB has been associated with adherence and pathogenicity during the early stage of pneumococcal colonization. Here, we have evaluated the interaction of the TCS08 proteins and their regulatory effect on the expression of PavB and other pneumococcal surface proteins.
Objectives: This study aimed to elucidate regulatory functions of the TCS08 with a special focus on PavB expression.
Methods: The isolation and purification of the TCS08 proteins were carried out using a maltose binding protein-tagged HK08 and RR08 and affinity chromatography. The interaction between the recombinant HK08 and RR08 were studied by phosphotransfer profiling and an EMSA has been established to investigate the interaction between the RR08 and the promoter region of *pavB*.
Results: The purity of recombinant MBP-HK08 and MBP-RR08 were determined by SDS-PAGE and immunoblot analysis. A phosphorylation interaction was demonstrated between the HK08 and its cognate RR08, suggesting autophosphorylation of RR08 and phosphatase activity of HK08. A shift in the electrophoretic mobility of a *pavB* promoter fragment was illustrated when the promoter interacts with the non-phosphorylated RR08. The *hk08*-mutant had a dramatically increased expression of the PavB protein, while the *rr08*- or the *tcs08*-mutant (negative for RR and HK) showed PavB levels similar to the wild-type, suggesting a repressor function of non-phosphorylated RR08 for the expression of PavB.
Conclusion: The results revealed that the TCS08 itself is not directly related with virulence and pathogenicity but regulates at least the expression of the PavB of *S. pneumoniae*.

GRP13
Functional analysis of regulatory circuits involving the TEA family transcription factor Tec1 in *Saccharomyces cerevisiae*
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Introduction: In *Saccharomyces cerevisiae*, the two transcription factors Tec1 and Ste12 are under control of a conserved MAPK signaling module, which controls mating, biofilm formation and filamentation in response to pheromone or nutrient signals. Previous studies revealed detailed insights into the wiring of many MAPK module components. A crucial regulatory circuit operates at the posttranslational level and causes rapid degradation of Tec1 in response to pheromone and MAPK phosphorylation, to prevent inappropriate activation of biofilm genes during mating. Two further transcriptional regulatory circuits act at the *TEC1* promoter and involve (i) several Tec1 consensus binding sites (TCSs) forming a potential auto-regulatory loop during biofilm formation, and (ii) a number of pheromone responsive elements (PREs), which are bound by Ste12 and are expected to confer regulation in response to pheromone.
Objectives: We have initiated a detailed analysis of the two transcriptional regulatory circuits that act at the *TEC1* promoter, because their precise physiological roles are largely unknown.
Methods: The functions of the transcriptional regulatory circuits were analyzed by using synthetic *TEC1* promoters lacking either TCS or PRE elements, or both. Ste12-mediated control of *TEC1* was further replaced by synthetic doxycyclin-mediated transcriptional control. Effects of synthetic manipulations were analyzed by quantification of Tec1 production and Tec1-mediated gene expression, biofilm formation and filamentation.
Results: We find that the PRE-mediated regulatory circuit not only confers induced *TEC1* transcription in response to pheromone, but is also essential for efficient Tec1 production during biofilm formation. Moreover, PRE-mediated control of *TEC1* expression can be fully uncoupled from mating control by synthetic doxycyclin-mediated transcriptional control. In contrast, TCS-mediated control was found to be dispensable during both biofilm formation and filamentation.
Conclusion: Our study suggests that a single PRE-mediated regulatory circuit is sufficient to appropriately control *TEC1* transcription during opposing cellular programs (mating versus biofilm formation), whereas potential auto-regulation via a TCS-mediated positive feedback loop seems not to be operative.

GRP14
Regulation of the rhamnose metabolism in *Bacillus subtilis*
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The gram-positive model organism *Bacillus subtilis* is a soil bacterium that degrades plant cell walls and is thereby confronted with a variety of complex, heterogeneous polysaccharides. To cope with this the genome of this bacterium encodes for a large number of carbohydrate degrading enzymes. An example is the degradation of rhamnose and rhamnogalacturonan. The enzymes for rhamnogalacturonan degradation are encoded by two separate gene clusters. A third gene cluster encodes enzymes for the degradation of L-rhamnose. Four enzymes convert the deoxy-hexose sugar to L-lactaldehyde and dihydroxyacetone phosphate. Interestingly, the L-rhamnose utilization gene cluster, consisting of five genes (*rhaEW, rhaR, rhaB, rhaM* and *rhaA*), does not include a rhamnose-specific transporter. Consistent with this *B. subtilis* only growths poorly in minimal medium containing rhamnose as the sole carbohydrate source. Our focus lies on the regulation of the degradation of rhamnose which is a major component of the cell wall from plants and many other organisms. Comparative genomics reveal YulB (RhaR) as the putative transcriptional regulator which belongs to the DeoR family of repressors [1]. Here we show by promoter studies that RhaR negatively regulates the *rha* gene cluster in the absence of rhamnose by binding upstream of the *rhaEW* gene. Moreover a functional cre (catabolite-responsive element) site is located adjacent to the -10 box of the *rhaEW* promoter leading to catabolite repression in the presence of glucose. For transcriptional activation the last intermediate of the rhamnose degradation pathway, L-rhamnulose 1-phosphate, is needed. Deletion of the regulatory gene *rhaR* established a strong promoter. However, in the wild type the induction level only reached about 10 % of the high constitutive level, indicating that there is no rhamnose-specific uptake system and that the rhamnose degradation pathway serves for further utilization of degradation intermediates descending from rhamnose-containing polysaccharides like rhamnogalacturonan.

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GRP15
Characterization of the heme d_1 biosynthesis enzyme NirDLGH
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In order to generate energy under anaerobic growth conditions some bacteria are able to use denitrification. In this process nitrate is stepwise reduced to nitrogen *via* the intermediates nitrite, nitric oxide and nitrous oxide [1]. In *Pseudomonas aeruginosa* the second step of denitrification is catalysed by the cytochrome cd_1 nitrite reductase NirS. This homodimeric enzyme carries two cofactors, the covalently bound heme *c* and the noncovalently bound heme d_1. The enzymes which are required for the biosynthesis of the isobacteriochlorin heme d_1 are encoded in the *nir* operon (*nirSMCFDLGHJEN*). The *nirFDLGHJE* genes are indispensable for the heme d_1 synthesis [2].
It was shown that the heterotetrameric enzyme NirDLGH catalyses the conversion of siroheme to 12,18-didecarboxy-siroheme [3]. In addition to its enzymatic function NirDLGH may possess a function in transcriptional regulation. Amino acid sequence alignments showed a great homology to the family of AsnC/LRP-transcription regulators. Moreover the crystal structure of the NirDL from *Hydrogenobacter thermophilus* also indicated

a role in transcriptional regulation by exhibiting an N-terminal helix-turn-helix domain [3] typical for DNA-binding proteins. Previous studies suggested the binding of NirL and NirH to a DNA region upstream of the *nirJ* gene thereby regulating the expression of *nirJEN*. To test this hypothesis, DNA-binding studies will be carried out by Band Shift Assays. Further the regulator binding motif, the DNA-binding region of the protein and the conditions that influence transcriptional regulation will be characterized.

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GRP16

Studies of *Rhodobacter sphaeroides* IscR confirm a predicted DNA binding motif and reveal a unique Fe-S ligation

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Iron-sulphur clusters are ensembles of iron and sulphide centres. Fe-S cluster are found in all life forms and are important components of many enzymes involved in diverse cellular processes, including respiration, DNA synthesis or gene regulation[1]. However, the increase in oxygen after the emergence of photosynthesis created a threat to Fe-S proteins and, consequently, to the organisms relying on them. In particular, ROS causing destabilization of Fe-S cluster, leading to release of Fe^{2+} ions that in turn fuel ROS production by Fenton chemistry. Therefore, bacteria have evolved mechanisms to maintain a precise intracellular iron concentration. Rodionov et al. hypothesized a potential major role of IscR in ***R. sphaeroides*** iron dependent regulation, and predicted a binding site in the upstream regions of several iron uptake genes[2]. Most IscR proteins have Fe-S clusters featuring $(Cys)_3(His)_1$ ligation[3]. However, Rhodobacteraceae IscR harbour only one cysteine residue and it is therefore unlikely that they can ligate an Fe-S cluster.
In this study, ***R. sphaeroides*** IscR was characterized ***in vivo*** and ***in vitro*** to expedite our understanding of IscR as a global transcriptional regulator and sensor of the Fe-S cluster status of the cell. The results presented lead to the proposal that ***R. sphaeroides*** IscR functions as transcriptional repressor of genes involved in iron metabolism by binding to the predicted DNA binding motif. Further the results indicate that IscR possesses a unique Fe-S cluster ligation scheme with only one cysteine involved.

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GRP17

Differential Regulation of Chitinase-3-like-1 in Bacterial and Viral Infections

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The human lung epithelium is exposed to various pathogens, including the gram-positive bacterium *Streptococcus pneumoniae*, Influenza A virus (IVA) or Human Rhinovirus (HRV). During inflammation several factors are required for proper regeneration, e.g. Chitinase-3-like-1 (Chi3l1) that is known as a biomarker for several diseases and that predominantly acts in tissue repair and remodelling. Studies have shown that *CHI3L1* is involved in bacterial clearance after *S. pneumoniae* infections but the exact function is still unknown. Furthermore, an indirect regulation of *CHI3L1* expression via Notch1, TNFα and NF-κB signalling was identified after Hepatitis C viral infections. Interestingly, there is evidence for the contribution of microRNAs to this signalling pathway. In this study, we investigate the underlying mechanisms that are crucial for function of the chitinase-like glycoprotein. We show that the bronchial epithelial cell line (Beas-2B) is capable of the expression and secretion of Chi3l1 following exposure to stimuli such as LTA, poly(I:C) and TNFα in a dose and time dependent manner. In time course experiments using both Beas-2B and primary human differentiated bronchial epithelial cells we observe differential expression of the *CHI3L1* transcript after infection with *S. pneumoniae,* IVA or HRV. Interestingly, components of known signalling factors as well as of microRNAs, that potentially target *CHI3L1* or *NOTCH1* 3`UTRs, are deregulated within the analysed infections. Further investigations are needed to clarify the role of Chi3l1 and to evaluate its great potential not only as a biomarker in various diseases but also for the development of therapeutic strategies.

GRP18

Signal integration at quorum sensing promoters

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Quorum sensing (QS) is a common regulatory strategy to control the production of common goods. The underlying molecular signaling networks used for QS show often puzzling complexity. Frequently several signals converge to regulate target gene expression. For example, in Bacillus subtilis strain-specific pherotype signals and species-specific signals converge to regulate the activity of the transcription factor ComA by modulating its phosphorylation state and its ability to bind DNA, respectively. An extra level of complexity is added by the architecture of the QS responsive promoters that comprise not only multiple but - as we recently discovered - also distinct types of DNA binding sites. Here, we use in vitro DNA binding studies to characterize the ability of the two alternative binding sites to differentiate between the phosphorylation status of the protein. We find that phosphorylation does not affect the binding to the consensus motif. However, phosphorylation markedly increases cooperative binding to two such sites regardless of their identity. Moreover, in vivo studies of synthetic and natural promoters show that the activity of promoters containing two or more binding sites is reduced in the absence of the kinase. Together these findings imply that a strain-specific quorum may be required to fully activate target gene expression thereby constraining maximal cellular investment into the production of public goods to homogeneous social situations.

GRP19

The Replication Initiator DnaA is Subject to Posttranscriptional Regulation in Starvation and Stationary Phase Conditions

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Introduction: 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 in stress conditions. Previous work in the model bacterium *Caulobacter crescentus* has shown that DnaA levels are downregulated in 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.
Objectives: While we know how DnaA abundance and DNA replication are controlled in proteotoxic stress conditions, the mechanism adjusting DnaA abundance upon carbon starvation and entry to stationary phase remain elusive. Here we genetically dissect the regulatory mechanism responsible for DnaA clearance and a cell cycle arrest in these conditions.
Results: We show that the conditions of carbon starvation and entry into the stationary phase lead to reduction of DnaA levels and cell cycle arrest in G1 phase. Furthermore, we observe a direct correlation between DnaA levels and nutrient composition of the growth medium. While Lon-mediated proteolysis is required for the clearance of DnaA and G1 arrest, we see no substantial change in DnaA degradation rate under these conditions. Interestingly, mRNA levels also do not change significantly compared to exponential growth, indicating that DnaA is controlled at a posttranscriptional level. In agreement with this result, we find that the downregulation of DnaA in stationary phase and starvation conditions strictly depends on the 5' untranslated region (UTR) of the *dnaA* transcript. Deletion of this region completely abolishes nutrient-dependent control of DnaA. Addition of the UTR to a constitutive promoter is sufficient to restore the nutrient-dependent modulation of DnaA levels.
Conclusions: Our data suggest a model in which constitutive DnaA degradation by the protease Lon allows for a rapid reduction in DnaA protein levels when translation is reduced by a mechanism involving the *dnaA* 5' UTR.

GRP20
The integration host factor (IHF) plays role on systemic infection potential of *Salmonella* Enteritidis in chickens
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Introduction: The integration host factor (IHF) is a DNA-binding and -bending protein with exerts roles in DNA structural organization and transcriptional regulation of *E. coli* and *Salmonella* spp. This protein is a heterodimer composed of two homologous subunits IHFα and IHFβ [1]. IHF was previously related to expression of genes required by bacteria undergo physiological changes associated to exponential-stationary phases adaptation. Afterwards, IHF was demonstrated influence expression of major virulence and cell invasion genes in *S.* Typhimurium. No earlier study has reported participation of IHF on virulence of *S.* Enteritidis (SE), the most prevalent serotype in poultry and human outbreaks.
Objectives: Verify if IHFα (*ihf*A), IHFβ (*ihf*B) or both IHFα and IHFβ are important during the systemic infection of SE in chickens, predisposing them to be targets for salmonellosis control.
Methods: Single mutants (SEΔ*ihf*A and SEΔ*ihf*B) were constructed by Lambda Red methodology, double mutant SEΔ*ihf*AΔ*ihf*B was constructed by transduction using the bacteriophage P22. Virulence was assessed by oral inoculation of one-day-old SPF chicks with 10^8 CFU of single and double mutants or wild type strain (WT). Samples from spleen and liver were collected at 2, 5, 7, 14, 21, and 28 days post-inoculation (dpi), macerate, diluted (1:10) in PBS, and the bacteria viable count (CFU/g) was estimated by plating aliquots onto Brilliant Green Agar. Samples when there was no growth were enriched and plated again. Media for the viable counts was analyzed by Tuckey's test ($p<0.05$).
Results: Counts of SEΔ*ihf*A, SEΔ*ihf*B and SEΔ*ihf*AΔ*ihf*B were considerably reduced by about 2.0-3.0 $\log_{10}$ CFU/g into liver and 3.0-4.0 $\log_{10}$ CFU/g into spleen of chickens in comparison of WT ($p<0.01$). Single mutants remained no longer than 7dpi into chicken's organs. Double mutant was found up to 14 dpi in liver, and remained into spleen as the WT, up to 28dpi.
Conclusion: IHF exerts effect on systemic infection ability of SE. Single and double mutants showed strong reduction in the capacity to invade liver and spleen, however, only single mutants showed impairment to persist in organs of chickens.

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GRP21
Non-conventional translation initiation in *Escherichia coli*
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Translation initiation is an important step in gene expression. It is the rate-limiting step and thus most regulatory mechanisms influence initiation efficiency. Several initiation mechanisms exist in bacteria. Best known is the so-called Shine-Dalgarno (SD) mechanism, which is based on the interaction between the SD-motiv and the anti-SD-motiv in the 16S rRNA. In addition to this conventional mechanism two further mechanisms exist, i.e. 1) initiation on leaderless transcripts, which requires the 70S ribosome and the initiator tRNA, and 2) initiation on leadered SD-less transcripts, which is not understood on the molecular level.
Bioinformatic genome analyses revealed that only about 60% of all genes in *E. coli* are preceded by a SD motif, and thus about 40% of *E. coli* genes use a non-conventional initiation mechanism. The fraction of non-conventional transcripts can be much higher in other bacterial species and exceed 80% [1].To enable the characterization of non-conventional initiation the 5'-ends of more than 40 *E. coli* transcripts were determined. The 5'-UTRs of 10 non-conventional transcripts and 2 SD-containing control transcripts were fused to the *gusA* reporter gene, and the translational efficiencies were determined under five different conditions. All non-conventional 5'-UTRs directed efficient translational initiation, albeit with very different efficiencies (some better than SD-containing controls). Unfortunately, a comparison of efficiencies under different conditions revealed that the non-conventional transcripts did not behave as a coherent group, but gene-specific regulatory behavior was observed.
For the *de novo* construction of a highly efficient non-conventional 5'-UTR a randomized sequence was fused to a novel reporter gene, *glpD*, and the plasmid library was introduced into a suitable *E. coli* strain. Thereby, growth rate was coupled to the intracellular GlpD concentration under selective conditions. Several rounds of growth indeed selected a specific sequence out of the random pool, which had a high translational efficiency in the absence of a SD motif.

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GRP22
The influence of various AHL systems on the gene expression of *Phaeobacter inhibens* T5
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Phaeobacter spp., which are affiliated to the *Roseobacter* clade, are often found associated with marine eukaryotic organisms or organic particles (Buchan et al 2005) suggesting that surface association and colonization are general aspects within the ecology of these group members.
In *Phaeobacter inhibens* DSM 17395 the switch to a biofilm formation mode together with the production of the antibiotic TDA and a brown pigment is regulated by the N-acyl-homoserine lactone (AHL) 3OH-C10-HSL (Berger et al. 2011, Beyersmann et al., unpublished). The genome of the closely related *Phaeobacter inhibens* strain T5 comprises three AHL synthetases of which one is homolog to the single synthetase of *P. inhibens* DSM 17395 (Dogs et al. 2013) and likewise the corresponding 3OH-C10-HSL was found to be produced in strain T5.
However, the production of two further AHLs is probable, as by means of qRT PCR it was demonstrated that all three AHL synthetase genes are expressed on a comparable level. While synthesis of C18-en-HSL by strain T5 was identified, the third AHL still remains unknown and is currently investigated using GC-MS. To assign the expressed AHLs to their respective synthetase, we construct AHL synthetase deficient knockout mutants and analyze them regarding their AHL production. Until now it is unclear how gene expression is influenced by the different AHLs in strain T5. To address this question, changes in gene regulation in these mutants will be observed by transcriptomics.
As one of the identified AHL synthetase genes is located in a genomic island, the altered gene expression pattern by the acquired AHL synthetase might give an insight into the impact of horizontal gene transfer and evolution within the *Roseobacter* clade.

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GRP23
Similar effects of mutations in the genes encoding RNase E and RNase J on quorum sensing in *Sinorhizobium meliloti*
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The nitrogen fixing plant symbiont *S. meliloti* harbours both RNase J and RNase E, two principal ribonucleases of *Bacillus subtilis* and *Escherichia coli*, respectively. To address the role of these RNases in *S. meliloti*, mutants with miniTn*5* insertions in the genes encoding RNase E and RNase J (ref. 1) were analysed by microarrays in comparison to the parent strain 2011. We found striking similarities between both mutants, which were confirmed by qRT-PCR analyses: We detected higher levels of *ndvA* mRNA encoding a protein important for glucan export, lower levels of the mRNAs of the flagella genes *flaA* and *flgB,* and growth-phase dependent differences in the levels of the mRNAs of the chemotaxis-related genes *cheR* and *mcpW*. Since the last four genes are under the control of quorum sensing, the production of the autoinducer molecules acyl-homoserine lactones (AHLs) was measured. We found that both RNase mutants produce higher AHL amounts at low population density, although RNase E (ref. 2) but not RNase J specifically cleaves the 5'-UTR of *sinI* mRNA encoding the autoinducer synthase. Further experiments revealed that the stability of the protein SinI is changed in a growth phase dependent manner in the two mutants. The similarities between the two mutants can be explained if one of the RNases controls the expression of the other one, a hypothesis which we are currently testing. Our results show the importance of the regulation of quorum sensing at the posttranscriptional level in *S. meliloti*.

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MCP02

Reductive activation of protein bound corrinoids - New insights into the reaction mechanism of corrinoid reducing metallo ATPases

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Introduction: Protein bound corrinoid cofactors play an essential role as methyl group carriers in the C1-metabolism of anaerobes. To bind methyl groups, the corrinoid cofactor has to be in its super-reduced [Co^{I}]-state, which is highly sensitive to autoxidation. Depending on the enzyme system, a re-activation of the inactive corrinoid cofactor ([Co^{II}]-state) is required every 100 or 2000 cycles of methyl transfer [1]. A common mechanism for re-activation seems to be the ATP-dependent reduction of the oxidized corrinoid cofactors catalyzed by RACE proteins, the **r**eductive **a**ctivators of **c**orrinoid-dependent **e**nzymes, which were found in archaea and bacteria [2].
Objectives: The reaction mechanism of RACEs will be elucidated by studying the enzymatic reduction of protein-bound corrinoid cofactors involved in the *O*-demethylation of phenyl methyl ethers in *Acetobacterium dehalogenans*.
Methods: Proteins of interest were produced as *Strep*-tag fusions in *Escherichia coli*. Corrinoid reduction activity was determined in anoxic quartz cuvettes by following the decrease in absorption at 475 nm ([Co^{II}]-state) concomitant with an increase at 386 nm ([Co^{I}]-state). ATP hydrolysis was measured using an NADH-coupled assay [3].
Results: The reductive activator that catalyzes the reduction of protein-bound corrinoids of *O*-demethylase enzyme systems of *A. dehalogenans*, termed activating enzyme (AE), has a molecular weight of 65 kDa and harbours an N-terminal [2Fe-2S] cluster. AE mediates two reactions: the hydrolysis of ATP and the reduction of the corrinoid cofactor. The AE activity strictly depends on the presence of potassium or ammonium ions. ATP hydrolysis was significantly stimulated by the corrinoid protein in the [Co^{II}]-state of the corrinoid cofactor. A co-assay was developed to measure both, the corrinoid reduction and ATPase activity in parallel in one reaction mixture [3]. The results of this assay indicate that 1 mol ATP is hydrolyzed per 1 mol corrinoid reduced. Flavodoxin of *A. dehalogenans* functions as electron donor for corrinoid reduction.

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MCP03

Kinetics and infrared spectroscopic analyses of inhibition of [Fe]-hydrogenase with Cu^{2+}, Fe^{2+}, O_2^- and H_2O_2

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Introduction: The third type of hydrogenase, [Fe]-hydrogenase, catalyzes reversible hydride transfer from H_2 to methenyl-tetrahydromethanopterin, which is involved in methanogenic pathway from H_2 and CO_2. The active site of [Fe]-hydrogenase is composed of the iron-guanylylpyridinol (FeGP) cofactor, in which two internal CO ligands are bound to the iron center.
Objectives: [Fe]-hydrogenase is known to be strongly inhibited not only with cyanide and isocyanides [1] but also with cupric ion and superoxide anion [2]. Here, we report the precise inhibitory properties of Cu^{2+}, Fe^{2+}, O_2^-, and H_2O_2 on [Fe]-hydrogenase from *Methanothermobacter marburgensis*.
Methods: Kinetics of inhibition of [Fe]-hydrogenase were analyzed by enzyme assay. Affects of the inhibitory compounds to the iron site of the FeGP cofactor were analyzed with infrared spectroscopy by measuring the peaks of two internal CO ligands.
Results: Cu^{2+} strongly and irreversibly inhibited [Fe]-hydrogenase (K_i = 1 µM); the inhibition was time dependent and the half inhibition time was 30 min in the presence of 0.3 µM Cu^{2+}. Fe^{2+} inhibited the enzyme reversibly (K_i < 1 µM). Other divalent metal cations tested (Mg^{2+}, Ca^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}) did not inhibit this enzyme. In the presence of Cu^{2+}, infrared peaks of [Fe]-hydrogenase were shifted but their intensities were not decreased, which suggested that the metal ion bound to the iron site of the FeGP cofactor but did not decompose the CO ligand. Contrary, irreversible inhibitions of O_2^- (K_i < 1 µM) and H_2O_2 (K_i = 20 µM) resulted in decomposition of the iron complex structure of the FeGP cofactor.
Conclusion: Our finding indicated unique properties of inactivation of [Fe]-hydrogenase by the inhibitory compounds. The inhibition must reflect the properties of the iron site of the cofactor, which is important for understanding the catalytic mechanism.

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MCP04

Constrains on the carbon isotope fractionation of homoacetogenic bacteria

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Introduction: Homoacetogenic bacteria are versatile microbes which use the acetyl-CoA pathway to synthesize acetate from CO_2 and hydrogen. Thereby many of them can homoferment monosaccharides completely to acetate. Likewise the acetyl-CoA pathway may be used to incorporate other one carbon substrates (e.g. methanol or formate) into acetate.
Objective: In this study we analyzed how different environmental factors impact the isotopic fractionation of acetogenic bacteria. The factors we analyzed were: different substrates and substrate rations, temperature and fractionation into biomass.
Material and Methods: We analyzed the natural abundance of ^{13}C in different acetogenic substrates (e.g. CO_2, glucose, methanol, and formate) and the product acetate for different acetogenic pure cultures as well as environmental incubations. We used this data to calculate the fractionation factor ε.
Results: All tested acetogenic pure cultures show a strong fractionation (average ε = -57.2‰) reducing CO_2 to acetate. We could confirm this strong fractionation for other C1 substrates (methanol, fomate). In contrast fermentation of e.g. glucose yielded only minor enrichments. While the substrate ration has an impact on the observed fractionation, temperature changes did not affect the apparent fractionation. Fractionation in to biomass was smaller than into the anabolic product acetate. The isotopic signal of acetogenically formed acetate in incubations with rice field soil under different conditions and temperatures could only be discriminated from the background acetate if an excess of H_2/CO_2 was applied.
Conclusion: Even though the acetyl-CoA pathway is characterized by a very strong fractionation which is robust under different laboratory settings, it is still a challenge to characterize the environmental impact of acetogenic bacteria solely by using isotopic data.

MCP05

Genome-wide transcriptome response of *Methylocystis* sp. strain SC2 to salt stress

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Introduction: Salinity has been shown to have a major effect on the composition and activity of methane-oxidizing bacteria (MOB) communities in dryland soil, affecting in particular the relative abundances of types Ia, Ib, and II MOB. However, the effect of salt stress on the genome-wide expression of a particular MOB has not yet been studied.
Objective: Assessment of short-term and long-term effects of salt stress on the transcriptome of the type II methanotroph *Methylocystis* sp. strain SC2.
Methods: Strain SC2 was grown to the mid-exponential phase and then treated with 0.75% NaCl. Exposure time to NaCl was 0 min (control), 45 min, and 14 hrs. Control and NaCl treatments were analyzed in triplicates by Illumina RNA-seq. Transcripts were mapped to the SC2 genome and expression levels were calculated using the CLC Genomics Workbench. Normalized gene expression levels were calculated and reported as RPKM (Reads Per Kilobase of CDS per Million mapped reads) values. Log_2 fold changes in RPKM values of ≤−2 and≥ 2 were considered significant.
Results: The expression level of genes involved in cell membrane and cell wall synthesis, stress response, and transcriptional regulation, but not those involved in methane oxidation, was significantly affected in both NaCl treatments, 45 min and 14 hrs. However, the expression patterns were remarkably different between short-term and long-term responses. Most genes that showed differential expression in short-term response (45

min) did not exhibit a significant response to the long-term treatment, suggesting that strain SC2 increasingly adapted to salt stress with treatment time. Genes that showed high up-regulation only in the 14 hrs treatment include the complete *VirB* operon. The 11 genes of this operon encode the regulatory VirB, which is hypothesized to form a membrane-localized T-DNA transport apparatus and to be required for processing and transmission of specific segments of a plasmid.
Conclusions: Significant changes in the genome-wide expression occurred in response to salt stress, with the exposure time to salt stress as a critical factor. The *VirB* operon is located on plasmid pBSC2-1, suggesting that this plasmid may play a major role in the response of *Methylocystis* sp. strain SC2 to salt stress.

MCP06
Two autotrophic pathways in *Ammonifex degensii*
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A thermophilic, strictly anaerobic denitrifying member of the low-GC, gram-positive bacteria, *Ammonifex degensii*, is the first example of a cultured and sequenced organism that uses two pathways for autotrophic carbon fixation in parallel. Genomic, metabolomic and biochemical as well as stable isotope labelling data provides proof that *A. degensii* fixes carbon dioxide via the Wood-Ljungdahl pathway as well as the Calvin-Benson cycle. The latter seems to have evolved convergently in this lineage and relies on two thermophilic enzymes with an archaeal origin: A form III Rubisco that is distinguished by its speed and its sensitivity to molecular oxygen[1] as well as a bifunctional fructose 1,6-bisphosphate aldolase/phosphatase[2]. Both enzymes have never been described as part of an autotrophic pathway. Furthermore, *A. degensii* uses an unconventional pathway to regenerate ribulose 1,5-bisphosphate, where sedoheptulose 7-phosphate is synthesized by the action of transaldolase instead of sedoheptulose 1,7-bisphosphatase. The evolution of a Calvin-Benson cycle in *A. degensii* demonstrates vividly how novel metabolic pathways are assembled in nature: Through lateral gene transfer and through recycling and readaption of enzymes from other metabolic pathways.

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MCP07
The physiological role of AtpI in *E. coli*
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The *atp* operon encodes the 8 structural subunits a, b, c, α, β γ, δ, and ε of the F_1F_o-ATPase and subunit i, the only non-essential and non-permanent part of the complex (Gay and Walker, 1981, Solomon et al., 1989). The F_1F_o-ATPase consists of a membrane integrated F_o part that is translocating protons and a soluble F_1 part containing the catalytic sites for synthesis of ATP from ADP and P_i using the electrochemical proton gradient (Capaldi and Aggeler, 2002; Okuno *et al*., 2011). The function of AtpI is still unknown although a chaperone-like function in c-ring assembly has been proposed in Na^+-translocating ATPases (Suzuki *et al*., 2007; Brandt *et al.,* 2013; Liu *et al*., 2013).
In this study we investigated the interaction of AtpI with the membrane-bound subunits of the F_o complex. For this we used the BACTH system which is based on the functional complementation of two fragments of the catalytic domain of the *Bordetella pertussis* adenylatcyclase (Karimova *et al*., 1998, 2001). If there is an interaction between the proteins of interest a functional complementation of the adenylatcyclase is achieved and enzymatic activity is restored. cAMP can be produced, binds CAP (*catabolite activator protein*) and allows expression of the *lac* operon. Interaction was studied using a following blue/white screen on selective medium and a β-galactosidase assay.
Our experiments revealed that AtpI interacts with all subunits of the F_o complex as well as subunit ε. To gain insight in the interface of the interaction we mutated several conserved amino acids in AtpI. The mutants show different and individual interaction behavior, giving further insight into the network of interactions. Taken together our results demonstrate that AtpI is part of the F_1F_o-complex at late stages of assembly and that it may interact with several other subunits at the same time. These results are in agreement with a chaperone-like function of AtpI but also allow for a more sophisticated model of role of AtpI during assembly of the complex.

MCP08
The oxidative pentose phosphate pathway in the haloarchaeon *Haloferax volcanii* involves a novel type of glucose-6-phosphate dehydrogenase
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The oxidative pentose phosphate pathway (OPPP), catalyzing the oxidation of glucose-6-phosphate to ribulose-5-phosphate that is required for nucleotide biosynthesis, is a well-studied ubiquitous pathway in eukarya and bacteria. Glucose-6-phosphate dehydrogenase (Glc6PDH) and 6-phosphogluconate dehydrogenase (6PGDH) are the key enzymes of this oxidative pathway. In contrast to bacteria and eukarya, the OPPP seems to be absent in archaea, with the exception of haloarchaea that encode a putative 6PGDH whose physiological significance has not been demonstrated yet. Further, Glc6PDH has not been identified in any archaea so far. Here we report the identification and characterization of a novel type of Glc6PDH in the haloarchaeon *Haloferax volcanii*. The enzyme was purified from cell extracts and the encoding gene was identified, followed by the generation and growth analysis of a chromosomal deletion mutant. Further, recombinant 6PGDH, encoded by *gndA*, [HVO_1830], from *H. volcanii* was characterized and a *gndA* deletion mutant was analyzed in growth experiments. Together, the data indicate that *H. volcanii* utilizes the OPPP for the generation of ribulose-5-phosphate from glucose-6-phosphate, involving 6PGDH and a novel type of Glc6PDH. This is the first report of the operation of the oxidative pentose phosphate pathway in the domain of archaea.

MCP09
The modified β-Oxidation: A new trait of carbon metabolism and virulence in *Candida albicans*
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Introduction: Propionyl-CoA is a toxic intermediate deriving from amino acid and odd chain fatty acid catabolism. A functional catabolic pathway for propionyl-CoA ensures unobstructed metabolism and virulence in many microbial pathogens. To date two major pathways for propionyl-CoA degradation are known: the methylmalonyl-CoA pathway and the methylcitrate cycle [1]. Although both pathways are absent in *C. albicans* it is capable of thriving on propionate as sole carbon source, indicating an alternative catabolic pathway for propionyl-CoA.
Objectives: The identification of enzymes responsible for propionyl-CoA catabolism in *C. albicans* and impact of the pathway on virulence.
Materials and Methods: Candidate genes and enzymes were identified by the use of 2D-proteomics, *in silico* gene analysis and gene deletion mutants. Metabolite accumulation was studied by HPLC, GCMS and NMR analyses. Heterologous protein expression and photometric assays allowed the first characterisation of the 3-hydroxypropionyl dehydrogenase CaHpd1p. Its impact on virulence was tested in a murine infection model of disseminated Candidiasis.
Results: The multifunctional enzyme CaFox2p from fatty acid oxidation is part of a peroxisomal branch of the modified β-oxidation leading to 3-hydroxypropionyl-CoA. Subsequently, 3-hydroxypropionyl-CoA is converted in mitochondria by CaEhd3p, CaHpd1p and Ald6p to 3-hydroxypropionate, malonate semialdehyde and acetyl-CoA. Ca*hpd1*Δ deletion strains accumulate 3-hydroxypropionate and are severely attenuated in virulence, indicating an important function of the modified β-oxidation for the infection process [1].
Conclusion: The modified β-Oxidation represents a new distinctive feature of *C. albicans* carbon metabolism and virulence.

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MCP10

Deletion of the *purL* gene abolishes the in vitro immune modulation of uropathogenic *E. coli*

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Introduction: Uropathogenic *Escherichia coli* (UPEC) are a major cause of urinary tract infections. These successful pathogens are equipped with many different virulence factors, such as toxins and adhesins. In addition, UPECs are able to modulate the host's innate immune response. Yet unknown factors contribute to the immune suppressive phenotype of UPECs. Here we identified that distinct purine synthesis mutants in UPECs elicited an increased cytokine response of macrophages in vitro.
Objectives: The goal of this project is the further characterization of the UPEC *purL* mutants and the putative impact of the purine biosynthesis on the inhibition of macrophage cytokine release.
Material and methods: Distinct transposon mutants of UPECs lack the immune suppressive phenotype of the parental wild type strain. Site directed knockout mutants of respective genes were created in the UPEC strain UTI89 and analyzed in a macrophage infection model (co-incubation with J774.A2 macrophages). TNF-α secreted from macrophages was measured by ELISA. Furthermore, cell toxicity and gentamicin protection assays have been performed. The metabolism of the mutant has further been functionally characterized by means of a Biolog™ assay.
Results: Deletion of the *purL* gene in the UPEC strain UTI89 caused a significant higher cytokine release in J774.A2 macrophages. The original UPEC phenotype could be restored by transient recomplementation. Likewise, a deletion of the purine synthesis gene *purM* caused increased cytokine levels. Differences in toxicity and intracellular bacterial counts between mutant and wild type could be excluded. Additionally, a biochemical characterization of the *purL* deletion mutant revealed 7 metabolites that were catabolized less efficient (<30%). The mutant's growth was impaired in purine poor medium (RPMI) used in the macrophage assay. When we supplemented the RPMI medium with IMP, a purine synthesis metabolite, wild type growth and the UPEC-specific immune modulation of the *purL*-mutant could be restored.
Conclusion: Beside a functional metabolic characterization of the UPEC purine synthesis mutant *purL*, our results underline the importance of a functional bacterial purine synthesis to establish full growth and virulence in a purine depleted environment.

MCP11

Auxin metabolism in denitrifying Betaproteobacteria

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The closely related denitrifying Betaproteobacteria species *Azoarcus evansii* and *Aromatoleum aromaticum* are capable of anaerobic growth with indole-3-acetate (auxin) as sole carbon and energy source. An hypothetical oxygen-independent metabolic pathway was recently 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 most of these proteins (*iaa* operon) (Ebenau-Jehle et al. 2012). This pathway suggested the initial hydroxylation of indoleacetate to oxoindoleacetate by a molybdenum enzyme, followed by an ATP-dependent hydrolytic cleavage of the heterocycle to aminophenylsuccinate, activation to a CoA-thioester, rearrangement via a coenzyme B12-dependent mutase and beta-oxidation reactions.
A peculiar observation in that study was the presence of genes coding for both a CoA ligase (*iaaB*) and a CoA-transferase (*iaaL*) in the operon, although the proposed pathway only contained one CoA-activation step. To resolve this question, we have overproduced the gene products of *iaaB* and *iaaL* from *A. aromaticum*, purified the proteins and analyzed their biochemical properties. In contrast to our expectations, the *iaaB* gene product was inactive with phenylsuccinate or other potential analogs of metabolic intermediates after ring cleavage, but turned out to be a highly specific indoleacetate-CoA ligase. Conversely, the *iaaL*-intermediate was characterized as a rather unspecific CoA-transferase for C4-dicarboxylic acids, including succinate, phenylsuccinate and benzylsuccinate. Based on the results of this study, we have re-evaluated the pathway of anaerobic indoleactate metabolism.

*Ebenau-Jehle et al. (2012). Anaerobic metabolism of indoleacetate. J. Bac. 194: 2894-2903.

MCP12

New insights in the reaction mechanism of the Benzylsuccinate synthase

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The degradation of toluene under anaerobic conditions is initiated by the addition of the methyl group of toluene to the double bond of fumarate, forming (*R*)-benzylsuccinate as the first intermediate. This reaction is catalyzed by a member of the glycyl radical enzyme family, (*R*)-benzylsuccinate synthase (BSS), which is posttranslationally activated by its own activating enzyme, BssD. The three different subunits of BSS, α, β, and γ, with molecular masses of 98, 8.5 and 6.4 kDa, respectively, compose a hexameric structure with an $\alpha_2\beta_2\gamma_2$ composition and a molecular mass of 220 kDa. The large α-subunits of BSS contain the essential glycine and cysteine residues that are conserved in all glycyl radical enzymes.
In our project, we pursue further information about the mechanism of the BSS reaction by biochemical and spectroscopic investigations of the activated enzyme both in wild type cells and with homologously overproduced mutant BSS variants. We identified new organic radical species via EPR spectroscopy originating from different substrate analoga and inhibitors which are highlighting mechanistic details of the reaction mechanism.
In order to acquire more information about the stereospecificity of the benzylsuccinate synthase reaction, we use chirally radiolabeled toluene with a proton, a deuteron and a tritium atom at the methyl group. This substrate is added to the double bond of fumarate in a BSS dependent reaction with either retention or inversion of the methyl group chirality. The product is converted to benzylsuccinyl-CoA and further to phenylitaconyl-CoA, which can be used as a proxy for finding the answer to the stereochemistry of fumarate addition, depending on the retention or loss of the tritium label.
We want to identify crucial amino acids forming the catalytic center of the BSS and taking part in the distinction between the substrate toluene and other substrate analoga.

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MCP13

Catalytic bias of the bifunctional tetrathionate reductase TsdA from *Campylobacter jejuni*

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In the human gut pathogen *Campylobacter jejuni* tetrathionate reduction is catalysed by TsdA, a diheme cytochrome *c* representing a novel class of bifunctional bacterial tetrathionate reductases/thiosulfate dehydrogenases [1]. In contrast, TsdA from the purple sulfur bacterium *Allochromatium vinosum* mainly acts as a thiosulfate dehydrogenase and barely catalyses tetrathionate reduction [2]. The proteins are closely related on the sequence level. Both contain an active site heme with rare axial His/Cys iron coordination (Heme 1). A conserved methionine is important for binding of the second heme iron, Heme 2. For CjTsdA these ligands were verified by nIR-MCD. We intend to elucidate the mechanism(s) that govern the catalytic bias of TsdA. As a first step, we used electrochemical approaches to determine the standard reduction potential of the tetrathionate/thiosulfate couple as 0.19 ± 0.02 V vs. SHE and to show that CjTsdA hemes 1 and 2 are redox active in the range -450 to -270 mV and -20 to 190 mV, respectively. We performed detailed enzyme kinetic studies and protein film voltammetry on CjTsdA and derivatives thereof carrying replacements of either Cys_{138} or Met_{255}, the sixth axial ligands of Heme 1 and 2, or of Asn_{254}. Spectroelectrochemistry revealed hysteresis in the redox properties of the wild-type and variant proteins consistent with a redox-driven change in the axial ligands of at least Heme 2. Wildtype TsdA exhibited much higher substrate affinity for tetrathionate (19 ± 1.7 μM) than for thiosulfate (440 ± 18 μM). Substrate affinities remained virtually unchanged when thiolate ligation of Heme 1 was removed by Cys_{138}Gly substitution. However, affinities for thiosulfate and tetrathionate

changed significantly in TsdA variants with altered ligation of Heme 2 iron, for example, an $Asn_{254}Gly$ replacement led to higher affinity for tetrathionate while the affinity for this substrate decreased when Met_{255} was substituted by glycine. Thus, changes in ligation of Heme 2 affect substrate affinity although the TsdA active site is at Heme 1. In summary, these findings lead us to conclude that cooperativity exists between the two hemes and that Heme 2 plays a pivotal role in defining the catalytic direction of this enzyme.

[1] Liu et al. (2013) Mol Microbiol 88, 173-188
[2] Denkmann et al. (2012) Environ Microbiol 14, 2673-2688

MCP14

Identification of oligosulfide enzyme inhibitors and metal substitution in the active site of the sulfur oxygenase reductase of *Acidianus ambivalens*

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Introduction: The sulfur oxygenase reductase (SOR) is the initial enzyme in the sulfur oxidation pathway of the thermo-acidophilic archaeon *Acidianus ambivalens*. It catalyzes the oxygen-dependent disproportionation of sulfur to sulfite and H_2S. The SOR consists of 24 identical subunits with a mononuclear non-heme iron in each active site. EPR experiments demonstrated a redox change of the active-site iron. A persulfurated cysteine is essential for enzyme activity and presumably the substrate-binding site.
Objectives: We aimed to find novel organic oligosulfide inhibitors for the identification of substrate/product-binding sites. SOR was produced with non-iron metals for crystallography and EPR spectroscopy in order to find out whether a redox change is essential for the catalytic cycle.
Methods: The *sor* gene was expressed heterologously in *E. coli* and purified by Strep-Tactin chromatography. Specific activities were determined in the presence of inhibitors. Metal substitution was performed by refolding of SOR inclusion bodies in the presence of high purity metals.
Results: Dimethyl disulfide, di-tert-butyl polysulfide, diallyl trisulfide and garlic oil had an inhibitory effect on the SOR. IC50 values were between 0.1 and 1.3 mM. Refolded SOR preparations with Co, Ga, Mn or Ni were biochemically active. First diffraction data did not point to significant structural rearrangements around the metal center. EPR spectra of Co and Mn-containing SORs showed signals for Co^{2+} and Mn^{2+} species, respectively. The metals remained in the same oxidation state during catalysis.
Conclusion: SOR-inhibitor complexes showed reduced activities. X-ray crystallography will confirm whether this is a result of inhibition and visualize the binding sites. Co^{2+} and Mn^{2+} in the same oxidation state before and after the SOR reaction may indicate that a redox change is not essential during catalysis.

MCP15

Marine metagenomes as a source for novel enzymes involved in phycobilin 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 [1], 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 includes scaffolds from the "global ocean survey" (GOS) that are considered to be of viral origin but contain microbial gene clusters. The discovered *pcyX* and *hemO* genes form mini-cassettes on viral scaffolds, suggesting them being new members of highly efficient cyanophage enzymes involved in bilin biosynthesis [2]. 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 both enzymes are functional. Furthermore, we identified the specific reaction product of HemO as biliverdin IXα. Also, first results showed that PcyX reduces BV in a two-step reaction to phycoerythrobilin (PEB) with 15,16-dihydrobiliverdin (DHBV) as an intermediate. To further characterize the PcyX reaction, we are currently working on time-resolved FDBR assays, mutagenesis experiments as well as crystallization studies.

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MCP16

Towards the understanding of metabolic channelling during phycoerythrobilin biosynthesis

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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 (3). 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-linker in *in vitro* as well as *in vivo* experiments.

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MCP17

Identification of potential Radical SAM methyl transferases responsible for the C-methylations of glutamine and arginine in the methyl-coenzyme M reductase of methanogenic archaea

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Biological methane formation is a process carried out by methanogenic archaea belonging to the phylum of *Euryarchaeota* [(1)]. The methyl-coenzyme M reductase (MCR) catalyses the final reaction step of methane formation where methyl-coenzyme M and coenzyme B are converted to the heterodisulfide of coenzyme M and B with concomitant release of methane [(2)]. The crystal structures of different MCRs revealed the presence of four methylated amino acids and one thiopeptide thioglycine near the active site of the MCR [(3)]. The unique modifications 2-(S)-methylglutamine and 5-(S)-methylarginine have been never observed before. This methyl groups are introduced most probably co- or post-translationally by Radical SAM methyl transferases [(4)].
Our goal is to identify and characterize the Radical SAM methyl transferases responsible for the methylation of glutamine and arginine in the McrA. For this purpose genome analysis and alignments of different methanogenic archaea were conducted. In the close proximity of *mcrA* two different gene candidates encoding two Radical SAM enzymes were observed that might be responsible for the methylations of glutamine and arginine. We aim to produce the respective candidate proteins from different methanogenic archaea heterologously and to establish an enzyme activity assay, analyzing the methylations of glutamine and arginine. Moreover, we aim to create knockout strains by deleting the candidate genes in distinct achaea and to analyze the modifications in the McrA by LC-MS/MS.

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MCP18

Growth of *Sulfolobus solfataricus* on ethanol and its metabolic pathway reconstruction

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Introduction: *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, sugar acids, alcohols and peptides. However, most of the metabolic pathways and their regulation in response to different carbon sources are still far from understood.
Objectives: The SulfoSYS^{Biotec} project is a collaboration of different academic and industrial partners, to unravel the complexity and regulation of the archaeal carbon metabolic network, using a functional, model driven system biology approach. Based on the results of a genome scale metabolic model [1], growth of *S. solfataricus* on different substrates, the underlying metabolic pathways, the enzymes involved and their regulation are analysed.
Methods: *S. solfataricus* was grown in minimal medium, containing ethanol as sole carbon and energy source. Ethanol metabolism was analyzed by modeling approaches [1], -omics studies [2], enzyme measurements and literature mining. Genes encoding enzymes involved in the predicted ethanol degradation pathway were cloned and expressed.
Results: *S. solfataricus* is able to grow with ethanol as sole carbon and energy source. Ethanol degradation likely proceeds via two oxidation steps, first to acetaldehyde, catalyzed by an alcohol dehydrogenase, and secondly to acetate by a dehydrogenase or oxidoreductase. Finally, acetate is activated to acetyl-CoA by means of AMP-forming acetate CoA ligase, which feeds into the citric acid cycle. Furthermore, first insights into growth of *S. solfataricus* on mixed substrates (glucose/ethanol) are presented.
Conclusion: *S. solfataricus* grows on ethanol as sole carbon and energy source and the enzymes involved are currently characterized. First experiments suggest diauxic growth on glucose/ethanol mixtures with glucose being preferentially utilized.

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MCP19

First insights into the crystal structure of a novel class of phosphotransacetylases

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Phosphotransacetylases catalyse the reversible conversion of acyl-CoA to acyl phosphate and play therefore an important role in the energy metabolism of anaerobic microorganisms. Up to now there are three known classes of phosphotransacetylases (class^{I}: EutD, class^{II}: Pta and class^{III}: PduL) that can be found in bacteria. Enzymes that belongs to class^{III} where found to be involved in the 1,2-propanediol degradation by *Salmonella enteric* Serovar Typhimurium LT2 [1] and recently it was found that they are also key enzymes for acetate formation during heterotrophic and autotrophic growth of *Moorella thermoacetica*. While comparing sequences of all three groups of phosphotransacetylases it was found that the C-terminal region of class^{II} is highly similar to the sequence of class^{I}, whereas the third class lacks any similarity [1]. Therefore it was difficult to reliably predict secondary and tertiary structures of this novel class^{III}.
In this work we will give first insights into the structure and stability of this recently discovered group of phosphotransacetylases. Therefore, PduL2 (Moth1181) of *M. thermoacetica* was over-expressed in *Escherichia coli* and purified by *Strep*-TagII® technology. Afterwards, the stability of PduL2 was investigated under different storage conditions revealing a high stability at RT or while storing the enzyme at -80 °C. For crystallisation, PduL2 was purified further by size-exclusion chromatography to remove streptavidin and then subjected to a range of crystallisation conditions. Optimisation of these conditions is currently ongoing.

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MCP20

Investigations into the mechanism of the coenzyme B_{12}-dependent rearrangement catalyzed by glutamate mutase from *Clostridium cochlearium*

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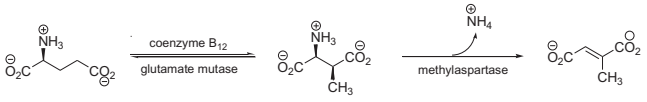
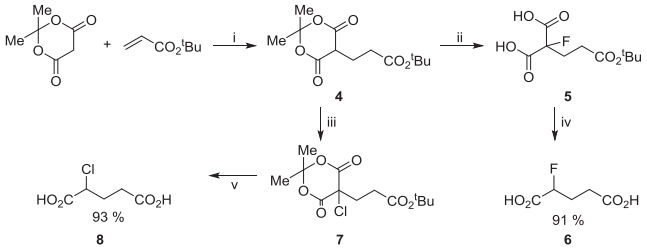
Coenzyme B_{12} (adenosylcobalamin)-dependent reactions proceed *via* the reversible homolytic cleavage of the Co-C-bond to form cob(II)alamin and the 5'-deoxyadenosyl radical, which initiates the rearrangements. The coenzyme B_{12}-dependent glutamate mutase catalyses the reversible conversion of (*S*)-glutamateto (2*S*,3*S*)-3-methylasparate, which is further deaminated to mesaconate during the fermentation of glutamate to ammonia, acetate, butyrate and hydrogen in *Clostridium cochlearium*.[1]



To enhance the understanding of the glutamate mutase and methylaspartase mechanisms, we synthesized 2-fluoroglutarate, 2-chloroglutarate, 2-fluoro-3-methylsuccinate and 2-chloro-3-methylsuccinate, to see whether these compounds could act as substrates or inhibitors.



Synthesis of both 2-fluoroglutaric acid and 2-chloroglutaric acid proceeded through halogenation of the Meldrum's acid derivative followed by acid catalysed hydrolysis and decarboxylation.[4] The proposed intermediate 2-fluoro-3-methylsuccinate has also been synthesized through a Meldrum's acid derivative. Initial experiments indicate that 2-fluoroglutarate and 2-fluoro-3-methylsuccinate are no substrates for glutamate mutase and methylaspartase. However, 2-fluoroglutarate and 2-fluoro-3-methylsuccinate are inhibitors of glutamate mutase and methylaspartase, respectively. Interestingly, already at low inhibitor concentrations 50% inhibition of these enzymes was achieved. Even at high inhibitor concentrations no further decrease in the enzymes' activities was observed.

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[2] Bothe, H. *et al*. *Biochemistry* **1998**, *24*, 4105.
[3] Buckel, W.; Golding, B. T. *Ann. Rev. Microbiol.* **2006**, *60*. 27.
[4] Drozdowska, M. P. *PhD Thesis*; Newcastle University: Newcastle upon Tyne, 2012.

MCP21
Importance of itaconate degradation in *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 have discovered that itaconate degradation pathway is present in *Yersinia pestis* (*5*). Furthermore, the *rip* (***r***equired for ***i***ntracellular ***p***roliferation) genes encoding enzymes involved in itaconate degradation can be found in many other pathogens including *Salmonella* Typhimurium (*5*). Now, we have detected the activities of the corresponding enzymes in *S.* Typhimurium cell extracts and characterized the recombinant Rip proteins, which were highly active with corresponding substrates. *S.* Typhimurium is capable to grow on itaconate as a carbon and energy source. As expected, the *ripB* and *ripC* knockout mutants were able to grow neither on itaconate nor on acetate in the presence of itaconate. Interestingly, the *ripA*, *ripB* and *ripC* mutants were severely attenuated in *cybb*$^{-/-}$ *nos2*$^{-/-}$ mice, further confirming the importance of *rip* operon for the pathogenesis.

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(2) A. Michelucci *et al.*, *PNAS* 110, 7820-7825 (2013).
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MCP22
Bioinformatic analyses indicate a novel multi-enzyme system for sulfur oxidation in prokaryotes
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Broad range database analysis of cytoplasmic sulfur-trafficking revealed that a wide array of chemo- and phototrophic sulfur oxidizing prokaryotes lacking the well-established Dsr pathway instead contains the gene cluster *hdrC1B1AhyphdrC2B2* encoding a heterodisulfide reductase (Hdr)-like protein complex [1]. Transcriptomic [2] and proteomic [3] analyses of *Acidithiobacillus* species support the notion that this Hdr-like complex is involved in a process functionally replacing the Dsr system in the generation of sulfite, a central intermediate *en route* to sulfate. Here, we established a core set of genes present in all sulfur oxidizers containing *hdr*-like genes. Just as the *dsr* genes, *hdr*-like clusters are inevitably linked to genes for proteins involved in cytoplasmic sulfur trafficking, i.e. TusA, DsrE and often also rhodaneses. The DsrE-related proteins fall into distinct groups depending on their genetic linkage to *dsr* or *hdr* genes [4]. Furthermore, genes for lipoate-binding proteins resembling glycine cleavage system component H and genes for several enzymes responsible for biosynthesis of liponamide-containing proteins are located in direct vicinity of *hdr*-like genes. Notably, a putative dihydroliponamide dehydrogenase is encoded immediately adjacent to the *hdr*-like genes in sulfur oxidizing archaea like *Metallosphaera cuprina* [4]. The intimate genomic linkage of genes for proteins involved in sulfur trafficking, Hdr-like systems, liponamide-binding proteins and in archaeal sulfur oxidizers also for proteins with the potential for NAD^+ reduction in conjunction with the established potential of HdrA for electron bifurcation [5] guided us to propose new models for Hdr-linked sulfur oxidation. These include the suggestion that part of the electrons arising from sulfane sulfur oxidation can be directly transferred to NAD^+, thereby decreasing the need for energy-requiring reverse electron flow and providing reducing equivalents at a redox potential negative enough to allow direct reduction of carbon dioxide during lithoautotrophic growth on reduced sulfur compounds.

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MCP23
D-galactose degradation in *Haloferax volcanii*
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The haloarchaeon *Haloferax volcanii* was found to grow on D-galactose as carbon and energy source. The pathway of D-galactose degradation in halophilic archaea has not been analyzed so far. Genome analyses (1) suggest the presence of several genes in *H. volcanii* encoding putative enzymes of a DeLey-Doudoroff pathway, which has been reported to be operative in few bacteria. So far, these enzymes have not been characterized in Haloarchaea and the functional involvement in D-galactose degradation has not been demonstrated. Here we report the elucidation of D-galactose degradation pathway in *H. volcanii*. The studies involve (I) transcriptional analyses of candidate genes, (II) enzyme measurements in cell extracts, (III) cloning, homologous overexpression, purification and characterization of enzymes and (IV) proof of functional involvement of genes and enzymes by analyzing the respective deletion mutants in growth experiments.
Together, the data indicate that in *H. volcanii* D-galactose is oxidized to glyceraldehyde-3-phosphate (GAP) and pyruvate, involving galactose dehydrogenase (GalDH), galactonate dehydratase (GalAD), 2-keto-3-deoxygalactonate kinase (KDGalK) (2) and 2-keto-3-deoxy-6-phosphogalactonate aldolase (KDPGalA). Transcript analyses and enzyme measurements indicate specific upregulation of GalDH, GalAD, KDGalK and KDPGalA during growth on galactose as compared to glucose. Analysis of deletion mutants of respective genes indicated GalDH, GalAD, KDGalK and KDPGalA to be essentially involved in galactose degradation. Further, molecular and kinetic properties of GalDH, GalAD, KDGalK and KDPGalA were determined. This is the first report on galactose degradation via a DeLey-Doudoroff pathway in the archaeal domain. This pathway differs from the classical Leloir pathway operative in galactose degradation in most bacteria and eukarya.

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MCP24
Multifuntionality of Fructose 1,6 bis-phosphatase in *S.cerevisiae*
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For decades the up-regulated glycolysis exhibited by tumours was the major focus of cancer carbon metabolism (Warburg et al, 1927).The role and regulation of gluconeogenesis in cancers was overlooked until recent years when downregulation of gluconeogenesis was linked to tumor aggressiveness through evidence that the rate-limiting enzyme, fructose 1,6 bisphosphatase (FBP1), is silenced in both liver and colon cancers (Chen et al. 2011). FBP1 has recently also been proven of central importance for maintenance of epithelial phenotype in breast cancers.Further investigation correlated FBP1-loss to reduced intracellular ROS levels, implying that ROS could be mediating FBP1's effects on cellular morphology (Dong et al. 2013).Our results suggests FBP has dual roles in yeast *Saccharomyces cerevisiae* akin to the apparent multiple roles of its homolog in cancer cells.We show that in addition to enabling growth on non-fermentable carbon sources, FBP promotes increased sensitivity to MMS, a DNA-alkylating agent (Kitanovic and Wölfl 2006). Furthermore, mutational analysis identified several point-mutations rescuing only one of the phenotypes attributed to FBP1. ROS levels in yeast cells were also positively correlated with FBP1 expression, thus suggesting that ROS could be acting as signalling molecules conveying FBP1's effects on sensitivity to DNA-alkylation.

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MCP25

Differential effects of exogenous benzimidazoles on cobamide biosynthesis and utilization in the tetrachloroethene-respiring *Sulfurospirillum multivorans*

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Introduction: The norpseudo-B_{12} cofactor of the periplasmic tetrachloroethene reductive dehalogenase (PceA) of *Sulfurospirillum multivorans* harbors an adenine (Ade) moiety as terminal base of the nucleotide loop (NL), which is positioned in the *base-off* conformation in the enzyme [1]. Guided cobamide biosynthesis, which led to the exchange of the Ade moiety by exogenous 5,6-dimethylbenzimidazole (DMB), hindered an effective incorporation of the cofactor into the apoenzyme and affected PCE-dependent growth [2]. A negative effect on the PCE metabolism was also observed for exogenous 5-methylbenzimidazole (5-MeBza), while other benzimidazole derivatives had no impact. The nicotinate mononucleotide:base phosphoribosyltransferase in *S. multivorans* (CobT*Smul*) was predicted to channel Ade and benzimidazoles into cobamide biosynthesis via formation of α-riboside monophosphates. A specific transporter for benzimidazole uptake was not identified so far.
Objectives: Deciphering the late steps of B_{12}-formation in *Sulfurospirillum multivorans*
Methods: The incorporation of exogenous benzimidazoles (up to 25 mM in the growth medium) into cobamides was tested by B_{12}-extraction and analysis via LC-MS. The CobT*Smul* enzyme was heterologously produced, purified, and characterized using an enzymatic assay previously described [3]. Product formation was monitored via LC-MS.
Results: All exogenous benzimidazoles efficiently replaced Ade in the cobamide produced by *S. multivorans* with one exception, the MeBza (only 30% incorporation). Besides the negative effect of DMB, only the presence of MeBza caused a reduction (about 50%) in the PceA activity in crude extracts. The CobT*Smul* enzyme converted Ade and benzimidazoles at different rates: OHBza > Ade > OMeBza > Bza > DMB > MeBza. The rates for DMB and MeBza conversion were comparably low.
Conclusions: The transfer efficiency of different cobamides into PceA of *S. multivorans* is dependent on the terminal base of the NL rather than on the CobT conversion rate. The inefficient incorporation of MeBza into cobamides is not limited by the CobT activity. It might be caused by a low uptake rate of MeBza into the cells.

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MCP26

The conserved BolA protein family functions in a medium stage of cellular iron-sulfur protein maturation

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Introduction: BolA proteins form a structurally conserved protein family that is found in bacteria and eukaryotes. Most eukaryotes harbor three BolA members, one in the cytosol and two in mitochondria. Despite their broad conservation, the precise functions of BolA proteins are unknown. In humans, mutations in the mitochondrial BolA3 cause a severe disease associated with defects in the Fe/S proteins lipoate synthase and respiratory chain complexes I and II [1]. These findings suggested that mitochondrial BolA proteins may play a crucial role in the maturation of Fe/S proteins.
Objectives: The synthesis of Fe/S clusters and their insertion into apo-proteins is a complex, highly conserved and essential biochemical process [2]. In this work we defined the function of the BolA proteins within mitochondria.
Methods: *Saccharomyces cerevisiae* strains with single, double and triple deletions of *BolA* genes were analysed for defects in cellular Fe/S protein maturation.
Results: Cells lacking both mitochondrial BolA1 and BolA3 proteins displayed moderate defects in enzymatic activity of and Fe/S cluster insertion into several mitochondrial Fe/S proteins. Most prominently, activities of lipoate-dependent 2-oxoacid dehydrogenases were reduced and cells displayed diminished protein-bound lipoic acid levels that were caused by an impaired activity of the Fe/S protein lipoic acid synthase. Additionally, these cells display moderate defects in the maturation of cytosolic Fe/S proteins. This latter defect was further enhanced in cells lacking all three BolA proteins.
Conclusion: The mitochondrial BOLA proteins are ISC assembly factors that are involved in a medium step of Fe/S cluster delivery from the core ISC system to Fe/S apo-proteins.

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MCP27

Structure-guided functional identification of Hcg enzymes involved in the biosynthesis of the [Fe]-hydrogenase bound FeGP cofactor

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Introduction: [Fe]-hydrogenase plays an important role in methanogenesis from H_2 and CO_2 by catalyzing stereoselective hydrogenation of methenyl-tetrahydromethanopterin to methylene-tetrahydromethanopterin via activation of molecular hydrogen. The [Fe]-hydrogenase harbors as its active site an iron-guanylylpyridinol cofactor (FeGP cofactor) which exhibits unique features such as a highly substituted pyridinol and an acyl-iron organometallic component. Its biosynthesis has been, so far, unclear. Comparative genomics study on genomes of methanogens suggested that seven conserved genes (*hmd co-occuring gene A-G*; *hcgA-G*), clustered together with *hmd* encoding [Fe]-hydrogenase, encode for enzymes involved in FeGP cofactor biosynthesis.
Objectives: The functional identification of three Hcg proteins by a "structure-guided approach".
Methods: This analysis is based on a comparison between the crystal structures of Hcg proteins and their structural homologs in the database with known functions, which allowed a prediction of possible functions of Hcg proteins.
Results: HcgB is structurally similar to inosine triphosphate hydrolase (ITPase), suggesting that HcgB could bind a nucleotide triphosphate like GTP. Based on this information we annotated HcgB as a guanylyltransferase which catalyzes the formation of guanylylpyridinol (GP) from GTP and the corresponding pyridinol.[1] More recently, HcgE and HcgF have been identified as enzymes involved in the activation of the carboxy group of GP. HcgE is structurally similar to ATP-dependent ubiquitin-activating E1 enzymes and catalyzes the adenylylation of the carboxy group of GP. The crystal structure of the HcgF-GP complex revealing a Cys (HcgF)-GP thioester suggested that this enzyme catalyzes the trans-esterification of adenylylated to thiolated GP. Both enzymatic reactions were confirmed by *in vitro* assays.
Conclusion: The found thioester-based carboxylate activation process is equivalent to the ubiquitin activation mechanism and provides, in addition, general clues about FeGP cofactor biosynthesis.

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MCP28

A metal-binding GTPase from *Cupriavidus metallidurans* is involved in metal homeostasis

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The heavy metal resistant bacterium *Cupriavidus metallidurans* contains genes for three putative members of the COG0523-subfamily of proteins, located on chromosome 1 in two gene clusters. This subfamily belongs to the SIMIBI class of G3E P-loop GTPases (2). COG0523-subfamily members are not very well characterized and usually annotated as proteins involved in metal metabolism such as biosynthesis of cobalt-containing cofactor cobalamin (1). One of the three COG0523 proteins from *C. metallidurans*, $CobW_3$, shows the Walker A and Walker B motives typical for these proteins, a base recognition and a metal-binding motive. To study the role of $CobW_3$ in metal-metabolism, a Δ*cobW*$_3$ deletion mutant was constructed. The Δ*cobW*$_3$ mutant was more resistant to EDTA and zinc but more sensitive to cadmium than the parent strain *C. metallidurans* AE104. This results indicated that the putative chaperone $CobW_3$ was involved in metal-homeostasis. ICP-MS measurements demonstrated a lower zinc content in the mutant cell (about 55,000 atoms per cell) compared to the parent (about 75,000 atoms per cell). A His-tagged version of $CobW_3$ was

produced in *Escherichia coli* and purified. This protein bound zinc ions with high affinity as shown with a metal-binding assay in the presence of Zincon. On the one hand, these results supported the hypothesis that proteins of the COG0523-subfamily of G3E P-loop GTPases have a function in metal-metabolism. On the other hand, they demonstrated that $CobW_3$ might be more likely connected to zinc homeostasis than involved in biosynthesis of cobalamin.

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MCP29

NirN from *Pseudomonas aeruginosa* is a Novel Electron-bifurcating Dehydrogenase Catalyzing the Last Step of Heme d_1 Biosynthesis

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Heme d_1 is the unique cofactor of the cytochrome cd_1 nitrite reductase NirS, catalyzing the second step of denitrification in *Pseudomonas aeruginosa* [1]. The final step of heme d_1 biosynthesis requires the dehydrogenation of one of the proprionate side chains of the tetrapyrrole to generate an acrylate side chain. The isobacteriochlorin is synthesized by enzymes encoded in the *nir* operon (*nirSMCFDLGHJEN*) [2]. NirN, encoded by the last gene in the *nir* operon, is a periplasmic *c*-type cytochrome with 24 % identity to NirS. NirN was shown to bind heme d_1 and transfer it to the NirS *in vitro*. It was therefore thought to be important for transport and insertion of the isobacteriochlorin into the NirS [3]. Furthermore, NirN was shown to interact with NirF and NirS during the maturation of the cytochrome cd_1 nitrite reductase [4]. Our evidence suggests that a precursor of heme d_1, dihydro-heme d_1, is formed in a *P. aeruginosa ΔnirN* mutant, as indicated by UV-Vis-, resonance raman spectroscopy and mass spectrometry. This precursor can be purified along with the NirS protein. In a NirN *in vitro* activity assay, NirN is able to convert dihydro-heme d_1 to heme d_1, as confirmed by mass spectrometry. For this conversion, NirN uses an electron bifurcating mechanism: one electron is transferred to the heme *c* cofactor and the other reduces the product heme d_1 to the ferrous state. Furthermore NirF is able to bind dihydro-heme d_1, but does not convert it to heme d_1 *in vitro*. Thus, NirN catalyzes the last step of heme d_1 biosynthesis before transferring the cofactor to the nitrite reductase [5].

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MCP30

Characterization of recombinant *Methanococcus maripaludis* strains lacking *hcg* genes responsible for biosynthesis of the [Fe]-hydrogenase cofactor

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Introduction: [Fe]-hydrogenase (Hmd) harbors a unique iron-guanylylpyridinol (FeGP) cofactor as the active site. The *hmd* co-occurring genes (*hcgA-G*) encode the enzymes for the FeGP cofactor biosynthesis. We have demonstrated that HcgB is a guanylyltransferase catalyzing ligation of the GMP and pyridinol moieties of the cofactor [1]. However, the biosynthetic functions of some other *hcg* genes are still elusive. *Methanococcus maripaludis* is a model organism for genetic analysis of methanogens without cytochrome. However, the strain used for genetic analysis (Mm901 strain) is not suitable for the analysis of the Hmd activity due to its very low and unstable activity although previous knock-out mutation studies have indicated that deletion of the *hcg* genes affected on the growth properties of *M. mariparudis*.
Objectives: This study aimed to identify functions of the *hcg* genes by gene-deletion experiments followed by Hmd activity assay and by characterization of the substrates and products of the reactions catalyzed by the Hcg proteins
Methods: The endogenous *hmd* gene of *M. maripaludis* was deleted from the Mm901 strain and the *hmd* gene from the hyperthermophilic methanogen, *Methanocaldococcus jannaschii* was introduced into the strain (designated as basal strain). The *hcgB* and *hcgC* genes of this basal strain were eliminated by markerless in-frame deletions.
Results: The basal strain exhibited thermostable Hmd activity. In the cell extract of the *hcg*B and *hcg*C deletion strains, the Hmd activity was not detected.
Conclusion: These findings are in agreement with the proposed functions of the Hcg proteins. To identify the precursors of the biosynthetic pathway, analysis of compounds accumulated in the cell extract of the *M. maripaludis* mutants and knock-out mutation of the other *hcg* genes are in progress.

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MCP31

The Frataxin dependent Iron Distribution System in *Bacillus subtilis*

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Introduction: Frataxin, a small acidic protein which is highly conserved among prokaryotes and eukaryotes, is suggested to act as a regulatory component as well as an iron chaperone in different cellular pathways. In *B. subtilis,* the deletion of the *fra* gene shows a drastic growth defect phenotype, caused by an accumulation of iron and a disrupted heme and Fe-S biosynthesis. Previously, we could show that Frataxin is able to bind iron and deliver it to the iron-sulfur cluster machinery[1].
Objective: We try to understand the role of Frataxin in the *B. subtilis* metabolism and investigate its role in iron homeostasis *in vivo* and *in vitro*. Furthermore, we also aim to elucidate the role of Frataxin in the heme maturation of *B. subtilis*.
Methods: Complementation of Δ*fra* mutants, *in vitro* reconstruction of iron containing cofactors, analytical gel filtration, pull-down experiments, microscale thermophoresis and Hydrogen/Deuterium Exchange (HDX)-MS Experiments.
Results: Here we demonstrate that we can rescue Δ*fra* mutant in by reintroducing *fra* homologs in the *amyE* site. We further investigated the effect of iron loaded Fra homologs (human, yeast, *E. coli*) on the Fe-S reconstruction assay and demonstrate that the homologs have an enhancing effect similar to the wild type Frataxin.
We characterize *in vitro* the delivery of ferrous iron from Frataxin onto the ferrochelatase HemH and specified the interaction epitope further by HDX-MS measurements. We found that the putative iron-binding site of Fra is facing towards the HemH active site, where the iron-binding site is located. Furthermore a conformational change of the chelatase could be observed.
Conclusion: We showed that *B. subtilis* Frataxin is involved in the heme maturation and gave structural evidence for the mechanism of the iron delivery. Furthermore we highlighted the importance of Frataxin in *B. subtilis* and showed that the organism is promiscuous towards the putative iron chaperon.

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MCP32
Biochemical characterization of the heme chaperone HemW
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Introduction: Heme is an iron containing tetrapyrrole that is associated with a diversity of biological functions. In spite of its biological importance free heme is cytotoxic. Thus, heme requires binding proteins, chaperones and transporters to reach its site of function. Despite its high DNA sequence homology to *hemN* coding for a coproporphyrinogen III dehydrogenase (CPDH), *Lactococcus lactis* HemW was proposed to be involved in heme trafficking. Moreover, HemW like proteins are present in animals, plants and bacteria [1].
Objectives: We aim to further characterize HemW in different organisms and elucidate its function.
Methods and Results: For *E. coli* HemW no CPDH activity was shown *in vivo*. However, incubation of overproduced and anaerobically purified *E. coli* HemW with ^{14}C-SAM and a subsequent chromatography revealed that SAM served as cofactor. Specific heme-binding was shown by a UV-VIS absorption spectrum of heme or heme analog supplemented HemW and a heme binding assay. In addition titration of HemW with increasing amounts of hemin demonstrated the equimolar binding of heme to HemW. Furthermore, the iron-sulfur cluster was analyzed. A size-exclusion chromatography after reconstitution of the cluster indicated a HemW dimer. Mössbauer spectra of HemW revealed a $[4Fe\text{-}4S]^{2+}$ cluster. *E. coli* HemW was also able to transfer hemin to heme-depleted quinol nitrate oxidoreductase (NarGHI) membrane vesicles and restored Nar activity. Immunohistochemistry analysis of *Danio rerio* fibroblasts revealed localization of HemW in mitochondria. Moreover, *in situ* hybridization of HemW in D. rerio embryos showed HemW localization in heart and gills, where heme is highly needed for the respiratory chain.
Conclusion: We identified *E. coli* HemW as a specific heme chaperone with an iron-sulfur cluster.

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MCP33
***In vitro* characterisation of Radical SAM enzymes involved in tetrapyrrole biosynthesis - NirJ and AhbC**
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Several fundamental processes in life, such as photosynthesis, oxygen and electron transport, or methanogenesis, rely on enzymatic cofactors with a tetrapyrrole structure (for example chlorophyll *a*, haem, cytochrome *c* or cofactor F_{430}). The biosyntheses of the most common tetrapyrroles are well understood, but there are still pathways with unrevealed reaction mechanisms. For example, the alternative haem biosynthesis pathway found in sulfate reducing bacteria and archaea, or the biosynthesis of haem d_1, a cofactor of the nitrite reductase NirS. These two pathways share identical steps, up to the point of 12, 18-didecarboxysiroheme (DDSH). In the route for haem d_1, DDSH is assumed to be transformed by the enzyme NirJ into 3, 8-dioxo-12,18-didecarboxysirohaem, the precursor of haem d_1. In contrast, in the penultimate step of the alternative haem biosynthesis, AhbC converts DDSH into iron-coproporphyrin III. The latter reaction was shown by *in vivo* experiments already. [1, 2, 3]
Our aim is to test the proposed activities of AhbC and NirJ by *in vitro* experiments. So far, the heterologously produced enzymes NirJ (from *Dinoroseobacter shibae*) and AhbC (from *Methanosarcina barkeri*) were characterised as Radical SAM enzymes, each bearing at least one [4Fe-4S]-cluster. Additionally an experimental strategy was established, in order to show their catalytic activity. Crude cell extracts containing the substrate DDSH are incubated with either NirJ or AhbC under strictly anaerobic conditions. Subsequent analysis of the extracted tetrapyrroles is done by HPLC and MS.

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MCP34
Crystal structure of the L-tryptophan 2-monooxygenase VioA from *Chromobacterium violaceum*
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Violacein is a purple pigment with a wide range of antibacterial, antiviral and antitumorigenic activities. This compound is synthesized by the Gram-negative β-Proteobacterium *Chromobacterium violaceum.*
Violacein biosynthesis starts with the enzymatic oxidation and coupling of two molecules of L-tryptophan. The resulting intermediate represents the scaffold for the formation of the final indole derivative. The initial step of tryptophan oxidation to indole-3-pyruvic acid (IPA) imine is catalyzed by the flavoenzyme VioA. Subsequently the hemoprotein VioB catalyzes the coupling of two IPA imine molecules to an IPA imine dimer.
Recombinantly overproduced VioA and VioB proteins were purified to apparent homogeneity. Then, the crystal structure of VioA was resolved by heavy atom soaking of VioA crystals and subsequent MAD (Multi-wavelength Anomalous Diffraction) phase determination. The structure of VioA belongs to the GR(2) family of FAD dependent oxidoreductases. The highest homology is given to the L-amino acid oxidase from *Rhodococcus opacus* (*ro*LAAO). Co-crystallization experiments of VioA in the presence of a newly synthesized tryptophan derivate revealed the amino acid binding site in close proximity to the isoalloxazin ring of the FAD cofactor. A hydride transfer in analogy to the LAAOs was concluded.
Initial experiments revealed the interaction of VioA and VioB. The direct channeling of the highly reactive IPA imine reaction product was deduced.

MEcP01
Epidemiology of nasal carriage of *Staphylococcus aureus* and methicillin resistant *Staphylococcus aureus* among patients admitted to two healthcare facilities in Algeria
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Aims: To evaluate nasal carriage rate and variables associated with *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) in patients admitted in two healthcare facilities.
Results: *S. aureus* was isolated from 159 (26%) of the enrolled patients. Methicillin-susceptible *S. aureus* (MSSA) was isolated from 150 (24.5 %) patients, and MRSA was isolated from nine (1.5 %). Cancer and previous hospitalization were associated with a significantly higher frequency of nasal *S. aureus* carriage among the patients admitted to the general hospital and the nephrology department, respectively. MRSA isolates were heterogeneous with respect to their Staphylococcal Chromosomal Cassette (SCC) *mec* type, sequence type (ST) and toxin genes (*pvl* and *tst1*) content. Four isolates were attributed with the ST80-MRSA-IV clone which is known to be predominant in Algeria.
Conclusions: This is the first assessment of *S. aureus* and MRSA nasal carriage and associated variables in Algeria. Our findings provide also a picture of the MRSA strains circulating in community in this geographic area. They can be useful as a guide for implementing screening and control procedures against *S. aureus*/MRSA in the Algerian healthcare facilities.

MEcP02
A stable microbial community influenced by a highly polluted river in the Gulf of Naples (Italy)
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The Gulf of Naples (Italy), which connects coastal waters with the open Tyrrhenian Sea, offers optimal conditions for a highly diverse marine environment, which has been investigated intensively since <140 years. The Gulf of Naples is affected by anthropogenic pollution, which may cause serious damage to the pristine ecosystem. The Sarno river is, beside the extensive ports of Naples, the main source of anthropogenic pollutants

introduced into the Gulf of Naples. The release of waste water from tanneries, agricultural regions, human conglomerations, and the pharma industry along the river causes high concentrations of heavy metals, hydrocarbons, and a broad variety of other pollutants in the Sarno river. These pollutants may have an immense impact on the ecosystem of the Gulf of Naples, in particular on the microbial community, which was rarely addressed in previous studies. Here, we present the first results of the investigations of the microbial community within the plume of the Sarno river and the Gulf of Naples. We analyzed four stations along a transect from the river estuary towards the open waters of the Gulf of Naples, using metagenomics, metatranscriptomics, flow cytometry, and fluorescence in situ hybridizations techniques. The aim of this study is to investigate the microbial diversity and activity of the Gulf of Naples and the response of this community towards the influences of the Sarno river. The bacterial and archaeal community was relatively stable with cell numbers ~ 1.1x106 cells ml-1. The overall community is dominated by marine organisms and shows only minute changes from the estuary to the open Gulf of Naples. A slightly higher abundances of Beta-, Delta-, and Epsilonproteobacteria was found in the vicinity of the estuary, while higher abundance of the SAR11 clade was found at distance. Genes for general cell processes, like nutrient uptake and energy production, were found in abundance. However, several genes related to pollutants from the Sarno river, such as mercury or cadmium resistance genes, were found in the station close to the estuary, where pollutant concentrations are highest. These first data suggest a relatively stable microbial community dominated by marine organisms. However, influences of the polluted fresh waters of the Sarno river on the diversity and genetic content of the microbial community were indicated.

MEcP03

Communication between the Host Organism and Symbiotic *Lactobacilli* – Production of Neuromediators (Biogenic Amines and Amino Acids) by Microbial Cultures

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An important subfield of microbial ecology focuses on the chemical dialogue within the host organism—microbiota consortium that presumably involves neurochemicals (biogenic amines, amino acids, etc.). Human microbial inhabitants, e.g., *Escherichia coli* [1], produce biogenic amines. The symbiotic microbiota includes lactobacteria, and some of them represent probiotics. Are neuromediators released by lactobacteria contained in fermented dairy items and starter cultures?

Materials and Methods: The contents of biogenic amines (BAs) and regulatory amino acids (RAAs) in dairy products and pure *Lactobacillus* cultures were measured using high performance liquid chromatography (HPLC) on a reversed-phase column.The BAs were determined amperometrically with a glass-carbon electrode (+0.85 V), and the RAAs were complexated with ortho-phthalic aldehyde and determined fluorometrically (the excitatory and emission wavelengths were 230 and 392 nm, respectively).

Results: A comparison of milk and yogurt samples revealed that the yogurts are enriched in noradrenaline, dopamine, DOPA, their precursor, and RAAs such as glycine, taurine, and GABA (contained in the fruit filler-lacking yogurt only). The pure cultures of *Lactobacillus helveticus* 100ash, *L. helveticus* NK-1, *L. casei* K_3III_{24}, and *L. delbrueckii* subsp. *bulgaricus* release into the milk- or pancreatic hydrolysate of milk-containing medium (sub)micromolar amounts of DOPA, dopamine, noradrenaline, serotonin (produced by *L. helveticus* 100ash only), GABA (at concentrations that are close to those in the human blood and spinal fluid), glutamate, glycine, and taurine.

Conclusions: The data obtained suggest that the bacteria that interact with the human organism, exemplified by several *Lactobacillus* species/strains, form neuromediators such as BAs and RAAs. They can exert a local effect and, with the gut-blood and blood-brain barrier-passing DOPA and GABA, a systemic influence, particularly on the functioning of the brain. These compounds presumably function as signal agents in the microbiota-gut-brain axis.

References: Shishov, V.A, Kirovskaya, T.A, Kudrin, V.S, & Oleskin, A.V. (2009). Amine neuromediators, their precursors, and oxidation products in the culture of *Escherichia coli* K-12. *Appl. Biochem. Microbiol., 45(5),* 494-497.

MEcP04

Seasonal dynamics of bacterial and archaeal methanogenic communities in flooded rice fields and the effect of drainage and crop rotation

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Introduction: Introducing non-flooded crops into rice-dominated ecosystems will lead to dramatic changes in field conditions, which will affect carbon cycling and the composition and activity of the present microbial communities.

Objectives: We studied the resident (DNA) and the active (RNA) members of soil archaeal and bacterial communities during rice plant development by sampling three growth stages (vegetative, reproductive and maturity) under field conditions in rice fields at the International Rice Research Institute in the Philippines. Additionally, the microbial community was investigated in two non-flooded fields (unplanted, cultivated with upland maize) in order to monitor the reaction of the microbial communities to non-flooded, dry conditions.

Material & Methods: Community profiling was conducted by the fingerprinting method T-RFLP and 454 pyrosequencing targeting the archaeal and bacterial 16S rRNA gene and 16S rRNA. The abundance of Bacteria and Archaea was monitored by quantitative PCR.

Results: Community profiling by T-RFLP indicated a relatively stable composition during rice plant growth. Pyrosequencing revealed only minor changes in relative abundance of few bacterial groups. Comparison of the two non-flooded fields with flooded rice fields showed that the community composition of the Bacteria was slightly different, while that of the Archaea was almost the same. Quantative analysis showed an increase in 16S rRNA genes during reproductive stage and stable 16S rRNA copies throughout the growth season. The abundance of bacterial and archaeal 16S rRNA gene copies was highest in flooded rice fields, followed by non-flooded maize and unplanted fields. In contrast, the abundance of ribosomal RNA (active microbes) was unchanged under the non-flooded conditions.

Conclusion: The maintenance of a high level of ribosomal RNA under the non-flooded conditions, which were unfavorable for anaerobic bacteria and methanogenic archaea, is possibly a stress response and serves as preparedness for activity when conditions improve. In summary, the analyses showed that the bacterial and archaeal communities inhabiting Philippine rice field soil were relatively stable over the season but reacted upon change in field management.

MEcP05

Comparison of sample preparation protocols for metaproteomic studies of the rumen microbiota

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Introduction: Various metabolic processes are expressed in the rumen, caused by a complex biocoenosis consisting of bacteria, archaea, protozoa and fungi. The description of the most active fraction composed of bacteria and archaea and the identification of functional pathways are of great interest for animal nutrition, biotechnology and climatology.

Objectives: Metaproteomic studies of the rumen microbiota are challenged by the need of optimized sample preparation protocols in order to retrieve an increased amount of prokaryotic instead of plant and bovine derived cells prior to protein extraction and subsequent LC-MS/MS analysis. The present study evaluates two protocols for the fibre-adherent communities and one for the liquid-associated species.

Materials & Methods: Solid and liquid rumen samples were subjected to various chemical and physical treatments. Prior to mass-spectrometric analysis, in-gel trypsin digest and 1D-SDS-PAGE, total protein was extracted from the enriched samples. Protein identification was performed using NCBInr database. Phylogenetic classification was done using Unipept and functional grouping of the identified proteins was determined by WebMGA.

Results: Our findings suggest the integration of cheesecloth-gauze filtration in sample preparation to achieve a better protein identification ratio. None of the protocols, respectively identified proteins, biased towards a specific phylogenetic distribution, subcellular localization or Clusters of orthologous Groups. Main protein functions were involved in carbohydrate metabolism and translation processes.

Conclusion: Although the identification ratio of prokaryotic to eukaryotic proteins was improved, the total number of identifications (~2300 prokaryotic proteins) still only depicts a sparse part of the actually active microbiota. Anyhow, technical progress in mass spectrometry and an increasing availability of reference sequences enhance appropriate application of metaproteomic approaches for rumen research.

MEcP06

Evaluation of commercial DNA extraction kits for analysing microbial diversity in the pig gastrointestinal tract

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Introduction: The gastrointestinal tract is a complex ecosystem and harbours a huge variety of microorganisms that take influence on metabolism of nutrients and health of the host. Molecular techniques based on the analysis of 16S rDNA genes are nowadays often used to study the complex gut microbiota. Therefore bacterial genomic DNA needs to be extracted out of gut samples. Since these types of samples are full of microbial cells, host cells and food components, that can inhibit downstream applications, the extraction of bacterial DNA is a crucial challenging step.
Objectives: In this study we compared 15 manual (commercial DNA extraction kits) and two automated procedures to extract DNA from pig feces and ileal digesta samples with regard to DNA quantity and quality and bacterial community structure.
Methods: Feces and ileal digesta sample from T-cannulated growing pigs were collected and stored at -80°C until DNA extraction. 15 different procedures with manual DNA extraction kits and two procedures with an automated DNA extraction kit were tested. The evaluated kits use different chemical, mechanical and magnetic techniques to lyse cells. The quantity and quality of extracted DNA was compared. Furthermore to study bacterial diversity coverage of each procedure 16S rDNA-based methods, terminal restriction fragment length polymorphism (TRFLP)-analysis and Illumina amplicon sequencing spanning the V1_V2 and V5_V6 regions, were used.
Results: In general the yield of extracted DNA was higher in feces than ileal digesta sample. The evaluated DNA extraction methods show differences in the microbial community diversity within each type of sample.
Conclusion: The method used to extract DNA from pig gastrointestinal tract samples affects the representation of the bacterial community. Thus, the choice of the DNA extraction method should be carefully evaluated according to DNA quality, quantity and wide coverage of bacterial species.

MEcP07

Microbial diversity during a strong deep winter convection year in an oligotrophic environment (south Adriatic)

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Introduction: The South Adriatic Sea is the deepest part of the Adriatic Sea representing a key area for both the Adriatic Sea and the deep Eastern Mediterranean. It has a role in dense water formation for the Eastern Mediterranean deep circulation cell and it represents an entry point for water masses originating from the Ionian Sea.
Objectives: The biodiversity and seasonality of bacterial picoplankton before, during and after deep winter convection in the oligotrophic South Adriatic waters were assessed.
Methods: A combination of 16S rRNA sequence analysis and catalyzed reporter deposition-fluorescence in situ hybridization (CARD-FISH) was used to determine the biodiversity and seasonality of bacterial picoplankton.
Results: The picoplankton communities reached their maximum abundance in the spring euphotic zone when the maximum value of chlorophyll a was recorded in response to deep winter convection. The communities were dominated by Bacteria while Archaea were a minor constituent. A seasonality of bacterial richness and diversity was observed, with minimum values in the winter convection and spring post-convection period, and maximum values during summer stratified conditions. SAR11 was the main constituent of the bacterial communities and reached the maximum abundance in the euphotic zone in spring. Cyanobacteria were the second most abundant group strongly depending on the convection event when minimal cyanobacterial abundance was observed. Euphotic zone in spring and autumn was characterized by Bacteroidetes and Gammaproteobacteria. The Bacteroidetes clades NS2b, NS4 and NS5 and the gammaproteobacterial SAR86 clade were detected to co-occur with phytoplankton blooms. SAR324, SAR202 and SAR406 were present in the deep layer exhibiting different seasonal variations in abundance.
Conclusion: The abundances of particular bacterial clades and the overall bacterial richness and diversity are greatly impacted by strong winter convection.

MEcP08

Effect of supplementing diets with phytase and mineral phosphorus on the composition of the microbiota in the gastrointestinal tract of chicken

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Introduction: Phosphorus (P) is essential for all animals. Plant feedstuffs contain mainly phytate bound P. Release of P from phytate involves the enzyme phytase, which is hardly present in the intestine of chicken. Therefore, mineral P and microbial phytases are usually added to broiler diets. These supplements affect phytate hydrolysis and P availability in the gastrointestinal tract (GIT). This may be mediated by shifts within the bacterial community composition and changes in hydrolysis of phytate initiated by the microbiota.
Objectives: We investigated the effect of phytase and mineral P supplements on the bacterial community composition along the GIT of broilers.
Methods: Two maize-soybean meal-based diets contained P only from plant sources (BD-) or additionally supplemented mineral P (BD+). Diets remained unsupplemented or a microbial phytase was added (500 or 12500 U/kg of diet). Diets were fed to broilers from day 15-25 of age for *ad libitum* consumption. On day 25 digesta was obtained from the crop, jejunum, ileum and caeca for DNA extraction. DNA was used for terminal restriction fragment length polymorphism analysis and pyrosequencing of 16S rRNA gene amplicons.
Results: Both methods used revealed an effect of mineral P in the crop. As detected by pyrosequencing *Flavobacteriaceae* were more abundant in BD- diets. Sequencing data also indicated an effect of mineral P in the caeca with *Clostridiales* being of higher and *Enterobacteriaceae* of lower abundance with mineral P. Phytase addition affected diversity and evenness in all GIT sections. It favoured the abundance of *Flavobactericeae* and *Aeromonadaceae* but lowered that of lactobacilli in the crop. In the ileum an increase of *Enterobacteriacea* and *Peptostreptococcaceae* was detected with phytase in BD- diets whereas lactobacilli were decreased. In the caeca phytase addition led to less *Erysipelotrichaceae* but more *Bacteroidaceae.* Sequencing data revealed no effect of mineral P or phytase on the bacterial community in jejunum that was mainly colonized by *Lactobacillaceae*.
Conclusion: Results suggest a considerable impact of the P source and phytase on the GIT microbiota. Further studies should differentiate between phytase- and non-phytase-producing bacteria and should consider the relevance of phytate hydrolysis products that are modulated by these supplements.

MEcP09

Evaluation of extraction methods for quantification and cultivation-independent analysis of viruses from soil

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Introduction: Viruses that infect specific host bacteria, known as bacteriophages, have a vast influence on their hosts mortality, evolution, physiology and community structure, and thus on biogeochemical cycles. Although viruses represent the most abundant entities on earth, research has focused mainly on marine ecosystems neglecting terrestrial habitats. Thus the implications of viruses for the genetic landscape of the terrestrial biosphere remain unclear. A first crucial step for the study of soil viruses is the need for reliable extraction protocols in order to either directly count virus-like particles (VLP) in soil samples by epifluorescence microscopy or to apply cultivation-independent fingerprinting tools.

Objectives: Here we show a study aiming at the evaluation of extraction efficiencies of 36 combinations of commonly used buffers and mechanical treatments for VLP extraction from soil.
Methods: Nine different buffers in combination with four mechanical treatments (i.e. vortexing, shaking, bead-beating and sonication) were tested to extract VLPs from a soil sample (7.5 g) taken at the Hainich National Park (Thuringia, Germany). The extracted VLPs were quantified using epifluorescence microscopy after staining with SybrGold and the community structure of dsDNA viruses was analyzed with RAPD-PCR (i.e. PCR of randomly amplified polymorphic DNA).
Results: The extraction efficiencies of the tested methods differed highly. Abundances of extracted VLPs ranged from 6 x 10^8 to 5 x 10^9 VLPs/g soil. The most effective extraction method was 1x saline magnesium buffer in combination with 20 min vortexing, while treatments using deionized water and potassium citrate buffer extracted the lowest numbers of VLPs. Fingerprinting analysis using random primers allowed community-level comparison of DNA fingerprints as a rough measure of virus diversity in the VLP extracts.
Conclusion: The most efficient method will be used to process future soil samples from the Hainich National Park and compare virus communities along a land-use gradient.

MEcP10
Activation of methanogenic communities by incubation under different moisture levels
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Introduction: Methanogenic communities are involved in the anaerobic degradation of organic matter in permanent water logged systems like rice fields. A controlled irrigation technique during rice cultivation is used in the Philippines for several years (Rejesus et al., 2011), which reduces the available amount of water in the field. However, it is still not clear how the moisture level affects the methanogenic community.
Objectives: The goal of this study is to investigate how the availability of water (expressed as % of maximal water holding capacity) is influencing methanogenesis and the composition of the methanogenic community.
Methods or Materials & Methods: Therefore incubation experiments with two types of rice field soils (from Italy and the Philippines) were performed. The soils were amended with different amounts of water and incubated under anoxic conditions at 25 °C for 70 days. Gas measurements and stable isotope analysis of CH_4 and CO_2 were routinely performed. In addition the abundance and community structure of the methanogenic community were analyzed by qPCR and Terminal Restriction Fragment Length Polymorphism.
Results: Our results showed that in the tested rice field soil water availability and CH_4 production rate were positively correlated. The early isotopic signature of CH_4 indicated a trend toward hydrogenotrophic methanogenesis, which became relatively more active with higher water concentrations. In the end CH_4 produced by the hydrogenotrophic pathway contributed 20-35% of total CH_4. The composition of the methanogenic community was rather similar at the different moisture levels. The abundance of these communities, which was determined by copy numbers of archaeal 16S rRNA genes and mcrA (functional maker gene of methanogens) was also similar.
Conclusion: These results suggest that the methanogenic community reacts to water changes by adjusting the activity, instead of changing the microbial community structure.

Rejesus, R. M., Palis, F. G., Rodriguez, D. G. P., Lampayan, R. M., and Bouman, B. A. M. (2011). Impact of the alternate wetting and drying (AWD) water-saving irrigation technique: Evidence from rice producers in the Philippines. *Food Policy* 36, 280-288. doi:10.1016/j.foodpol.2010.11.026.

MEcP11
Insights into the lifestyle of a thermophilic electrolithoautotrophic consortium
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Microbes that are able to utilize electrical energy provided from a cathode as a source for metabolic electrons are performing a recently discovered lifestyle that is known as microbial electrosynthesis. The uptake of cathodic electrons can be coupled to the fixation of carbon dioxide, which results in electrolithoautotrophic growth. So far the biochemistry behind this process is unknown, but some methanogens as well as homoacetogens were shown to thrive using this strategy. It is the aim of this work to isolate novel electrolithoautotrophs from environmental samples with a particular focus on thermophiles. Hence, the inoculum for our enrichment was a mixed sample from hydrothermal systems. The enrichment was performed in a 2 L self-designed three electrode setup reactor that was incubated at 60°C and continuously purged with a N_2/CO_2 gas mixture. A carbon cloth cathode was inserted into the reactor and was poised to a constant potential of -350 mV vs. SHE. The system runs for nine months and meanwhile shows a constant current of about -1 mA. By the use of 454 sequencing to analyze the taxonomic community composition we could show that the community in the electrosynthesis reactor comprises a mixture of bacteria as well as archaea. In a subsequent metagenomic illumina approach we searched in the metagenome for evidence of CO_2 fixation and we were able to detect several CO_2 fixation pathways, including the Wood-Ljungdahl pathway, the 3-hydroxypropionate/4-hydroxybutyrate pathway and the reductive citric acid cycle. Currently we conduct ^{13}C-CO_2 fixation experiments to quantify the carbon dioxid fixation rates of the cathodic community. With the results of the meta-analysis we aim for a reverse engineering of an electroautotrophic growing reactor by imitating the community with distinct isolated organisms.
For future perspectives an ultimate goal would be to biotechnologically engineer both process and organisms in a way that allows a targeted production of chemicals of industrial value.

MEcP12
Drying effects on the bacterial and archaeal community composition and methanogenesis in bromeliad tanks
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Introduction: Tank bromeliads are highly abundant epiphytes in neotropical forests. Their densely arranged leaves form tanks that efficiently collect wind-borne particles, leaf litter and rainwater. Diverse communities of macro- and microorganisms inhabit these tanks and are responsible for the breakdown of tank organic matter, the release of plant-available nutrients and of substantial amounts of CH_4. Bromeliad wetlands are very susceptible to within-day variations of evaporation and precipitation and single tanks can dry out completely within few days upon drought. The effect of water availability may be further increasing since increasing droughts are anticipated in the tropics as a result of anthropogenic climate change.
Objective: Therefore, we investigated the effect of different moisture levels on the bacterial and archaeal community composition and the methanogenic pathway in bromeliads tanks.
Material and methods: Greenhouse experiments were established to investigate the resident and active bacterial and archaeal community targeting the 16S rRNA gene and the 16S rRNA in composition (T-RFLP, 454 pyrosequencing) and abundance (qPCR) in the tank slurry of bromeliads at three different moisture levels. Methane production and stable carbon isotopic signatures of CH_4 formed in the tank slurry were measured under different moisture levels to determine the methanogenic pathways.
Results: Our study provides evidence that moisture in the tank slurry is a controller of the microbial community composition, the size and the methanogenic pathway. With decreasing moisture level the methanogenic community shifted from a hydrogenotrophic dominated community, represented by *Methanobacteriales*, to an aceticlastic dominated community, represented by *Methanosaetaceae.* This shift was accompanied by an increase of aceticlastically produced CH_4. With decreasing moisture the bacterial diversity decreased and the relative abundance of the *Burkholderiales* almost tripled.
Conclusion: We suggest that microbial community composition and CH_4 cycling in neotropical bromeliad wetlands are immediately affected by drying.

MEcP13
Address Action of *Lactobacillus* Multispecies Pools Towards Extended *Candida albicans* and *Candida tropicalis* Pool
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Introduction: *Candida albicans* and *C.tropicalis* form main group (I: Ia+Ib) of infections. The coupled system "*Candida*(I)+*Lactobacillus*(II)" (SCL) is highly sensitive one to study interactions involving urogenital microcenoses. SCL reveals antagonism between probiotic like and relatively pathogenic compartments. Earlier we described algorithms of screening and identification of leader strains of biofilm forming (BF)

influence between pools I and II. SCL allows evaluation of direct and opposite relationships between I and II [1].
Objective: Further study of urogenital SCL direction II—I.
Materials & Methods: Clinical urogenital strains of *L.acidophilus* (106, 124 [leader, probiotic-like, low BF], 183a), *L.brevis* (104, 109, 143), *L.casei* (124b [leader, probiotic-like, low BF], 183), Ia (3, 23, 26, 45, 116, 147, 161, 320 [leader, antimycotic-resistant]), and Ib (*97, 112, 144, 162, 417, 433, 438, 897*) were isolated. Mono- and mixed cultures in MRS were grown in micropanels (48 h, 37°C). BF was evaluated by staining, stain extraction and measurement at 620 nm. The influence of pool II on BF of each strain of pool I was calculated and ranged. Blocks in rows were compared. Architectures distribution and colony morphology were registered in micropanels, in special standard cultures.
Results: A. Blocks Ia1(bolded), Ia2 and Ib in direction II(pools:7-8 strains)—I(pool:16 strains) were calculated and ranged. A1. Case "II as all strains": **23 > 161 > 320 > 147** > *144* > *97* > 45 > *438* > *897* > *112* > *417* > *162* > *433* > 116 > 3 > 26. A2. Case "II without 124": **23 > 161 > 320** ≥ *144* > 97 > 147 >*112* ≥ *438* ≥ *897* > *417* > *433* > *162* ≥ 116 > 3 ≥ 26 > 45. A3. Case "II without 124b": **23 > 45 > 320 > 147 > 161** ≥ *438* > *144* > *97* ≥ *897* > *162* ≥ *417* > *433* > *112* ≥ 116 > 26 > 3. Leaders of II influenced expression of block Ia1 in opposite manner (A2, A3). Strain 124 regulated coupled expression of Ia1 and Ia2 (A2), and strain 124b increased expression of Ib (A3). B. Early germ tubes appearance and their fast growth were registered in case of strains 23, 147 and 320. Universality of block analysis of *Candida* pools status in the presence of bacteria is demonstrated.
Conclusion: Results indicate therapeutic address potential of II possessing low BF.

1. M. Lakhtin, V. Lakhtin, A. Bajrakova and S. Afanasiev, BioSpektrum (2014), p. 296.

MEcP14
Temporal and spatial patterns of nitrifying organisms in grassland soil
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Interrelated processes, such as successive transformation steps in nitrification, are performed by distinct microbial groups with differing dependencies on environmental parameters, raising the question of how networks of interacting organisms are distributed at different spatial scales. We aimed to monitor those patterns at the spatial scale of square metres at a grassland site over an entire season (Regan et al., 2014) within the frame of the Biodiversity Exploratories (www.biodiversity-exploratories.de). We focused on the interaction between nitrite- and ammonia-oxidizers, the latter generating the substrate for the subsequent nitrite-oxidation step.
We used the abundance of the *amoA* gene as a proxy for ammonia-oxidizing bacteria (AOB) and archaea (AOA), *nxrA* as a proxy for *Nitrobacter*, and 16S rRNA genes for *Nitrospira*, both belonging to the nitrite-oxidizers (NOB). We observed high temporal variability in the abundance of AOA with highest values in May and November, but contrasting abundance patterns for AOB. Interestingly, the temporal dynamics of *Nitrospira* abundance were similar to those of AOA. We determined a positive correlation between AOA and NOB genera for different time-points.
Only *Nitrobacter* and *Nitrospira* were found to exhibit distinct spatial distributions, likely due to specific requirements for substrate concentrations that varied with season. These patterns ranged from distinct patchy clusters indicating niche differentiation to gradient-like structures where both genera were found to co-occur. Phylogenetic data, based on 16S rRNA gene analyses selected for OTUs affiliated with NOB genera, will be presented to reveal putative community shifts and to complete the picture of their interactions at the diversity level.

MEcP15
Denitrifier communities in tank bromeliads and prospected N_2O emissions from tank substrate upon increasing N-deposition
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Introduction: It is well known that tropical rainforest soils with total emissions of 1.34 Tg N/yr from the tropics, play a significant role in the global N_2O emissions scenarios. Significant contributions were reported particularly for tropical rainforest soils in South and Central America due to the large areas covered by rainforest in this region. In tropical rainforests of the Americas tank bromeliads constitute a prominent group of plants and were shown to significantly contribute to production the production of the greenhouse gas methane from tropical forests.
Objectives: It is, however, essentially unknown whether and how bromeliads which may contribute to the production of N_2O, another important greenhouse gas. It is also unknown whether N_2O emissions relate to atmospheric N-deposition and whether an increase in emissions is to be expected upon the prospected increase in N-deposition.
Method: We studied the propensity of tank substrate of the bromeliad *Werauhia gladioliflora* to emit N_2O and how this potential is related to the underlying denitrifier communities. In tropical forests of Costa Rica *Werauhia gladiofolia* is very abundant with 9.85 specimen m^{-2}.
Results: Incubation of the tank substrate with increasing amounts of fertilizer to reflect predicted N-deposition scenarios resulted in proportionally increasing net N_2O production. Based on the abundance of *Werauhia gladiofolia* we estimated annual emissions of 395 µg N_2O-N m^{-2} day^{-1} for N-deposition levels to date which is in the range of tropical soils. At a surplus of N 70% of N_2O produced were not reduced leading to accumulation of N_2O which agreed well with the finding that 95% of the denitrifiers detected lacked a gene encoding a N_2O-reductase and are therefore unable to reduce N_2O to dinitrogen. Generally, denitrifiers were highly abundant and ready to denitrify immediately after provision of a nitrogen source because carbon is non-limiting in tank substrate.
Conclusions: Our results suggest that tank bromeliad substrate may be a significant source of N_2O in neotropical forests and that with prospected increasing future N-depositions annual N_2O emissions might increase.

MEcP16
Influence of root derived carbon on CH_4 emission and methanogenic community structure in the rhizosphere of rice
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Introduction: The roots of rice plants represent a suitable surface for colonization by several lineages of soil methanogenic archaea. Atmospheric CO_2, photosynthetically fixed by the rice plant is allocated to the roots and continuously released as root exudates, which serve as precursors for formation of CH_4 from the rhizosphere.
Objectives: Aim of this study is to investigate whether the rice roots can select particular species or groups of methanogenic archaea and how selection depends on the pool of microorganisms in a certain environment. Furthermore we want to understand how far the colonization of the rice roots is of concern for the emission of CH_4.
Methods: Defined plant-soil model systems, with 20 % of environmental sample as inoculum and 80 % of inert matrix (sand and vermiculite), were used to minimize the influence of the physical and chemical conditions of the tested environmental samples. Rice field soil-, as well as a mixture of rice field soil and digested sludge were used as inocula, while microcosms with 100 % rice field soil served as control. The rice plants in the microcosms were pulse-labeled with $^{13}CO_2$. Labeling of root derived substances was followed by HPLC-MS and formation of $^{13}CH_4$ by GC-MS.
Results: All rice plants assimilated ^{13}C into their above- and below-ground biomass. Labeling of pore water substances (e.g. propionate, CO_2) was 4-18 times higher in rice soil-based microcosms compared to those containing digested sludge and compared to the control. Incorporation of ^{13}C into pore water substances occurred faster in samples containing digested sludge. Emission rates of $^{13}CH_4$ from rice field soil were higher (~16 nmol $g_{dw,root}^{-1}$ h^{-1}), while those containing digested sludge (~4 nmol $g_{dw,root}^{-1}$ h^{-1}) were lower than in the control (~9 nmol $g_{dw,root}^{-1}$ h^{-1}).
Conclusion: Our results show that CH_4 emission derived from freshly assimilated CO_2 was dependent on whether the rice plants were grown with digested sludge or rice field soil as source of methanogenic archaea. Analysis of the microbial community composition of the colonized roots will follow. Lower content of soil organic matter in the microcosms apparently also enhanced CH_4 emission from root derived substances.

MEcP17
Functional and structural responses of methanogenic microbial communities in Uruguayan soils to intermittent drainage
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Introduction: Intermittent drainage is one of the most promising approaches to mitigate CH_4 emission from paddy fields. However, how the methanogenic bacterial and archaeal communities adapted to such conditions is poorly known. Rice fields in Uruguay may serve as a useful model system for studying the influence of intermittent drainage, since they are only temporarily established on soils that normally are used as cattle pastures.
Objectives: We studied soil from rice field of the pasture-rice rotation (UR) as well as soil from a permanent cattle pasture (UT) in Uruguay hypothesizing that activity and structure of the bacterial and archaeal communities involved in production of CH_4 change systematically with intermittent drainage.
Methods: We characterized the function of methanogenic communities by the path of CH_4 production, and the structure of the communities by utilizing quantitative PCR, T-RFLP and pyrosequencing.
Results: Drying significantly decrease rates of CH_4 production in UT pasture and UR rice soil. CH_4 was mainly produced from acetate both in UR rice (73-86%) and UT pasture (51-65%) soils. Drying slightly enhanced CH_4 production from hydrogenotrophic methanogenesis in UR soil, while it had a negative effect on UT soil. Methanogenic archaeal gene copy numbers were much lower in UT than UR soil. However, intermittent drainage weakened the differences between the two soils. Intermittent drainage had an effect on the composition of the archaeal and bacterial community in UT pasture soil, but to a less extent in UR rice soil. Intermittent drainage resulted in dramatic changes of the relative abundance of *Clostridiales* in both of the two soils.
Conclusion: We conclude that intermittent drainage resulted in a relative stable methanogenic microbial community in rice field compared with pasture field despite that the function of the methanogenic microbial community had been impaired both in the two soils.

MEcP18
Is Reptile-Exotic-Pet-Associated-Salmonellosis (REPAS) a Public Health Problem?
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Introduction: Numerous reports exist on the prevalence of *Salmonella* (*S.*) *enterica* in captive reptiles [1]. Fatal outcomes following reptile-associated salmonellosis in babies have been reported [2]. Thomas et al. [3] concluded that the potential of captive and pet wildlife to transmit *Salmonellae* to humans should not be underestimated, and that epidemiological studies on sources for human salmonellosis should simultaneously investigate both the human cases and the wild and domestic animals in contact with them.
Objectives: Reptiles are suspected to be a source for salmonellosis in humans, and numerous case reports exist on salmonellosis in infants related to reptiles. It was therefore the aim of the study to obtain data on reptiles kept in households with children diseased from an exotic *Salmonella* serovar, including the *Salmonella* status and possible transmission paths, and to compare the isolates with those found in the respective child.
Methods: 79 affected families were contacted, and in 19 households and a total of 36 reptiles, samples of the oral cavity, the cloacae, the skin and the stomach (lizards) were collected. *Salmonella* isolation was conducted using a strict protocol with repeated enrichment and serotyping which was followed by pulsed-field gel electrophoresis (PFGE) identification in cases of identical serovars found.
Results: Almost 50% of the households answered that they kept at least one reptile. 68% of the examined reptiles were bearded dragons (Pogona vitticeps). Altogether 319 isolates were investigated and 44 different serovars identified. In 79% of the households, in at least one reptile the identical serovar was found and confirmed by PFGE. In 84% of all bearded dragons examined, the identical serovar was confirmed. In most reptiles several serovars were found.
Conclusion: The results demonstrate that reptiles and especially bearded dragons shed different serovars of *Salmonellae* including those serovars that were isolated from diseased children in the respective households. Hygiene protocols and parent's education is therefore highly necessary to reduce the risk of REPAS.

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MEcP19
Identification of metabolically active microbial communities in sediments by two independent RNA-based *in vivo* labeling techniques
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Metabolically active bacteria in microbial communities are commonly identified by the analysis of 16S rRNA gene transcripts. A well-established method to determine the community fraction that actively degrades specific substrates is RNA-based stable-isotope probing (SIP). The method depends on the degradation and incorporation of ^{13}C-labeled substrates which are not always commercially available. To circumvent this, we tested a universal and substrate-independent *in vivo* labeling approach. The technique is based on the incorporation of the modified nucleotide digoxigenin-11-uridine-5`-triphosphate (DIG) into *de novo* synthesized RNA. It was initially established for biofilms and was now adapted for sediments. To compare both labeling techniques, experiments were carried out on tidal-flat sediments from oxic and anoxic layers (0-1 cm and 20 cm below sea floor). Sediment slurries for SIP were amended with ^{13}C-labeled glucose and samples for the DIG approach with unlabeled glucose in the presence of DIG. To identify active degraders of organic sulfur compounds by DIG, dimethylsulfoniopropionate and dimethylsulfoxide + glucose were used as substrates. RNA from all experiments was extracted after 48h of incubation. Labeled and unlabeled RNA were separated by density-gradient centrifugation or by using anti-DIG-antibody-coated magnetic beads, respectively. RNA was transcribed to cDNA and the community structure was analyzed by DGGE targeting the 16S rRNA. The same active glucose degraders were identified by both methods. Similar DGGE banding patterns indicated high accordance of the DIG with the SIP approach. Additionally, distinct bacterial communities for the oxic and anoxic layer and a unique banding pattern for samples amended with the sulfur compounds were observed. In conclusion, DIG-labeling turned out to be a valid method for the identification of metabolically active bacteria in oxic and anoxic sediments. Advantages of the DIG method over SIP are short incubation times and the universal incorporation of the label into RNA, independent from the substrates of interest.

MEcP20
Indication for unexpected high diversity of 1-methyl alkyl succinate synthase genes in marine hydrocarbon seep sediments
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Alkanes comprise a substantial fraction of crude oil and refined fuels. As such, they are prevalent at marine hydrocarbon seeps and petroleum-contaminated sites. These environments are typically anoxic, and host diverse microbial communities that can potentially use alkanes as substrates. Anaerobic alkane biodegradation has been reported to occur under nitrate-reducing, sulfate-reducing, and methanogenic conditions in *Betaproteobacteria, Gammaproteobacteria, Deltaproteobacteria* and *Firmicutes* (Widdel, 2010). Most widely distributed mechanism of anaerobic alkane activation is the addition of alkanes across the double bond to fumarate by 1-methyl alkyl succinate synthase (Mas; Heider, 2007).
We studied the sequence diversity of a 460 bp fragment of the gene encoding subunit D by parallel 454-tagged sequencing in 12 marine sediments sampled at 7 hydrocarbon seeps differing in hydrocarbon composition, temperature, water depth, associated chemosynthetic fauna, and geographical position. A comprehensive gene database for MasD and other glycyl radical enzymes was established and used for subsequent phylogenetic analysis.
We obtained a total of 420 MasD $OTUs_{0.96}$, and a surprisingly high MasD richness and evenness, suggesting a more diverse alkane-degrading community than known by now. The MasD diversity was highest in sediments with a broad range of crude oil components, like at Guaymas Basin, known for its unique microbial communities. 75% of MasD

$OTUs_{0.96}$ were present only at a single site. Comparison of the alkane-degrading communities at the different stations by NMDS showed highest similarity of MasD at sites with similar hydrocarbon composition like present at Hydrate Ridge, Tommeliten and Hikurangi Margin methane seeps.
Phylogenetic analysis revealed 4 MasD clades ($OTU_{0.60}$) of which one was closest affiliated with *Deltaproteobacteria,* another one with *Firmicutes.* The remaining two clades were only distantly related to known alkane-degrading strains and showed undetected divergent gene lineages of MasD homologues. These findings suggest the existence of yet unknown groups of alkane degraders.

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MEcP21
Effect of oxidized fat on the gut microbiom of rats
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The prevalence of obesity is increasing dramatically. One parameter that causes obesity is a high fat diet often with deep-fried products which is typical for the western style diet. Studies analysing the gut microbiota of obese and normal weight persons showed different microbial community structures in both groups. A high fat diet is thought to cause a higher prevalence for the development of obesity. Moreover deep fried products contain distinct lipid peroxidation products due to chemical reactions of the oil. The products are known to have a potent biological activity and thus may influence the microbial gut community.
To reduce the individual diet effect of persons we analysed the microbiota of rats fed with a standard diet supplemented with fat. Beside lean (fa/+) rats also obese diabetic (fa/fa) rats were analysed. 36 male obese diabetic rats (fa/fa) were divided into three groups: group 1 received a standard diet and fresh palm fat, group 2 the standard diet and oxidized palm fat, group 3 the standard diet and oxidized palm fat + a 10fold higher dose of vitamin E. The lean (fa/+) sugar rats received the standard diet and fresh palm fat. At the beginning of diet changes and 4 weeks after the respective diet rat feces of each animal was collected and the DNA was extracted out of it. The 16S rRNA gene was amplified using a universal bacterial primer set modified for deep sequencing by Ion Torrent. Phylogenetic diversity, taxonomic relative abundance and the short-chain fatty acids profile was compared between the different diet regimes. The taxonomic abundance of OTUs from the lean (fa/+) rats was clearly separated from the diabetic (fa/fa) rats. The effects of oxidized fat on phylogenetic diversity and abundance of OTUs will be further analysed as well as the short-chain fatty acid profile.

MEcP22
Microbial communities in deep biosphere sediment of the El'gygytgyn Crater Lake
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Introduction: Despite the progress in the field of deep subsurface microbiology, we still know little about the diversity and function of microorganisms in deep biosphere lake sediment. The El'gygytgyn Crater Lake was formed by an impact 3.6 million years ago. Since its formation, the lake was unglaciated and thus led to a continuous accumulation of sediment deposits. These chronological sediments contain information about the climate history, and the evolution of life since the meteorite impact.
Objectives: Molecular ecological investigations of the El'gygytgyn lake sediment enable a unique view into microbial life over the last 3.6 million years.
Methods: A sample series, which includes one sample every 100.000 years of lake history,was analyzed for geochemical and geophysical properties as well as for the microbial composition and abundance.
Results: Moisture contents ranged between 16.4 and 55.0 %, whereas total carbon (0.16 to 1.89 %) and total nitrogen contents (mainly < 0.10 %) were low. The DGGE patterns are showing high differences between the sediment depths. In general the number of DNA bands appeared high, even in sediment layers with low total carbon and nitrogen contents (TOC 0.2-0.4%, TN < 0,1%). It is remarkable that we revealed a high archaeal diversity in a sediment sample of the largest cooling of the mid-Pliocene. Most acquired sequences were related to *Methanobacterium* or *Thermoprotei*, a class of *Crenarchaeota*. Bacterial representatives belong amongst others to *Herminiimonas* and *Janthinobacterium*. Quantitative PCR revealed higher bacterial abundance than archaeal abundance in all samples. Interestingly, an increase of bacterial as well as archaeal copy numbers in very deep sediment layers (nearly 3.59 million years old) was observed. This result is confirmed by higher DNA concentrations in deeper El'gygytgyn Crater Lake sediment, but is in contrast to studies of marine sediment.
Conclusion: Our results show the successful application of our combined methodical approach to obtain insights into the deep biosphere of the El'gygytgyn Crater Lake sediment. Based on this unique sediment material, we were able to present the first microbiological dataset of such an old lake ecosystem. Further steps include next generation sequencing on selected sediment samples.

MEcP23
Molecular characterization of aerobic methane oxidizing bacteria in groundwater treatment
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Methane is a water constituent often neglected in drinking water production from anaerobic groundwater. After oxygenation methane can affect the treatment negatively since it enables the growth of methane oxidizing bacteria (MOB). Biomass and metabolic activities of MOB in rapid sand filters can cause i.e. incomplete manganese and ammonium removal, declined filter hydraulics, formation of slimy biofilms and hygienic problems.
Until now, fluorescence in situ hybridization (FISH) has been applied as a standard method for quantitative analysis of MOB [1]. Major drawbacks of this microscopic enumeration method are the provision of only relative results (percentage of MOB on total cell counts) and the great labor intensity and time consumption. Quantitative real-time PCR (qPCR) has the potential to overcome these problems by delivering absolute results and allowing a higher sample throughput leading to increased precision and reduced workload.
The objectives of the project are the quantitative analysis (qPCR vs. FISH) of MOB in drinking water treatment plants in conjunction with diversity and activity studies. The poster depicts the concept of the DVGW-funded project.
First, a SYBR Green based qPCR assay for the quantification of MOB in drinking water treatment (filter sand, backwash water, biofilms) should be established. Therefore, different PCR primers (A189F, mb661R, MethT1R, MethT2R) targeting *pmoA* and 16S rRNA genes of methanotrophs should be evaluated with regard to their applicability to quantify MOB in this particular habitat. The impact of potential influence factors (DNA extraction method, inhibitory sample components) onto quantification should be investigated and specificity of the assay verified by phylogenetic analysis of amplified gene fragments. The results of the qPCR based quantification should be compared to the results obtained with specific FISH probes. Quantitative and phylogenetic findings should be completed by methane oxidation rates obtained by activity measurements as well as stable isotope probing.
A qPCR based quantification of MOB in drinking water treatment plants will represent a fast and direct method to provide crucial knowledge for the detection and elimination of methane-induced treatment problems.
Bendinger et al., **2002**. Proceedings AWWA-WQTC, Seattle, USA

MEcP24
Establishing new parameters for monitoring anaerobic digestion
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Production of biogas *via* anaerobic digestion is a microbiological multi-step process. In this process organic biomass is degraded to methane and carbon dioxide, the main components of biogas. The very complex microbial community involved can be divided into hydrolyzing, fermenting, syntrophic and methanogenic. Only methanogenic archaea are able to generate methane using H_2+CO_2 (hydrogenotrophic pathway), acetate (aceticlastic pathway) or methylated substances (methylotrophic pathway). Therefore, the methane forming archaea are key players of the biogas process. Biogas production can become unstable as, for example, the accumulation of volatile fatty acids can lead to acidification of the digester, and eventually to failure of the process. Thus, more performance parameters need to be identified for a better control of the biogas process. In this study, we compared the general performance of a commercial

biogas plant in Germany (assessed by its physico-chemical status) with its microbial composition (assessed by DGGE, qPCR and CARD-FISH), with H_2-dependent methane formation, and with the activities of total (viologen-dependent) hydrogenase and methanoarchaeal F_{420}-dependent hydrogenase. Over the course of six months, changes of the methanogenic capacity of the plant correlated with changes of the enzyme activities and - with some delay- with the microbial composition. Thus, activities of key enzyme may be suitable parameters for monitoring anaerobic digestion.

MEcP25
Diversity and function of microbial communities in permeable sublittoral marine surface sediments
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The benthic microbial community in permeable 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]. In contrast, little is known about degradation processes in sublittoral advective sediments [2], and therefore we have initiated a study addressing benthic microbial diversity, abundant bacterial populations and their biogeochemical functions.
Samples were collected in the southern North Sea during spring (2 sites) and late summer (7 sites) 2014. Metagenomic data was constructed for one site to assess the community's genomic potential. The microbial community of oxic surface sediments and bottom water was phylogenetically characterized via Illumina tag sequencing of the V3-V4 region of the 16S rRNA gene and supplemented with CARD-FISH based in situ quantification of selected clades. Based on highest sequence abundances, sediments were dominated by *Gammaproteobacteria* (JTB255, *Pseudoalteromonas*), *Deltaproteobacteria* (Sva0081, *Sandaracinaceae*, Sh765B-TzT-29), *Alphaproteobacteria*, Chloroplasts and *Planctomycetes*. The relative cell abundances, as determined by CARD-FISH, of *Pseudoalteromonas* and *Planctomycetes* were with 5.6% and 17%, respectively, five and two times greater in summer than in spring samples. To assess the metabolic carbon degradation potential, packed bed sediment flow through columns were percolated with sea water or artificial sea water (ASW) amended with dissolved organic carbon (DOC) derived from *Spirulina* sp. or *Thalassiosira pseudonana*. To monitor DOC consumption, DOC and oxygen concentrations were measured in the in- and outflow. The percolation experiment with sea water showed no net consumption of DOC. In contrast, ~40% of the fresh DOC from ASW was consumed. This raises the question why such an uptake is not observed for in situ DOC.
Work on diversity analysis (correlations with metadata, comparative in situ quantification) and metabolic function (identification of DOC, metagenome) will be presented and discussed.

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[2] Huettel, M., *et al*., Ann Rev Mar Sci **6** (2014) 23-51

MEcP26
Targeted cultivation and enrichment of bioactive compound producing Planctomycetes
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Planctomycetes are a group of bacteria widely and ubiquitously distributed in the environment. In aquatic habitats, enabled by their complex life cycle, Planctomycetes form biofilms and attach to the surface of higher organisms such as marine algae, the shells of crustaceans or organic debris, so-called marine snow, where they are major players in remineralization processes. Our previous research showed that marine, as well as limnic Planctomycetes are active producers of novel bioactive compounds with chemical properties yet to be described. Thus, Planctomycetes are a promising source for the discovery of novel small molecules with potential application in human medicine, husbandry or food and plant protection. Planctomycetes were long considered as unculturable bacteria, while only in the last ten years improved cultivation methods have led to the isolation of several novel Planctomycetes from environmental samples, but also from mammal associated communities. A recent study has shown, that Planctomycetes also colonize the gut of humans and other mammals. Given the resistance of Planctomycetes against many antibiotics, members of this bacterial phylum can even act as pathogens in humans.
Here we present novel, targeted cultivation methods, which have led to the successful isolation of a variety of novel Planctomycetes strains from various locations. We sampled, the surface of different marine macroalgae, a limnic cyanobacterial bloom, marine hydrothermal vents as well as marine eukaryotes such as corals and sponges. Employing our novel enrichment procedure we were able to selectively isolate more than 250 strains including 12 novel planctomycetal genera from our environmental samples. We further transferred these methods to explore mammal-associated planctomycetal strains.
Our novel strains represent a huge repertoire for secondary metabolite discovery and extracts of these novel isolates will be screened for biological activity in the future.

MEcP27
Microbial analyses of C-/N-dynamics in the rhizosphere under elevated atmospheric CO_2 concentration
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Prospective elevated atmospheric CO_2 concentration positively affects photosynthetic activity and leads to an increased above-ground plant growth as well as to an enhanced production of root biomass. As a possible consequence higher amounts of carbon are added to soil due to increased litterfall and rhizodeposition, respectively, which may lead to an alteration of soil microbiome and increased microbial activity. Higher microbial activity, which also could be triggered by future elevated air temperatures, in turn could foster soil respiration, resulting in an amplified CO_2 flux from soil into the atmosphere possibly causing a positive feedback on global warming. Enhanced inputs of carbon under elevated CO_2 on the other hand may increase the microbial nitrogen demand inducing an intensified competition between plants and soil microorganisms for available nitrogen. In this study the effects of elevated atmospheric carbon dioxide concentrations (+ 20 %) and higher air temperatures (+ 2 °C) on microbial diversity and activity in the rhizosphere as well as on changes in carbon and nitrogen pools in soils under grassland, grapevine and vegetable cultures will be investigate. Microbial functional and phylogenetic diversity of rhizosphere soil will be investigated by molecular analyses of functional genes involved in microbial carbon and nitrogen metabolisms, and phylogenetic markers, by applying quantitative real-time PCR and next generation sequencing. Additionally, for determining microbial activity and the effect on C- and N-dynamics in rhizosphere soil, we apply a variety of analytical methods for quantification of microbial processes (nitrification, denitrification, N-fixation, methanogenesis, methane oxidation, respiration), microbial biomass, and C/N pools.

MEcP28
Molecular ecological characterization of microbial communities involved in the carbon transformation in permafrost soils in Northeast Siberia
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Introduction: A substantial amount of the global organic carbon is stored in permafrost soils of the northern hemisphere. In terms of rising temperature, huge amounts of the formerly frozen organic matter become bioavailable for the microbial carbon transformation. The end products are relevant greenhouse gases such as carbon dioxide and methane.
Objectives: The aim of this study is to reveal the role of past and recent microbial communities involved in the transformation of soil organic carbon in permafrost environments and to understand the link between substrate composition and the microbial metabolic pathways within the carbon cycle.
Methods: We characterized the microbial community of a terrestrial permafrost core from the Siberian Laptev Sea Shelf region with respect to their abundance, diversity and function using a broad set of biological techniques including gas chromatography, q-PCR and PCR-DGGE.
Results: The highest abundance of the microbial community occurred within the active layer, ranging from $9.5*10^5$ - $1.1*10^9$ copy numbers g^{-1} sediment. The bacterial diversity and methane concentration (155 nM) were highest within the Holocene deposits. Besides, a second zone with an

increased methane concentration (55 nM) and a rising bacterial diversity was observed in deeper Late Pleistocene permafrost deposits. PCR-DGGE exhibited moreover a clear shift in the relative diversity of the bacterial community along depths with a strong correlation to the sedimentary organic matter content.
Conclusion: Our results indicate a transition in the microbial community structure along the permafrost chronosequence, suggesting that microbial communities in permafrost deposits are driven by climate conditions and the bioavailability of soil organic carbon. Further investigations of the prokaryotic diversity via next generation sequencing will help to reconstruct microbial carbon cycling in glacial and interglacial periods.

MEcP29
Abundance and community composition of microbial groups involved in denitrification and anammox in two superimposed limestone aquifers differing in oxygen availability
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Denitrification and anaerobic ammonia oxidation (anammox) result in the formation of gaseous nitrogen compounds and thus play a key role in nitrogen losses from ecosystems. Despite the increasing problem of groundwater pollution with nitrate, microbial groups involved in nitrogen loss have rarely been studied in aquifers. We carried out comparative investigations of denitrifiers and anammox-bacteria in two superimposed limestone aquifers in the Hainich region (Thuringia, Germany) which differed strongly in oxygen availability. Oxygen saturation ranged from 0 - 20% in the upper, suboxic aquifer and from 50 - 80% in the lower, oxic aquifer. Groundwater samples were obtained from eight wells with sampling depths ranging from 12 to 88 m in monthly intervals. Abundances of 16S rRNA genes of anammox-bacteria and genes encoding nitrite reductase (*nirK*, *nirS*) determined by quantitative PCR ranged from 1.5×10^4 L^{-1} to 5.9×10^7 L^{-1} for anammox-16S rRNA and from 2.1×10^3 L^{-1} to 6.4×10^5 L^{-1} for *nirK* with about ten times higher abundances in the upper, suboxic compared to the lower, oxygen-rich aquifer. Maximum abundances of anammox-16S rRNA genes coincided with the co-occurrence of up to 70 µmol L^{-1} nitrate and 20 µmol L^{-1} ammonium under anoxic conditions. The detection of [5]-ladderane FAME at concentrations of up to 2.6 ng L^{-1} further indicated the presence of active anammox bacteria in the groundwater. Our results point to the potential contribution of both denitrification and anammox to nitrate removal in the aquifer system, which will further be evaluated using activity assays.

MEcP30
The temperature gradient forming device – TGFD – a simple apparatus allowing high temperature light microscopy
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Introduction: Light microscopic analyses of hyperthermophilic microorganisms should be performed at their growth temperature, e.g. 100°C. A first "thermomicroscope" [1] used a plexiglass housing, with all operation functions extended to the exterior. One preset temperature could be used with this device after a heating time of ca. 30 min.
Objectives: A simple apparatus should be constructed, which can be added to an existing light microscope; it should allow rapid heating and even establishment of a temperature gradient.
Methods: In cooperation with the electronic workshop of the Faculty for Biology of the University of Regensburg a circuit board was constructed which contains a stainless steel plate into which a rectangular groove is milled. The latter holds a glass capillary (tightly closed on both sides) containing the cells to be analyzed; 5 observation holes allow microscopic detection of cells. Two heating elements allow analyses at up to 110°C; in addition temperature gradients of up to 40°C can be established over 2 cm.
Results: Analyses using the TGFD can be performed at least 5 times faster compared with the original thermomicroscope. The TGFD has heating rates of > 5°C/s; thereby we could show that cells stored for 9 months at 4°C react within a few seconds to high temperature by swimming [2]. The establishment of a temperature gradient allowed for the first time to demonstrate thermotaxis for a prokaryote other than E. coli [2].
Conclusions: We have constructed a simple apparatus which can be added onto any light microscope, converting this into a thermomicroscope.

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2. M. Mora, A. Bellack, M. Ugele, J. Hopf and R. Wirth. Appl. Environ. Microb. **80** (2014) p. 4764-4770

MEcP31
The exometabolome of *Phaeobacter inhibens* DSM 17395 depends on substrate source and growth stage
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Dissolved organic matter (DOM) is a central component of marine element and energy cycles. Pelagic microbes play an important role in consuming marine DOM and thus shaping its composition and the extraordinary chemodiversity of the marine geometabolome. Especially organisms of the *Roseobacter* clade consume major fractions of labile DOM released during phytoplankton blooms but little is known about whether they release DOM compounds as their exometabolome during this process. We hypothesize that exometabolomic signatures are a function of the substrate condition and growth stage of any marine bacterium, including those of the *Roseobacter* clade. To test this hypothesis we analysed the exometabolome of the *Roseobacter* clade model organism, *Phaeobacter inhibens* DSM 17395, and related it to its physiology and metabolic profile by transriptomic analyses. *Phaeobacter inhibens* was grown on 3 (glucose, glutamate, acetate) single substrates in low carbon ASW-medium and DOM, amino acids and carbohydrate were analysed during lag, exponential and stationary phases by ultra high resolution Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) and HPLC. Samples for transcriptomic analyses were collected as well. Distinct patterns in the composition and concentration of released amino acids and carbohydrates reflected a clear effect of substrate and growth stage on the exometabolome. FT-ICR-MS exometabolome analyses detected 328 (acetate), 1230 (glutamate) and 1246 (Glucose) assigned molecular formulas for different substrate conditions and only 101 compounds were shared among the different substrate and growth conditions. The substrate dependent comparison of assigned molecular formulas indicated 134 (acetate), 187 (Glutamate) and 208 (Glucose) substrate unique compounds. Growth phase influence on the exometabolome was also identified with a Bray-Curtis dissimilarity analysis using the molecular formulas detected with the FT-ICR-MS and by transcriptomic patterns. Altogether, the composition of the exometabolome of *P. inhibens* depends on the utilized substrate and growth stage. The high numbers of different molecules released by this prominent bacterium indicates the large influence pelagic microbes have on the chemodiversity of DOM.

MEcP32
Transport of plant-associated bacterial populations in an agricultural soil upon rainfall
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Introduction: High fluxes of mobile organic matters are transported from upper to deeper soil layers after extreme precipitation events, which can mobilize considerable amounts of carbon from plant-associated top soil layers to deeper mineral soils and also to groundwater. Microbes constitute part of the mobile organic matter pool in soils, and it was speculated that translocated microbes from top soil could be an important source of biomass for subsoils [1]. We have previously shown that specific rhizosphere-associated bacterial populations were transported to deeper horizons upon snowmelt in winter [2]. The flux mechanisms and seasonal dynamics of this bacterial transport, however, are still unclear.
Objectives: We now aimed to quantify *Bacteria* mobilized from top soil layers in plant-associated soil upon strong precipitation in summer, and uncover potential differences in transport behaviour.
Methods: We applied at a maize field artificial rain experiments to soil above lysimeters, with fluorescently-labelled *Arthrobacter globiformis* added to top soil. The amount of transported labelled *A. globiformis* in seepage water, sampled at 35 cm depth, was determined by flow cytometry. Ongoing work uses T-RFLP fingerprinting and pyrotag sequencing to compare the transported bacterial communities and track

interesting populations over time-resolved seepage water sampling.
Results: Only < 0.1 % of added labelled *A. globiformis* was retrieved in seepage water. Consistent with our previous findings [2], a specific subset of plant-associated bacterial populations was mobilized, and a more pronounced shift in mobilized bacterial community structure in summer emerged due to fresh plant-derived labile carbon inputs.
Conclusion: The contribution of vertical bacterial transport to carbon flux in soil was quantitatively not prominent, but the translocated bacterial populations can potentially impose great influence on the subsoil communities and microbial activities. These results extend our understanding of the organismic connectivity between soil compartments and the role of *Bacteria* in carbon flux in soil.

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MEcP33
Impact of climate change on the diversity and abundance of culturable bacteria living in the phyllosphere of grasslands
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A high number of bacterial species is inhabiting the aboveground part (phyllosphere) of plants. Global climate changes lead to an increasing atmospheric CO_2 concentration which can directly or indirectly (by increasing plant growth) affect the abundance and community structures of the phyllosphere-inhabiting bacteria and in consequence influence plant-microbe-interactions.
The aim of this study was to gain a first insight into the effects of elevated atmospheric CO_2 (eCO_2) on the composition and diversity of phyllosphere inhabiting bacteria. Two different plant species, *Arrhenatherum elatius* and *Galium mollugo,* were investigated at two time points (spring/summer 2014). Samples were collected at the permanent grassland of the Giessen Free Air Carbon Dioxide Enrichment (GiFACE) system, which is continuously exposed to eCO_2 since more than 15 years. Phyllosphere bacteria collected from plants of control FACE-rings (ambient CO_2, aCO_2) were compared to those of FACE rings exposed to eCO_2 (+20% CO_2). A cultivation-dependent approach was applied for the quantification of culturable bacteria with the focus on two abundant functional groups, heterotrophic and pink pigmented facultative methylotrophic bacteria (PPFM). Abundant bacteria were isolated to investigate the diversity of phyllospheric bacteria using genomic fingerprinting (BOX-PCR) and phylogenetically identified by 16S rRNA gene sequencing. The localization of bacterial cells at the leaf surface was investigated by scanning electron microscopy (SEM). Total cell numbers were additionally determined by SybrGreenI staining.
Significant differences were obtained for the concentrations of culturable heterotrophic and methylotrophic phyllosphere bacteria among some FACE rings exposed to aCO_2 and eCO_2 treatments within a plant species and between the two plant species, respectively. A total of 444 isolates were identified. First data indicate a high diversity of abundant heterotrophic bacteria (including representatives of *Actinobacteria, Bacteroidetes, Firmicutes* and different *Alphaproteobacteria*) and different PPFM depending on the plant species and CO_2 treatments. An effect of eCO_2 on the abundance and diversity of phyllosphere bacteria is obvious but needs to be confirmed by more detailed analyses in ongoing studies.

MEcP34
Analysis of raw milk microbiota by next-generation-sequencing
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Introduction: Raw milk microbiota are complex communities that are influenced by many factors such as housing and feeding conditions of cows as well as the hygienic conditions of milking and the milking equipment. Next generation sequencing techniques are powerful tools for the analysis of such complex microbiota, however, bacterial counts in fresh raw milk are comparatively low and there is a high amount of accompanying eukaryotic DNA originating from somatic cells of the cow. The extraction of sufficient amounts of bacterial DNA is therefore a difficult task.
Objective: The aim of this work was to establish a protocol to extract and enrich bacterial DNA out of raw milk samples and to apply the technique for analysing the change of raw milk microbiota collected over a one week cold storage period at 6°C.
Materials & Methods: The optimized protocol included an initial enrichment of cells by centrifugation and dissolution of casein micelles by EDTA. Somatic cells are lysed by ultrasonification and proteinase K treatment followed by a digestion of released somatic DNA. Following extraction of bacterial DNA, ddPCR and a 2-Step-PCR approach were used to amplify 16S rRNA gene fragments and to generate the sequencing library. Sequencing was performed using an Illumina MiSeq platform.
Results: 96 raw milk samples were analysed and 1.7 million reads of the sequencing run passed quality filtering. The sequences were assigned to 1877 OTUs of which 239 had a higher proportion of 0.5% in at least one sample. The number of detected OTUs per sample varied between 7 and 396 and decreased with increasing storage time. Most fresh milk samples were dominated by gram-positive bacteria, of which *Actinobacteria* and *Clostridiales* made up the main part, but these almost disappeared towards the end of storage. An exception to this were lactic acid bacteria, that increased in some samples to up to 80%. At the end of storage, *Acinetobacter* and *Pseudomonas*, but in some cases also *Serratia* were the dominant genera.
Conclusion: NGS data reveal a tremendous microbial biodiversity in raw milk, however, after prolonged storage at refrigerated temperatures almost no organisms are detected that could not be found by cultivation dependent techniques.

MEcP35
Evaluation of marine phages as markers of hydrological flow and reactive transport
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Introduction: Even though phages represent the most abundant biological entities on earth, research has mainly focused on marine ecosystems neglecting terrestrial habitats. Marine phages and their bacterial hosts are naturally absent in the Earth's critical zone (CZ), and thus can be used as sensitive tracers of hydrological flow and reactive transport of colloidal particles in our PHAGE project; In the frame of the DFG Collaborative Research Center 1076 - AquaDiva. The variability of phages in terms of size, shape and physicochemical properties can be used to elucidate nano-particulate matter transfer in the CZ.
Objectives: presented work intends to physicochemically characterize marine phages and to evaluate them as suitable tracers (i.e. in response to hydrological, geological and biological parameters in defined laboratory experiments).
Materials & Methods: A selection of marine phages differing in properties relevant for transport is characterized by their size, surface charge and surface hydrophobicity (as measured by TEM, dynamic light scattering and water contact angle analysis (CA)). Laboratory percolation columns filled with geo-materials and a modified plaque assay techniques are used to quantify deposition and transport of phages.
Results: Preliminary data show that (i) marine phages exhibit distinct differences in their morphology and physico-chemical surface properties (e.g. Myoviridae PSA-HM1 phage of *Pseudoalteromonas* H7 has a size of 60 nm and CA of 52 ± 1 vs. H3/49 *Myoviridae* phage of *Shewanella baltica OS195 with* 70 nm and CA of 32 ± 3), (ii) phages and particularly marine ones can be sensitively detected by the modified plaque assay up to a sensitivity of 1 pfu/ml (iii) the marine phage (HM1) exhibits lower retention in sand filled columns relative to a terrestrial model phage T4 (*Myoviridae* phage of E.Coli *DSM 613 has a* size of about 80 nm and CA of 72.8 ± 4).
Conclusion: Our preliminary results demonstrate that marine phages have a high potential for the use as highly sensitive tracers also in terrestrial habitats.

MEcP36
A new gnotobiotic mouse model to study colonization resistance against enteropathogens
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Russell W. Schaedler was one of the first to colonize germfree mice with selected murine bacterial strains, known as the "Schaedler Flora" [1]. A few years later, the consortium was modified and termed the "Altered Schaedler Flora" (ASF) [2]. Composed of 8 murine isolates, it´s been widely used as a low complexity microbiota model but the strains are currently not available in public strain collections [3]. Moreover, the ASF has some limitations with regard to a lack of colonization resistance provided against intestinal pathogens [4] and the representation of only few bacterial phyla found in a conventional microbiota [5]. Therefore, we established a novel defined consortium of bacteria, the Oligo-Mouse Microbiota (Oligo-MM), which includes 12 cultivable murine isolates representing 5 major intestinal phyla: *Actinobacteria*, *Bacteroidetes*, *Proteobacteria*, *Firmicutes* and *Verrucomicrobia*. We developed a system using frozen Oligo-MM mixtures to reproducibly colonize germfree mice. In order to characterize this defined consortium, we generated draft genome sequences which were assembled and automatically annotated. To further study gut microbiota dynamics, a strain-specific quantitative multiplex PCR assay was established. We show that the majority of the Oligo-MM strains stably colonize gnotobiotic mice for at least 4 mouse generations and provide colonization resistance against oral infection with the enteric human pathogen *Salmonella enterica* serovar Typhimurium. Fluorescence *in situ* hybridization (FISH) probes for the Oligo-MM strains were designed and validated to quantify and localize single bacteria on intestinal cross-sections. Interestingly, the abundance of some of the Gram positive strains was significantly higher when detected by FISH analysis as compared to high throughput sequencing and qPCR. This result points at a systematic under-estimation of intestinal Gram-positive bacteria by current DNA-based detection methods. In conclusion, we expect that the Oligo-MM model and its analytical tools will be a useful tool for the scientific community in order to address specific aspects of bacteria-host interactions and to understand the role of single species in a complex microbial consortium.

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MEvP01
Lipids in the origin and evolution of cellular systems
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A key step in the evolution of living cells is the controlled separation of the inner contents from the external environment. This is achieved by the structure that is often referred to as the cytoplasmic membrane. Based on our knowledge of the structure all modern cytoplasmic membranes it is possible to detect a common theme, albeit with different solutions. Typically the major components are compounds referred to as lipids and a large variety of proteins that may be either attached to or inserted into the hydrophobic lipid layer. Lipids are a key driving force in the evolution of the cytoplasmic (and other membranes) and the formation of a barrier that essentially consists of a layer with defined physical constraints. While it is easy to demonstrate that lipids may self-assemble modern membranes are highly complex organised structures. Although the origin of cellular life and the evolution of the cell are inseparable from the role of lipids little attention is usually given to this class of compounds. A fundamental aspect of membrane evolution is the dichotomy between the lipid building blocks of the membranes of Bacteria and Archaea that remains to be linked in a satisfactory way to the early evolution of the cell. An appreciation of this fundamental dichotomy also needs to be coupled with an understanding of the consequences.

MEvP02
Parasexual gene transfer from the mycoparasitic fungus *Parasitella parasitica* to its host *Absidia glauca* – Characterization of transferred DNA and sequence analysis of the *P. parasitica* genome
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Introduction: Infection of the mucoralean fungus *Absidia glauca* by the fusion parasite *Parasitella parasitica* is accompanied by mycelial fusion, nuclear transfer and, as a consequence, by transfer of DNA from the parasite to the host. This foreign DNA is able to complement genetic defects of the host [1]. The system is highly efficient and may be used as a tool for manipulation of biotechnically important zygomycetes. It is also important to evaluate the contribution to gene spreading during evolution, provided by this system.
Objective: The intention of this study is to analyze the molecular background of naturally occurring horizontal gene transfer in this fungal system with *P. parasitica* as gene donor and *A. glauca* as recipient.
Methods: Gene transfer was analyzed by PCR, Southern blotting and sequencing following infection on Petri dishes. Genomic sequencing was performed by 454 and Illumina sequencing.
Results: The transfer of DNA from a prototrophic donor to a methionine-deficient recipient, carrying non-revertible rearrangements in the *Met-2* gene for homoserine transacetylase, was studied at the molecular level for the corresponding *Parasitella* wildtype gene. This heterologous gene for homoserine acetyltransferase proved to be able to complement the genetic defect of the host [2].
In order to understand the relevance of *P. parasitica* as gene donor during evolution, we sequenced its genome. Details of this genome [3] and of information presumably involved in the fusion process with the host will be presented.
Conclusions: Transferred chromosomal DNA is essentially propagated in the recipient as autonomously replicating element. The *P. parasitica* genome is much larger than other fungal genomes and contains many repetitive elements.

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MEvP03
Intraclonal genome diversity of the major *Pseudomonas aeruginosa* clones C and PA14
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Introduction: *Pseudomonas aeruginosa* is an important causative agent of nosocomial infections. The two most prevalent clonal complexes clone C and PA14 were analyzed by sequencing the genomes of 58 clone C and 42 clone PA14 isolates from environment, acute infections and chronic airway infections.
Objectives: Investigation of the intraclonal genome diversity of the most common clonal lineages in the *Pseudomonas aeruginosa* population.
Methods: Next Generation Sequencing (NGS), RNA-seq, bioinformatics, fitness experiments
Results: We completely sequenced a phenotypically characterized clone C strain (NN2) (Cramer et al., 2011) by DNA- and RNA-seq which was not done yet. The 6,902,967 bp large NN2 genome encodes 6,601 open reading frames and at least 898 non-coding RNAs.
Intraclonally, the length of syntenic segments with 100% sequence identity of two clone C or two clone PA14 strains was large. Blocks of a median size of 99 kb (clone C) or 163 kb (clone PA14) shared DNA were identified. The majority of strains forms a star-like structure of closely related independent singletons with very few outliers. The ratio of synonymous to non-synonymous substitutions was higher than the expected value of 2.9/9.1 in all strains. Comparing intraclonal sequence diversity against a clonal reference genome, neutral substitutions are more likely to be fixed than amino acid substitutions. This trend of purifying selection against non-synonymous substitutions increases with the total number of SNPs. Intraclonal diversity was not only caused by sequence variation and a variable composition of the accessory genome but also by deletions in the core genome up to 144 kb.

Fitness winners in nutrient rich and mineral media carried additional genes from phages and plasmids as well as for DNA protection and modification and Type VI secretion.
Conclusions: Most sequence variants had emerged in the most recent clades and had not been fixed in the global population. The major clones C and PA14 are endowed with distinct repertoires of functional protein variants. Clone C is more versatile in genetic elements that confer the adaptation to a particular niche.

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MEvP04
Salinity structures bacterial communities at contrasting seasons in the Baltic Sea
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Freshwater and saltwater ecosystems are inhabited by evolutionary distinct bacterial lineages. The Baltic Sea exhibits a long stretched salinity gradient from saltwater (salinity 32-9) to brackish water (salinity 3-9) conditions. The major part of the Baltic Sea resembles brackish water conditions, with a water residence time exceeding 30 years. The bacterial community is strongly structured along the salinity gradient of the Baltic Sea, but also strongly influenced by seasonal changes. In this study we used the Baltic Sea as a model system to examine the impact of salinity and seasonality on the bacterioplankton community composition. Abundances of 16S rRNA gene ampliconsequencing reads were assessed from samples taken on similar geographic locations in summer (June/July) and winter (February/March) along the salinity gradient. We found major differences in the bacterial community composition at the different salinity regimes and seasons. Principle coordinate analysis revealed that salinity explains most of this variability, whereas temperature, as proxy for seasonality, was the second most important factor. By changing the phylogenetic distance (dissimilarity radius) defining a bacterial phylotype gradually from 1% to 25%, the impact of temperature on bacterial community composition decreased drastically at a phylogenetic distance of 16% whereas the influence of salinity remained high until a distance of 24%. This indicates that the shift from saltwater to brackish conditions acts on large 16S rRNA gene phylogenetic distances, reflecting a long evolutionary distance between saltwater and brackish water phylotypes. Moreover, the results show that salinity and season act on different phylogenetic levels and that the brackish bacterial community is inhabited by evolutionary distinct bacterial phylotypes.

MEvP05
Genomic insights into species affiliation and propagation of virulence determinants in *B. cereus* sensu lato
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Introduction: *B. cereus* sensu lato contains eight species that possess a variety of toxin genes. The most prominent members *B. anthracis, B. cereus* and *B. thuringiensis* are differentiated only by the presence/absence of plasmid-bound virulence factors. Their cytotoxicity is attributed to chromosomally encoded enterotoxins CytK, Nhe and HBL, and varies from innocuous to lethal.
Objective: This study aims to i) investigate the prevalence of enterotoxin genes within different phylogenetic groups, ii) characterize the extent of lateral gene transfer, and iii) to determine the phylogenetic history of *B. cereus* virulence determinants on the basis of a reviewed species phylogeny.
Methods: 26 *B. cereus* strains were sequenced and assembled *de novo*. Multilocus sequence analysis, ANI calculation and phylogenetic comparison of virulence genes and entire genomes of *B. cereus* sensu lato were performed.
Results: The distribution of virulence genes was identified in a set of 142 *B. cereus* sensu lato strains. All strains possess the *nhe* genes, 69 % contain *hbl* genes. Out of these, 47 % possess a second truncated *hbl* operon and four strains contain a surplus, less conserved *nhe* operon. A species tree, built from seven concatenated housekeeping genes, perfectly represented *B. cereus* sensu lato phylogeny as predicted from whole genome ANI data. Comparison of the species tree topology with phylogenetic trees of virulence genes revealed that *cytK, hbl* and *plcR* are laterally transferred, while *nhe* transmission is completely clonal.
Conclusion: The presented study confirms that actual *B. cereus* strain relationships are represented best by the seven phylogenetic groups suggested previously [1]. Duplications of *nhe* and *hbl* might be functional and contribute to a strains` cytotoxicity. Therefore, they could be the starting point for the evolution of new pore-forming toxins in *B. cereus*.

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MEvP06
Evolving growth strategy phenotypes in *Bacillus subtilis*
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Bacterial cultures under certain conditions are heterogeneous. Often a metabolically stable phenotype gets selected and dominates the population. There is a constant tradeoff between individual benefiting optimization of growth rate (Rate strategists- RS) and population aiding optimization of growth yield (Yield strategists-YS). Conditions in well mixed environment favor RS as the nutrients are well distributed, whereas spatially arranged environments favor the cells utilizing the resources and nutrients economically that leads to a lower growth rate but higher biomass per substrate utilized, hence YS.
This study aims to select phenotypes with different growth strategies using adaptive laboratory evolution. Periodic serial transfer in liquid medium favors RS phenotype and hence the subsequent generations gives rise to dominant RS strain. We aim to select YS candidates using a novel emulsion-droplet based approach where each clonal population is confined in one droplet and thus minimizes the competition among linages [1]. The selection force in this technique was reported to boost the selection of candidates that might be delayed in growth rate but eventually and importantly manage to have a higher growth yield.
The emulsion-based laboratory adaption system was adjusted for *B. subtilis* and YS clones were found after 50 serial propagations. Inspection of the fitness as well as spatial arrangement of the evolved isolates in mixed and spatially arranged environment will give us better understanding of the metabolic strategies arising in the population. The genotypic differences and metabolic profiling with respect to the ancestor will conclude on the basis for such strategies.

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MEvP07
The *Sulfolobus acidocaldarius* archaellum – characterization of the archaellin-like proteins FlaG and FlaF
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Introduction: 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. The hyperthermophile crenarchaeon *Sulfolobus acidocaldarius* comprises an archaellum gene cluster of seven genes namely *flaBXGFHIJ*, that are all essential for motility [1].
Objectives: Periplasmic archaellum components have yet to be identified. The small, monotopic membrane proteins FlaG and FlaF harbor an archaellin domain, making them good candidates for periplasmic localization. The objective of the project was to confirm periplasmic localization of FlaG/FlaG and further characterization of a potential periplasmic complex.
Methods: Heterologous produced soluble domains of FlaG (sFlaG) and FlaF (sFlaF) 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 interaction. Dot-far western blot visualized interactions with S-layer. Finally, point mutated proteins were used for complementation *in trans* and their effect on motility was checked employing *in vivo* assays.
Results: Purified soluble domains of FlaG and FlaF are highly stable at pH 3, are able to form oligomeric species *in vitro* and interact with a K_D of

10 - 14 µM. Point mutations in the β-sandwich protein sFlaF disrupt the native dimer and result in monomeric sFlaF that does not interact with sFlaG anymore. Furthermore monomeric FlaF leads to non-motile cells. Additionally, it was shown that sFlaF interacts with the S-layer, the major cell wall component of *S. acidocaldarius*.
Conclusions: Given the facts that FlaG and FlaF are not only very stable at pH 3, but also harbor a predicted archaellin domain, a periplasmic localization is highly probable. Moreover, interaction with S-layer indicates that FlaF is involved in the required remodeling of the cell wall, thus allowing the growing filament to penetrate the rigid S-layer. Taken all results together FlaG and FlaF might form a periplasmic complex that tethers the archaellum machinery to the S-layer.
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MEvP08
A novel rhythm in archaellum assembly- analysis of motor ATPase, FlaI and KaiC homologue, FlaH
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Introduction: The archaellum is a functional homologue of the bacterial flagellum, but it shares structural homology with type IV pili. The archaellum is the sole structure responsible for rapid and directed movement in archaea. It is composed of only seven different proteins in *Sulfolobus acidocaldarius* and all the genes present in the archaellum gene cluster are indispensable for the assembly and function of the archaellum.
Objectives: To elucidate the role of the dual functionality of FlaI and functional dissection of the KaiC homologue, FlaH and their involvement in the motor complex.
Methods: We have performed a systematic, in depth analysis of the N-terminal amino acids and their involvement in the switch of FlaI activity in assembly and rotation of the archaellum by *in vivo* motility assay. For comparison of euryarcheal and crenarcheal FlaI we performed *in vitro* ATPase assays to check their activity. Microscale thermophoresis was performed to show interaction of FlaH and FlaI, Moreover*in vitro*auto-phosphorylation assay, MANT-ATP binding assay and western blot was performed to illustrate the role of FlaH.
Results: The hexameric structure of the nucleotide bound FlaI from *Sulfolobus acidocaldarius* revealed a unique inward movement of the flexible N-terminus. The flexible and unstructured region in the first 29 amino acids of the N-terminus holds the key for its dual function in terms of archaellum assembly and function. Furthermore a comparative analysis revealed 100 folds increase in activity of euryarchaeal FlaI in comparison to the crenarchaeal counterpart. Moreover, the KaiC homologue FlaH was shown to auto phosphorylates and bind ATP, but ATP hydrolysis could not be demonstrated.
Conclusion and hypothesis: KaiC homologues are found in all three kingdom of life and it is important for maintaining the circadian rhythm. The result suggest that the KaiC homologue, FlaH might control the activity of the archaellum motor ATPase FlaI in a phosphorylation dependent manner during archaellum assembly and/or function.

MEvP09
Origins of major archaeal clades correspond to gene acquisitions from bacteria
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Genome evolution in prokaryotes entails both tree-like components generated by vertical inheritance and network-like components generated by lateral gene transfer (LGT). The relative contribution of these two processes during the formation of prokaryotic species is the subject of intense debate. While it is clear that LGT within prokaryotic groups such as cyanobacteria, proteobacteria, or halophiles is an important factor for genome evolution, its role, if any, at the origin of such groups still remain as an open issue. To investigate the role of vertical and horizontal evolutionary processes underlying the origin of higher taxa in archaea, we have performed phylogenomic analysis of 134 archaea in the context of their homologues from 1,847 reference bacterial genomes. Our results show origins of archaeal higher taxa unexpectedly correspond to 2,264 group-specific gene acquisitions from bacteria. Interdomain gene transfer is highly asymmetric, transfers from bacteria to archaea are more than 5-fold more frequent than vice versa. These findings uncover a pivotal role for lateral gene transfer in major evolutionary transitions among prokaryotes and implicate bacterial gene acquisitions as key innovations en route to the origin of archaeal higher taxa.

MEvP10
Host specificity in smut fungi – insights from evolutionary comparative genomics
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Insights in the processes how pathogens interact with their hosts allow to understand molecular events involved in the determination of host range, processes underlying host shifts or process by which pathogens specialize. Smut fungi are biotrophic pathogens infecting mainly grasses, including important crop plants like maize, barley, sugar cane and sorghum. They constitute a particularly interesting model to unravel the basis of host specificity, since they have typically a narrow host range and because five annotated genome sequences (*Ustilago hordei*, *U. maydis*, *Sporisorium scitaminuem*, *S. reilianum* f. sp. *zeae* and *S. reilianum* f. sp. *reilianum*) as well as tools for genetic manipulations are available.
We present a computational approach to identify genes showing an evolutionary history that suggests a contribution to virulence and/or host specificity.For our predictions, we hypothesize that three major mutational events underlie the determination of host range: single amino acid substitutions, frame shifts or indels, or alteration of the gene repertoire by gene acquisition or loss. To identify protein coding genes showing signatures of these mutations, we reconstructed families of homologous genes using clustering techniques. From this data set, we called frame shifts using dedicated codon alignment tools. Genes showing single substitutions were scanned for positive selection with non-homogenous models of sequence evolution. Gene gains were inferred by detecting orphan genes. The resulting candidate sets were tuned with respect to the prediction of secretion.
Genes under positive selection were found frequently in the two closely related pathovariants of *S. reilianum*, whereas orphan genes could be mainly identified in the more distantly related species *U. hordei* and *U. maydis*. Deletion of one positively selected gene in *S. reilianum* f. sp. *zeae* led to a strong reduction of virulence on maize. In future experiments, we will investigate if this gene contributes to host specificity by exchanging its homologues between the two pathovariants of *S. reilianum* and assessing the resulting virulence phenotype on maize and sorghum.

MEvP11
Towards elucidation of the genetic basis of host adaptation
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The biotrophic smut fungus *Sporisorium reilianum* causes head smut of the economically important crops maize and sorghum. The fungus exists in two host-adapted varieties that cause disease either on maize or on sorghum. On sorghum, the maize-infecting variety induces a strong plant defense response which halts fungal proliferation. The sorghum-infecting variety can colonize but does not result in disease symptoms on maize. To identify genes contributing to host adaptation, we make use of the fact that *S. reilianum* varieties can mate with each other and produce viable meiotic progeny containing a different genome complement each of their parental strains. To associate parental genomic regions to virulence or avirulence on sorghum and maize, we compare the origin of genomic regions of virulent and non-virulent progeny. To this end we have generated a mapping population of 450 independent progeny of equal mating type that are currently tested for their virulence on sorghum. Selected strains are sequenced to correlate phenotype with genotype and identify genes associated with host adaptation. In a proof-of-principle study, we have sequenced 10 progeny either fully virulent or non-virulent on sorghum. Genome comparison identified one region with 100% linkage that contains a gene cluster with reduced conservation between *S. reilianum* varieties. In addition to increasing the size of the mapping population and to phenotyping of progeny, we currently test the contribution of this region to host adaptation by gene cluster deletion and virulence analysis of *S. reilianum* on sorghum and maize.

MEvP12
Demographic noise and the evolution of cooperation in bacterial populations
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Introduction: A pivotal question in evolutionary biology is the emergence of cooperative traits and their sustainment in the presence of free-riders [1, 2]. Production of a public good causes metabolic costs, which are saved by free-riders. Based on the selection advantage of free-riders, the entire population is destabilized and in danger of collapsing. The dilemma can be overcome by social behavior as demonstrated by various theoretical frameworks [e.g., 3]. We ask the question whether cooperation can arise without sophisticated social behavior, e.g., without recognition and memory. A positive answer is provided by recent theoretical models combining the growth dynamics of a population and its internal evolution [2, 4].
Objectives: We set out to experimentally test conditions for the development of cooperation in a bacterial population. We focused on the impact of demographic noise on population development based on the prediction that stochastic events can ease the dilemma of cooperation [2, 4].
Materials & Methods: We used the soil bacterium *Pseudomonas putida* KT2440 and its production of the iron scavenging siderophore pyoverdine as experimental model. We investigated the development of metapopulations consisting of a producer and a non-producer (free-rider). Groups with a random distribution of initial cell number and producer fraction were formed, grown for given periods of time, merged, and cell number and producer fraction were determined.
Results: Pyoverdine producer strains had a lower fitness than non-producers strains, while higher producer fractions caused faster group growth. Analysis of the merged groups revealed an increase of the producer fraction indicative of the development of cooperation. Extent and duration of cooperation was depend on the initial cell number and producer fraction and environmental conditions.
Conclusion: Deterministically the level of cooperation is declining. However, demographic noise for small population bottlenecks can lead to a transient increase in the level of cooperation as predicted [2, 4].

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MEvP13
Diversification of the host keeps the *Red Queen* running in communities of bacteria and bacteriophages
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Introduction: Previous studies on the co-evolution of bacteria and phages focussed on particular viruses in chemostats as well as on agar plates or in resting liquid cultures. They showed that the bacteriophages could persist over time periods between several 100 to 1000 host generations [1]. A frequently invoked argument to explain this phenomenon is the *Red Queen* hypothesis. Indeed, some studies on *Pseudomonas fluorescens* [2] and *in silico* simulations [3] suggest that spatial and genetic heterogeneity accelerate the co-evolution and population dynamics, allowing the co-existence of several host variants and thus the survival of the virus
Objectives: This work examines the hypothesis that the opportunities for genetic diversification of the host are the key factor driving co-evolutionary dynamics.
Materials / Methods: Population densities and patterns of growth and resistance of discontinuous and continuous carbon-limited cultures of *E. coli* and the viruses λ and T7 with or without biofilm formation were compared with theoretical reflections based on a computer model.
Results: While λ binds LamB of the host and only survived in discontinuous cultures allowing *E. coli* to form a biofilm, T7 uses the versatile lipopolysaccharides (LPS) for adsorption, surviving in every case. The LPS composition changed and lysozyme was inhibited. Furthermore, the bacteria formed mucoid colonies and no biofilm became visible in continuous cultures.
Conclusion: The described observations show that the opportunities for diversification of the host are crucial for the survival of the virus and drive co-evolutionary dynamics.

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MEvP14
Chemical clues in metabolic evolution
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Since Charles Darwin and Ernst Haeckel theories on evolution, the idea of a "tree-like" evolution, where nascent cells retain information from their predecessors, has been explored to address the nature of the metabolism of the first life forms. Unequivocally, some of this heritage is still conserved in modern genomes as the universality of the code, amino acids and chemiosmotic coupling show. But the major problem of this approach is caused by evolution itself. Throughout the million of years that separate us from that moment, massive changes in earth environment and in the availability of biological useful chemical species have occurred. This lead to a myriad of replacements and losses, inventions of new pathways and gene exchanges that make modern life as a whole, a chimeric frame of niches exploration. Evolution has favored diversity at the expenses of weakening the signal from the primordial metabolism remains. To address the metabolism of the first organisms, we are looking for distant connections that might represent fossils of the primordial chemistry that financed the appearance and evolution of life. From this point of view, methylations and the role of methyl groups in biological reactions stand out. Here we present an analysis on the current impact of biological methylation on the overall cell metabolism, with emphasis on energy and carbon related pathways. This represents an additional link supporting the ancestry of methanogenic-like forms of life at the start of biological evolution.

MEvP15
Insight into the evolution of TALEs
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Introduction: Transcription-activator-like-effectors (TALEs) are bacterial proteins that operate as transcription factors in eukaryotic cells. Diverse plant pathogenic *Xanthomonas* species adapted TALEs as tools to manipulate the host plant cell to the benefit of the pathogen. *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) and *Xanthomonas oryzae* pv. *oryzicola* (*Xoc*) are the causal agents of bacterial leaf blight (BLB) and bacterial leaf streak (BLS), respectively, two devastating rice diseases in different areas of the world. Although *Xoo* and *Xoc* strains employ a huge repertoire of *TALE* genes their role in virulence is different and often unknown. Additionally, only little is known about the evolution of TALE repertoires in different *X. oryzae* strains.
Objectives: We analyzed the *TALE* gene repertoire of *X. oryzae* strains from various world-wide locations to gain insight into the evolution of TALE-based pathogenicity. We investigated which promoter regions can be targeted by artificial TALEs to activate target genes.
Materials and Methods: The *TALE* genes of different *X. oryzae* strains were analyzed by southern blot and the activation of potential target genes was analyzed by qRT. The analysis of the TALE-dependent activation of a target gene was done by transient reporter assays, *Xoo*-rice infection studies and the analysis of transcriptional start sites.
Results: Our analysis shows that *X. oryzae* strains originating from different areas differ in their TALE repertoire. Nevertheless, they can employ TALEs that evolutionary converge on the same or related virulence genes. Certain TALEs include single aberrant repeats that provide an evolutionary solution to overcome resistance at allelic promoters. Our analysis of a collection of artificial TALEs that covered various positions in a given promoter shows that there are positional restraints for TALE activity.
Conclusion: *Xoo* strains have acquired different TALE sets but individual TALEs are evolutionary adapted to target an important class of virulence targets. Experimental studies suggest that TALEs activate a target gene from proximal as well as distal positions in the promoter but show that the activation efficiency is position dependent.

MEvP16

Organohalide respiratory gene region of epsilonproteobacteria reveals an exceptional high conservation grade

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Introduction: Organohalide respiration is a form of anaerobic respiration, in which (often harmful) chlorinated organic compounds are reductively dehalogenated. It is found only in four groups of bacteria, Dehalococcoidia, several Firmicutes and a few δ- and ε-proteobacteria. Genome sequencing of *Sulfurospirillum multivorans*, one of only two described organohalide-respiring ε-proteobacteria, revealed a ~50kbp region with genes encoding proteins involved directly or indirectly (i.e. maturation or cofactor biosynthesis) in organohalide respiration (OHR region). The single genes as well as the whole structure of the OHR region are fundamentally different than that of other organohalide-respiring bacteria, raising the question of the source and evolution of this region.
Objectives: Genetic structure and evolution of the organohalide-respiratory gene region in ε-proteobacteria.
Methods: Genome sequencing and comparative genomics of dechlorinating and non-dechlorinating *Sulfurospirillum* spp.
Results: Besides the already sequenced *S. multivorans*, the genome of *S. halorespirans* was sequenced, annotated and compared to that of *S. multivorans*. It has a similar size of about 3.2 Mbp and a nearly identical OHR region (amino acid sequence identity of 99 to 100%). This is remarkable, as the rest of the approximately 3,300 gene products have an identity to the corresponding *S. multivorans* sequences of only about 80 to 95 %. The only gene product in the OHR region which is not conserved is PceA, the PCE reductive dehalogenase and terminal reductase in the organohalide respiratory chain (amino acid sequence identity of about 92%). Clear evidences for the OHR region being a genomic island were not found, though several hints point toward this possibility.
Conclusion: The high conservation grade of the OHR region is surprising and rarely the case even in genomic islands. This is discussed against the background of the evolution of organohalide respiration in ε-proteobacteria, as the transfer of this putative genomic island might have occurred very recently. While the source of the *pceA* gene is unknown, cofactor biosynthesis genes are likely acquired from Fusobacteria.

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MEvP17

The manifold functions of genomic DNA in polyploid species as genetic material, structural material, nutrient, and phosphate storage polymer

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The first and most important function of genomic DNA is to faithfully pass on the genetic information from generation to generation. This is true for all species, irrespective of the ploidy level. All species of haloarchaea that have been investigated have been found to be polyploid. In polyploid species genomic DNA offers additional advantages compared to monoploid species. These include genetic advantages that depend on the presence of homologous recombination, including a low mutation frequency, a high resistance to conditions that induce double strand breaks, and gene conversion. Haloarchaea have been described to survive over geological times in ancient salt deposits or to survive under very extreme conditions, e.g. within desert rocks, under simulated Martian conditions, or during space flights. Polyploidy might well be essential for the ability to survive these conditions. Another advantage that polyploidy offers is a relaxed replication control, and in accordance with that, *Halobacterium salinarum* does not possess a S-phase. Some species export DNA and integrate it into biofilms, for which it is a structural component and important for stability. The concentration of external genomic DNA can be quite substantial in some environments, and it has been shown that *Haloferax volcanii* can use external genomic DNA as a source for carbon, nitrogen, and phosphorous. Very recently it has been shown that internal genomic DNA serves also as a phosphate storage polymer for *Hfx. volcanii*. The cells can divide several times in the total absence of an external phosphate source, and during this condition genomic DNA is degraded to liberate phosphate for other phosphate-containing biomolecules. The ability to grow in the absence of phosphate overrides genetic advantages of polyploidy, which are diminished. However, the re-addition of phosphate to phosphate starved cells lead to a very rapid synthesis of many genome copies, as can be expected for a pool of a *bona fide* storage polymer.

MEvP18

Correlating *B. subtilis* cell fate during starvation to the nutrient upshift response on a cell-by-cell basis

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Isogenic populations frequently adopt a phenotypic diversification strategy to cope with unpredictable changes in their natural environments. *B. subtilis* adapts to changes in nutrient abundance by switching between a vegetative and a spore state. Both sporulation and germination are known to occur heterogeneously on the population level. Here we investigate correlations between the differentiation history of a spore during nutrient down-shift and its outgrowth probability in a subsequent up-shift. To this end, we subjected the cells to sporulation permissive conditions and then simulated an environmental change by applying different nutrient upshift conditions (L-alanine, Valine, CH-medium and LB-medium) and follow the dynamics by single cell timelapse microscopy. We show that the commitment time to sporulation is strongly correlated to the subsequent outgrowth probability of the spore. For L-alanine the responding spores are confined to a subpopulation that had engaged into sporulation in a small window of less than 2 hours from the on-set of sporulation. In contrast, spores emerging from cells that delayed sporulation seem to require a stronger nutrient stimulus to respond. We also investigated the effect of starvation duration on the upshift response: as expected the vegetative subpopulation responds more efficiently to the induction compared to spores when starvation is short. As the starvation was gradually extended to 90 hours, the vegetative population declines and loses its capacity to respond to an upshift. Now spores become the major contributor to the next generation. Overall these results show that cell history can be a major player to generate phenotypic heterogeneity. Moreover, the diversification strategy adopted by *B. subtilis* appears to be more sophisticated than previously thought. Next to the well-known strategy to delay sporulation to cope with unpredictable timing of a future upshift, advancing sporulation could likewise present a bet-hedging element to cope with the variable strength of the upshift.

MEvP19

Neutrophil influx during *Salmonella* Typhimurium colitis induces a population bottleneck and stochastic diversity loss for the pathogen

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Several protective barriers prevent infection of a healthy individual by enteric bacterial pathogens, e.g. the acidity of the stomach, gut luminal competition by the microbiota and the innate and adaptive immune defenses of the host. In many cases it is poorly understood whether these obstacles impose population bottlenecks for the pathogen, which could generate stochasticity in spatio-temporal representation of bacterial subpopulations. Here, we identify and elucidate the mechanisms behind such a population bottleneck in an oral mouse model of *Salmonella* Typhimurium (*S.* Tm) -induced colitis. C57Bl/6 mice were infected orally with mixtures of differentially tagged (WITS, wild-type isogenic tagged strains) but phenotypically identical *S.* Tm strains which can be individually tracked using real-time PCR-based quantification. Systematic dilution experiments of these tagged strains revealed a substantial loss in diversity within the gut luminal *S.* Tm population. This effect was shown to be inflammation-dependent and could be reduced by depletion of Gr-1$^+$ cells (i.e. predominantly neutrophils). Furthermore, we show that a transient, Gr-1$^+$ cell-dependent reduction in the cecal *S.* Tm population size at day 2 post infection is responsible for the observed diversity loss, demonstrating that neutrophil influx constitutes a drastic population bottleneck to the pathogen within the cecal lumen, which only 0.001% of the original population survives.

In conclusion, population dynamics revealed by bottleneck analyses offer valuable insights in host immune defense mechanisms and pathogen within-host evolution, as they highlight Achilles` heels of the infection process, which can be targeted by therapeutic strategies.

MIP01

Diversity of endophytic bacteria associated with root-nodules of *Medicago sativa* L. growing in Al-Ahsaa region, Saudi Arabia

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Introduction: alfalfa represent 64% of the total area devoted to fodder cultivated area in Saudi[1-2]. Rhizobacteria have roles in sustaining agriculture via nitrogen fixation and plant-growth promoting activities [3]. Reports on bacterial endophytes of alfalfa nodules are few.
Objectives: Characterization of endophytic bacteria from root-nodules of Alfalfa and assessment their effects on growth of three economically-important crop legumes.
Materials and Methods: Endophytic bacteria were isolated on yeast-extract mannitol agar, identified using 16S rDNA sequencing and characterized phenotypically using API20E and Ch50 strips, IAA production [4] and solubilize inorganic phosphate [5]. The effects of the strains on *Lens esculentus*, *Phaseolus vulgaris* and *Pisum sativum* were assessed.
Results: Sixty-five strains were obtained and utilized 50% of the different chemical substrates contained in the API20E strip and API50CH. Interestingly, 65% of the strains produced acetoin, which plays an important role in induced systemic resistance. Twenty five strains IAA and solubilize inorganic phosphate. Few strains exhibited antibacterial or antifungal activities. All the strains had positive effects on one or more of the growth parameters (dry weights of roots, shoots and nodules) for tested plants. The strains were identified using 16srDNA sequencing as *Enterobacter cloacae*, *Bacillus megaterium, Bacillus* spp., *Staphylococcus aureus* and *Sinorhizobium meliloti*.
Conclusion: The root-nodules Alfalfa harbored diverse bacteria that are interacting in one way or another to improve plant growth via direct or indirect mechanisms. The strains belonged to plant growth promoting rhizobacteria and could have significant agricultural applications to increase plant productivity and reduce the use of synthetic fertilizers and pesticides.

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MIP02

Co-evolution of *S. thermophilus* and *L. bulgaricus* reveals the genetic and physiological bases behind their collaborations in the yoghurt consortium

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Introduction: The two bacteria *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* co-ferment milk into yoghurt, where they stimulate each other's metabolism and thereby influence important product characteristics such as viscosity and acidity.
Objectives: To identify the exact nature and evolution of several modes of interaction between the two species and to create a genome-based metabolic model of the mixed culture in order to explain or predict these interactions.
Methods: Two strains that have no history together were co-evolved for 1000 generations in repeated batch fermentations in milk. Of the parental cultures, the evolved cultures and several in between, physiological parameters (growth, acidification rate, acid resistance, metabolite production and consumption, viscosity, exopolysaccharide (EPS) production) as well as genetic parameters (full genome sequence, gene expression) were determined.
Results: It was found that the evolved strains had higher growth and acidification rates, were more acid resistant and produced higher amounts of EPS. These effects could be linked with mutations and altered gene expression, notably in pathways identified as playing a role in cross-feeding. Remarkably, the changes in gene expression led to exactly the changes in metabolic fluxes that were predicted as more optimal in the metabolic model.
Conclusion: Evolution of separate members drives the optimization of a microbial consortium and directed co-evolution can be applied to improve starter cultures. Where dairy companies now screen for new cultures with desired properties (e.g. a certain viscosity[sasie1]), directed evolution can be applied to improve these properties for already used starters.

MIP03

Computational analysis of quinolone resistance in clinical isolates of *Salmonella* Typhi from India

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Introduction: Enteric fever is rampant in the Indian subcontinent and is one of the major reasons of morbidity. The treatment for typhoid fever majorly includes the fluoroquinolone group of antibiotics. Excessive and indiscriminate use of these antibiotics has led to development of acquired resistance in the causative organism *Salmonella* Typhi. This resistance in the mutants has been seen to be associated with substitutions in the target gene DNA Gyrase.
Objectives: The analysis of the molecular basis of differential behaviour of protein - drug interaction for fluoroquinolones in drug resistant *Salmonella* Typhi DNA Gyrase mutants with the susceptible protein.
Methods: The Minimum Inhibitory Concentration (MIC) of commonly used fluoroquinolone representatives from three generations, ciprofloxacin, ofloxacin, levofloxacin and moxifloxacin was estimated for clinical isolates of *Salmonella* Typhi from patients in the Indian subcontinent. Consequently these fluoroquinolones were computationally analysed with respect to the quinolone binding pocket (QBP) of wild type as well as mutant DNA Gyrase and assessed by molecular docking for analysis of the differences in their binding behaviour.
Results: The MICs were found to lie between 0.032 to 8 µg/ml. Subsequently the sequencing of the gene encoding DNA Gyrase revealed point mutations at two specific locations comprising Ser83Phe/Tyr and Asp87Tyr/Gly in the quinolone resistance determining region of the target protein DNA Gyrase. The modeled structures of the wild type and mutant proteins were essentially similar with minor variations in the loop regions where the mutations were located. These structures indicated that the mutations in the protein led to local perturbations with alterations in both the chemical environment of the protein and neighbouring geometry of the QBP in terms of size and shape.
Conclusions: This study has revealed that mutations in DNA Gyrase alter the characteristics of the binding pocket resulting in the loss of crucial molecular interactions which as a result decrease the binding affinity of fluoroquinolones with the target protein. The study has provided an insight into the underlying structural and molecular mechanism for the decreased susceptibility of fluoroquinolone in the DNA Gyrase mutants.

MIP04

Mapping of uptake motif in Tin2 effector of *Ustilago maydis*

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The secreted Tin2 effector protein of the corn smut fungus *Ustilago maydis* is taken up by plant cells and stabilizes a maize kinase that transcriptionally induces the anthocyanin pathway. This is likely to deplete p-coumarate that would otherwise serve the lignin biosynthetic pathway and negatively affect fungal spreading and nutrition (Tanaka et al., 2014, eLife). How Tin2 protein is taken up by host plant cells is still unclear. Here we attempt to map the putative uptake motif in Tin2. When we compared the N-terminal region of Tin2 to a 20 amino acid stretch implicated in host uptake of Cmu1, another translocated effector of *U. maydis*, we failed to detect significant homology. However, in both cases a patch of charged residues was found downstream of the signal peptide. When the negatively charged residues were substituted by alanine, the mutant protein was less active. On the other hand, the substitution of positively charged residues did not affect the activity. We are currently assessing whether this results from altered stability or reduced uptake and will discuss our findings in the context of possible uptake mechanisms.

MIP05

The repetitive secreted protein Rsp4 is required for full virulence in *Ustilago maydis*

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Ustilago maydis, the causative agent of corn smut disease, is a biotrophic plant pathogen that relies on living plant tissue to complete its life cycle. This lifestyle requires an efficient suppression of the plant immune system which is accomplished by the secretion of so-called effector proteins. Among those, fifteen proteins with at least three internal sequence repeats have been identified in the secretome of *U. maydis*. They can be divided into two subgroups depending on the presence or absence of a putative Kex2-like cleavage site [1]. Rsp4 (repetitive and secreted protein 4, Um00466) belongs to the subgroup lacking such cleavage sites [1].
We have generated targeted gene deletions of *rsp4* and assayed their virulence in plant infection assays. *rsp4* deletion mutants were still able to penetrate and colonize host plants but displayed attenuated virulence. This was in line with qRT-PCR data revealing an induction of *rsp4* expression especially during late infection stages associated with tumor formation. Furthermore filaments of *rsp4* mutants showed altered hydrophobicity.
As the majority of repetitive proteins in fungi are either integral cell wall proteins or are surface-exposed [2] Rsp4 might influence cell wall functions and could therefore be important for pathogenic development. Future experiments will concentrate on the localization of Rsp4 as well as identification of putative interaction partners.

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MIP06

Conception of On Duty Molecular-Cellular Probiotic Lectins Systems in Human Stabilized Microcenosis Biotope

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Introduction: Probiotic lectins (PL) alone and/or in complexes possess useful properties in net assembling and degradation processes that support human organism [1-3].
Objective: Own data based conception of biotope on duty cofunctioning systems of cell surface and soluble PL.
Conception: 1. Basis for PL participation in on duty protection systems of organism. Molecular properties of PL: PL of human probiotic *Bifidobacterium* and *Lactobacillus* strains are represented as multiple multifunctional forms. Mosaic PL distributed in cell surface multilayers reveal capability to be reversibly further assembled and/or transferred in surroundings as truncated signals (switching and appearance of new PL activities are possible). Cell surface PL forms serve as cell navigators (PL imitate cells). Properties of PL as lectin systems: PL function as on duty (ranging glycoconjugates and/or simple antigens)-recognizing systems possessing impulsive adaptive vector of action. PL as metabolomebiotics: Net fenomena features of PL are due to directed PL assembling and PL cascade siganls involving. PL molecule can be represented as mini cascade reversible net. **2.** For example, on duty PL systems based on probiotic bacteria (*Bifidobacterium* and *Lactobacillus*) reveal multifunctional influence towards relatively pathogenic yeast like fungi (*Candida*) and bacteria (*Staphylococcus*) of biotope microcenosis. On duty leader probiotic strains reveal synergistic actions towards relative pathogenic metabolome net knots (planktonic yeast like fungal growth suppression; yeast like fungal localization and conservation; involvement in yeast like fungal and staphylococcal biofilm lysis).
Conclusions: Conception proposed allows study, monitoring, constructing and prediction of biotope net relationships between probiotic and relatively pathogenic compartments involving expected on duty directed action of molecular-cellular PL systems. Such on duty probiotic systems counteract diseases initiating factors.

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MIP07

Role of motility in *Escherichia coli* biofilm formation

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Introduction: Biofilm formation in *Escherichia coli* involves the transition from motile planktonic cells to sessile biofilm cells. This transition brings along changes in cell morphology and transcription patterns. Here we show that the regulation of motility plays a central role at several stages of *E. coli* biofilm formation.
Objectives: We aim to better understand the role of flagella-regulated motility in *E. coli* biofilms in a time-resolved manner during the formation of biofilms but also spatially in three-dimensional static biofilms both on population- and single-cell level.
Methods: We systematically tested knock-out strains with defects in motility to follow the role of motility throughout biofilm formation. To understand the gene regulation underlying the motility control in biofilms, we have created a library of fluorescent reporters, which are known to play an important role in bacterial motility. Biofilms were grown in microtiter dishes and analyzed by crystal violet staining or widefield and confocal microscopy.
Results: We show that flagella and flagella-regulated motility are required for the formation of *E. coli* biofilms. Motility allows trapping of smooth swimming cells at the surface, thus promoting initial attachment. In addition to this function in attachment, flagella also have a structural role in three-dimensional biofilms along with other components of the biofilm matrix. We show that the levels of second messenger c-di-GMP that negatively regulates motility need to be tightly controlled throughout the transition to the biofilm state, with both low and high levels of c-di-GMP being detrimental for the biofilm formation. We further demonstrate heterogeneous expression of motility genes within *E. coli* biofilms, with the microcolony-like structure that may reflect both stochasticity in the regulation of flagellar genes and the existence of different physiological zones within a biofilm.
Conclusions: We show that flagella and motility play several different roles throughout multiple stages of *E. coli* biofilm formation and have to be stringently regulated by the second messenger c-di-GMP. Furthermore, the expression of motility genes in mature biofilms is highly heterogeneous, likely reflecting the antagonistic regulation by different global regulators of gene expression.

MIP08

Cell-cell communications in *Bacillus subtilis* mixed-species biofilms

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Bacteria are known to form complex multicellular communities that are made of specialized cell types and can be formed on biotic or abiotic surfaces. These communities, known as biofilms, confer high resistance to several adverse environmental conditions. Prevention of biofilm formation can be important from a biotechnological and clinical point of view, but beneficial biofilm formation could be desired during plant root colonization and crop protection. The process of biofilm formation in diverse bacteria has been shown to specifically involve the response to self-generated secreted small molecules, i.e. quorum-sensing, but it can also be initiated by diverse signals produced by other organisms living in the vicinity, thus creating an interspecies communication network [1].
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. We aim to identify organisms producing novel signaling molecules that are able to modify the biofilm development of *B. subtilis*. The chemical nature of the signaling molecules and their signaling pathway is also studied.
Bacteria isolated from soil samples were screened for their ability to produce signaling molecules able to modify the structure of *B. subtilis* biofilms. The culture supernatants of selected bacteria that affect biofilm development were submitted to chromatography, enzymatic and biochemical analysis to discern the nature of the signaling molecules. The isolated bacteria were identified through 16S DNA sequencing.
Five soil isolates were selected for further characterization due to their ability to modify *B. subtilis* biofilms. 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 *Acinetobacter* sp. which is a Gram-

negative organism and thus likely to affect *B. subtilis* biofilm development via a distinct mechanism.

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MIP09

The role of Stp1, a secreted effector of *Ustilago maydis* essential for host colonization

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Secreted protein effectors play crucial roles during successful establishment of pathogens in their host plants. In the corn smut fungus *Ustilago maydis*, one of these essential effectors is *stp1*. *stp1* mutants are non-pathogenic and arrest shortly after penetration. Deletion analysis revealed that the N- and C-terminal domains of Stp1 are essential for function while the large central region is dispensable. In addition, co-expression of separated N- and C-terminal domains of Stp1 could restore pathogenicity of a Δ*stp1* strain. To elucidate the function of Stp1, we have identified 20 putative maize interactors of Stp1 by yeast two-hybrid screening. The activity of Sip3, a secreted maize cysteine protease, can be inhibited by both $Stp1_{\Delta 136\text{-}432}$ lacking the central domain and the C-terminal domain of Stp1, $Stp1_{433\text{-}515}$. However, the biological relevance of this inhibition is still elusive as we could not complement the *stp1* mutant with Pit2, another effector targeting the same cysteine protease (Mueller *et al.*, 2013). Additionally, the activity of Sip19, a serine/threonine-protein kinase isolated as putative cytoplasmic interactor of Stp1 could be inhibited by both $Stp1_{\Delta 136\text{-}432}$ and $Stp1_{433\text{-}515}$. When transiently expressed in maize, Stp1 localized to the nucleus. However, a Stp1 protein fused to a nuclear export signal could complement the *stp1* mutant, suggesting that the nuclear localization is not essential for the function of Stp1. A newly established uptake assay based on transgenic maize expressing cytoplasmic BirA biotin ligase suggests that Stp1 is an apoplastic effector. These latter experiments raise serious doubts on the presumed cytosolic function of Stp1. We are currently attempting to localize Stp1 via immuno-EM and discuss possible reasons why yeast two-hybrid screening may have failed to detect genuine interactors of Stp1.

MIP10

The effector protein Ten1 of *Ustilago maydis*

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To establish a biotrophic interaction with maize, ***U. maydis*** makes use of a large set of secreted effector proteins, many of which are encoded in gene clusters. The deletion of cluster 10A consisting of 10 effector-encoding genes results in strongly reduced virulence after seedling infection (Kämper ***et al***., 2006). By generating subdeletions and by complementing the full deletion of cluster 10A with individual genes we could show that ***um03744*** (termed ***ten1***) had a major contribution to virulence in seedling infections with the respective strains. This was further supported by showing that the single deletion of ***ten1*** yields a virulence phenotype comparable to that of the 10A cluster deletion. We thus consider this effector to play a major role in pathogenicity. By qRT-PCR is was determined that two days after inoculation the expression of ***ten1*** is about 40-fold increased in comparison to axenic culture and that eight days after inoculation the expression is highest with an approximate 100-fold increase. Through a yeast 2-hybrid screen, potential plant interaction partners have been identified. Besides two vesicle-associated-like (VAP-like) proteins, a putative protein phosphatase 2C (PP2C) of ***Z. mays*** could be identified as interactors of Ten1. We are currently confirming interaction through Co-IP after co-expression of Ten1 and the candidate interactors in ***Nicotiana benthamiana*** and are directly testing translocation of Ten1 with a newly established uptake assay system.

Keywords: Effector, plant-pathogen interaction

References: Kämper, J. *et al.* (2006) Insights from the genome of the biotrophic fungal plant pathogen Ustilago maydis. Nature 444, 97-101

MIP11

Virulence determinants of the human pathogenic fungus *Aspergillus fumigatus* are effective against soil amoeba

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The human pathogenic fungus *Aspergillus fumigatus* lives as a ubiquitous saprophyte, but can cause fatal infections in immunocompromised individuals. The evolutionary origin of its multifactorial virulence determinants like phagocytic escape or secretion of mycotoxins is not well understood, but has most likely emerged long before the appearance of innate immunity. We hypothesize that selection pressure imposed by amoeba predation in natural environments could have led to the genesis of traits that later supported virulence in higher organisms.

To test this hypothesis we are studying the interactions of *A. fumigatus* with the professional phagocyte *Dictyostelium discoideum.* For an *in-vivo*-support of our *in vitro* model system we initially screened several environmental sites and could demonstrate that these two microorganisms share the same microhabitats.

When confronted with amoeba, fungal conidia covered by a DHN-melanin layer were ingested but phagocytosis was much more effective with non-melanised conidia of an *A. fumigatus pksP*-mutant. Both wild-type and mutant conidia were capable to survive the phagocytic uptake and within only a few hours initiated intracellular germination, which resulted in loss of amoeboid morphology and subsequent cell lysis. Besides physical interactions, both amoeba and fungus secreted cross inhibitory metabolites, which suppressed fungal germination or induced amoeba aggregation, respectively. Using LC-MS analysis we identified gliotoxin as the major amoebacidal metabolite produced by the fungus.

Our current data supports the idea that two major virulence determinants, escaping from phagocytosis and secretion of secondary metabolites could actually have originated in environmental school of virulence.

MIP12

Genetic complementation of *Campylobacter jejuni* serine protease HtrA confirms its important role in heat tolerance, oxygen stress resistance, host cell adhesion, invasion and transmigration

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Introduction: *Campylobacter jejuni* is a highly important bacterial pathogen involved in foodborne illness. Transmigration across the host intestinal epithelial barrier and cellular invasion are primary reasons for tissue damage triggered by *C. jejuni*, but the involved molecular mechanisms are widely unknown. The serine protease HtrA (high temperature resistant protein A) of *C. jejuni* is important for stress tolerance and physiology, but is also secreted in the extracellular space, where it can cleave host cell proteins such as E-cadherin.

Objectives: Aim of the present study was to develop a genetic complementation system in two *C. jejuni* strains in order to introduce the wild-type *htrA* gene *in trans*, test all known *htrA* phenotypes and perform mutagenesis across the *htrA* gene.

Material & Methods: We complemented the Δ*htrA* mutant with wild-type *htrA* gene by introduction in the *C. jejuni* pseudogene downstream of Cj0208. Growth of *C. jejuni* under stress conditions was tested on MH agar plates. For HtrA secretion assays *C. jejuni* was grown in BHI broth medium followed by fractionation. Casein zymography was done with bacterial lysates, culture supernatants or recombinant HtrA as separated under non-reducing conditions in gels containing casein. To study bacterial transmigration across polarised MKN-28 epithelial cells, infection experiments were done in a transwell filter system (pore size 3.0 µm) and determination of CFU. Tight polarized cell monolayers were confirmed by measuring the transepithelial resistance (TER) and by immunofluorescence against E-cadherin. Cell-attached and intracellular bacteria were determined with conventional gentamycin protection assays.

Results: We confirmed that re-expression of the *htrA* wild-type gene in Δ*htrA* mutants restored the following phenotypes: (i) *C. jejuni* growth at high temperature (44°C), (ii) growth under high oxygen stress conditions, (iii) expression of proteolytically active HtrA multimers, (iv) secretion of HtrA into the supernatant, (v) cell attachment and invasion as well as (vi) transmigration across MKN-28 cells.

Conclusions: These results establish a genetic complementation system in *C. jejuni*, exclude polar effects in the *ΔhtrA* mutants, confirm important *htrA* functions and permit further dissection of HtrA functions *in vitro* and *in vivo*.

MIP13

The invisible organ – the human microbiome

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Introduction: Approximately ninety percent of all cells in the human body are microorganisms. They reside on our skin, in mouth, nose, ears, intestinal tract and genitals. The microbial material living in our gut weighs about 1-2 kg, and there are more bacteria on our skin than human beings on the Earth. Regarding these numbers, it is not surprising that the human microbiome (the entity of all microorganisms living with us) has an enormous power: Most species (out of approximately ten thousands of them) are essential for our survival, health and well-being. Health problems such as inflammatory bowel diseases, obesity, diabetes and even autism and depression have been linked to altered composition and diversity in human-associated microbial community. The human microbiome interacts closely with the environmental microbiome- from our indoor homes or plants, which are an important source of healthy microorganisms - as food and environmental component.
Objectives: The Microbiome Initiative GraZ (MIGrobeZ) is a consortium of research groups from all three local universities: the Technical University Graz (TU), the Karl-Franzens-University Graz (KFU) and the Medical University Graz (MUG) with the major aim to analyze, understand and control the microbiome of the human body, our environment and plants.
Material methods: A powerful assortment of diverse techniques is used by the Microbiome Initiative, including all available omics technologies, bioinformatics and biostatistics.
Results/Conclusion: We will present an overview of the areas of expertise, the types of the different projects and the goals of the Initiative with respect to the human health. By presenting our activities, we are seeking collaborations and partners all over Europe.

MIP14

Structural and functional characterization of the PA14/Flo-type adhesin family in *Saccharomyces cerevisiae*

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Introduction: *Saccharomyces cerevisiae* contains a small family of cell wall-associated adhesins, the flocculins (Flo), which confer cooperative cell-cell interactions by calcium-dependent binding of mannoproteins on neighboring cells and thereby promote aggregation of individual cells into protective flocs, a process known as flocculation. By performing high-resolution structural and functional analysis, we have previously shown that the adhesion (A) domain of one family member, Flo5, consists of a conserved PA14 domain for efficient ligand binding and an additional subdomain, that is not found in other PA14-type proteins.
Objectives: In this study, we aimed to structurally and functionally characterize and compare different PA14/Flo-type adhesin A domains with a special focus on the function of the Flo-specific subdomain.
Methods: Analysis of *in vivo* functions of the Flo1, Flo5, Flo9 and Flo10 A domains was performed by using a standardized *S. cerevisiae* expression system allowing direct functional comparison. *In vitro* functional and structural analysis was performed by production and purification of selected A domains from *E. coli* followed by fluorescence titration spectroscopy and crystal structure analysis.
Results: Our *in vivo* functional analysis reveals that the A domains of the four investigated Flo orthologs confer cell-cell adhesion and flocculation with variable efficiency, and that Flo10A is additionally able to confer adhesion to agar surfaces. We further find that the Flo-specific subdomains of Flo5A and Flo10A are essential for functionality and confer specificity *in vivo*, but are not required for efficient mannose or mannobiose binding *in vitro*. We also solved the crystal structures of Flo10A and of a variant of Flo10A carrying the Flo-specific subdomain of Flo5A in complex with mannoside ligands, which allow rationalizing the functional differences between the orthologous Flo5A and Flo10A domains.
Conclusion: Our data indicate that *S. cerevisiae* has developed a number of related flocculins with diverse PA14/Flo-type domains that enable variable cell-cell interactions, and that the Flo-specific subdomains of different orthologs have evolved to confer functional specificity.

MIP15

Field evaluation of *Bradyrhizobium* and *Pseudomonas* strains to improve growth and nodulation of soybean under under saline soil condition

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Introduction: Salinity is a major production problem for crops as saline conditions are known to suppress plant growth, particularly in arid and semi-arid regions. Co-inoculation of rhizobia with plant growth promoting rhizobacteria (PGPR) plays an important role in both promotion of nodulation and plant growth of soybean under saline conditions.
Objectives: Co-inoculation of soybean with *Bradyrhizobium* and *Pseudomonas* strains can enhance salt tolerance, nodulation, plant growth, pod yield and grain yield under saline soils.
Methods or Materials & Methods: The effects of bacterial inoculation on growth, nodulation, pod yield and grain yield of soybean plant grown under saline soil conditions were studied in field experiments. Plants were grown in saline soil of Sherobod district, Surkhandarya province, Uzbekistan.
Results: The results showed that co-inoculation of with *B. japonicum* NU1 *and Pseudomonas* sp. NUU8 gave more benefits in nodulation, plant growth, pod yield and grain yield of soybean compared to plants inoculated with *B. japonicum* NU1 alone and the uninoculated control. Co-inoculation of *B. japonicum* and *Pseudomonas* sp. can increase the number of root length and the number of nodulation of soybean compared to single Bradyrhizobium inoculation. Under field condition, co-inoculation of *B. japonicum* NU1 with *Pseudomonas* sp. NUU8 strains significantly improved shoot dry weight by 38% and root dry weight by 58% of soybean compared with the uninoculated control. Co-inoculation of with *B. japonicum* NU1 and *Pseudomonas* sp. NUU8 significantly increased the number of seeds per plant by 30% and the number of pods per plant by 31%, by the 100 seeds weight and seed weight per plant by 38-48 % compared to uninoculated control.
Conclusion: The synergistic use of *B. japonicum* NU1 and *Pseudomonas* sp. NUU8 also improved the nodulation, plant growth, pod yield and grain yield under salt-stress. The results suggested that these strains could be used to formulate a biofertilizer for sustainable production of soybean under salt stressed field conditions.

MIP16

Interkingdom Microbial Interactions

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Introduction: With the development of higher eukaryotes microorganisms were forced to co-evolve and to develop an array of specific interaction and sensing mechanisms that enable both individual and microbial communities to recognize and respond to eukaryotic signals with high specificity. We selected two different model systems to identify microbial signals and communication strategies, and to analyse their structure activity relationships. In our 1st example we investigate the bacterial-induced metamorphosis of larvae of a colonial marine hydropolyp (*Hydractinia echinata*). In the 2nd systems we analyse the ecological role of symbiotic gut microbes of fungus growing termites. Here we want to find important bacterial players which help to maintain the fungus garden healthy. In both cases we are also interested in the production of new secondary metabolites, which can be used as antibiotics for human welfare.
Objectives: Aim of our studies is to analyse and characterise interspecies communication signals as well as microbial secondary metabolites.
Methods: We use culture dependent and culture independent methods to investigate the role of secondary metabolites as chemical mediators. Important microbial species were isolated and characterised**.** We also collected important environmental RNA and DNA samples which will be analysed using transcriptomic, metabolomic and proteomic methodologies.

Results: In preliminary studies, we found in our 1st system that biofilm components produced by *Pseudoalteromonas sp.* induce metamorphosis of *H. echinata* larvae. In the 2nd system we are establishing a method to interlink transcriptomic and metabolomic data to analyse the contribution of the gut microbiome to the termite garden homeostasis.
Conclusion: Microbial commensals most likely contribute to the host homeostasis and defence system by secretion specific chemical mediators and antimicrobial compounds.

MIP17
Metabolic activities of strictly and facultatively anaerobic gut bacteria and their interactions in a germ-free cockroach model
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Introduction: The diverse microbial communities in the guts of termites and cockroaches represent a complex metabolic network of individual populations. In view of the special microenvironmental conditions within the gut and possible interactions among the microbiota, it is essential to investigate in how far the metabolic properties of pure cultures reflect their activities in their natural environment.
Objectives: To develop a protocol for the inoculation of germ-free cockroaches with defined cultures of autochthonous gut bacteria and to compare the metabolic profiles of the isolates in pure culture (*in vitro*) with their activities and interactions in the gut environment (*in situ*).
Methods: Pure cultures of gut bacteria were isolated from the cockroach *Shelfordella lateralis* and characterized using standard techniques. After inoculation of germ-free cockroaches, the strains were quantitated and localized *in situ* using qPCR, GFP-fluorescence and fluorescence *in situ* hybridization (FISH). Gut conditions and metabolites were analyzed using microsensors, HPLC and GC.
Results: The strictly anaerobic *Fusobacterium* sp. (strain FuSL) and the facultatively anaerobic enterobacterium (strain EbSL) exclusively colonized the hindgut of germ-free cockroaches. Both strains showed high cell densities in mono-association, but the abundance of strain FuSL was much lower when co-inoculated with strain EbSL. Although the fermentation products of each strain differed strongly *in vitro*, they were quite similar under *in situ* conditions, including the production of molecular hydrogen. Oxygen strongly influenced the metabolic products both under *in vitro* and *in situ* conditions.
Conclusion: The germ-free cockroach model provides first insights into the factors affecting the metabolism of the gut microbiota in their native environment. The observed differences in the fermentation products under *in vitro* and *in situ* conditions are at least partially caused by the constant influx of oxygen into the gut. The availability of oxygen would also explain why the anaerobic strain FuSL is outcompeted by the facultatively anaerobic strain EbSL, which should achieve higher growth yields under the microoxic conditions at the gut wall.

MIP18
Evolution and interactions of co-colonizing *Staphylococcus aureus* and *Pseudomonas aeruginosa* cystic fibrosis isolates
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Introduction: Cystic fibrosis (CF) is the most common inherited lethal disease among Caucasians caused by a defect CF transmembrane conductance regulator leading to a restricted mucociliary clearance within the airways. In consequence, the lung epithelium of CF patients gets colonized by opportunistic bacterial pathogens like *Staphylococcus aureus*, *Pseudomonas aeruginosa* and other species, being able to establish chronic infections.
Objective: In order to shed light on evolution and molecular interactions of *S. aureus* and *P. aeruginosa*, early and late isolates of one CF-patient have been investigated in this study.
Methods: Besides phenotypical characterization, mixed species biofilms of fluorescently tagged isolates, cultivated in artificial flow chambers have been analyzed by confocal laser scanning microscopy. Moreover, molecular interactions of selected co-cultivated *S. aureus* and *P. aeruginosa* strains have been investigated by gel-free semi-quantitative proteomics.
Results: Determination of virulence factors (protease production, motility, hemolysis and biofilm-formation) revealed that late *P. aeruginosa* isolates were less virulent and motile than early ones; in contrast, only minor differences could be observed between early and late *S. aureus* isolates. Proteomic analyses of both co-cultured species indicated that the presence of *P. aeruginosa* leads to an induction of *S. aureus* proteins involved in anaerobiosis and to a decreased expression of proteins involved in ROS detoxification. In *P. aeruginosa* potential virulence factors (e.g. alkaline protease AprA) seem to be induced during co-culture.
Conclusion: Elucidation of molecular interactions during polymicrobial infections in the CF-airway will significantly contribute to a better understanding of niche adaptation and is thus an essential prerequisite for personalized therapies.

MIP19
An *Ustilago maydis* repetitive secreted protein Rsp3 is required for full virulence
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The biotrophic fungus *Ustilago maydis* causes smut disease in maize. Hallmarks of the disease are the formation of tumor and anthocyanin induction. Here, we show that a repetitive secreted protein Rsp3 is required for virulence and anthocyanin accumulation. Rsp3 is highly expressed during the biotrophic stage and can be easily detected in culture supernatants when expressed from a constitutive promoter. Interestingly, *rsp3* alleles obtained from field isolates of *U. maydis* differ in size ranging from 1.8 to 2.5kb. These size differences are caused by reduced or expanded numbers of certain repeats. Introducing the shortest *rsp3* allele from a strain collected in Toluca valley in Mexico into the *U.* maydis *rsp3* mutant, could fully restore virulence, illustrating full functionality. Rsp3 showed highly anomalous migration behavior on SDS-PAGE most likely due to multimerization via a Cys-rich domain. Furthermore, deletion of the Cys-rich domain of Rsp3 prevented tumor induction but anthocyanin induction was still observed. This suggests that Rsp3 could be a dual function effector.

MIP20
Inhibition of competing microbes by bacteriocins is a dominant and highly diverse trait among staphylococci of the human nasal microbiome
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The human nasal microbiota is highly variable and dynamic often enclosing major pathogens such as *Staphylococcus aureus*. Mechanisms allowing certain bacteria to prevail in the nutrient-poor nasal habitat are largely unclear. Occasional reports suggest that some nasal bacteria can produce bacteriocins but it has remained unknown if bacteriocins are major fitness trait and how variable their structures and activities are.
The capacities of 90 nasal staphylococcal strains to inhibit other nasal bacteria were analyzed. Unexpectedly, the vast majority produced inhibitory substances bacteriocins in particular under specific stress conditions. The activity spectra were generally narrow but highly variable with activities against certain Gram-positive, Gram-negative, or both groups of bacteria. A representative staphylococcal bacteriocin was identified as a nukacin-related peptide whose inactivation strongly reduced the producer's capacity to compete with other nasal bacteria. Notably, the bacteriocin gene cluster exhibited signs of extensive previous recombination events.
Specific bacteriocins may become important agents for eradication of notorious endogenous pathogens.

MIP21

Horizontal gene transfer and genetic engineering of linear plasmid pLMA1 in *Micrococcus luteus* Fleming strain

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Micrococci are Gram-positive, G+C-rich, nonmotile, non-spore-forming actinomycetous bacteria. *Micrococcus* strains have proven to play important roles in the biodegradation of xenobiotics, bioremediation processes, production of biotechnologically important enzymes or bioactive compounds, as test strains in biological assays for lysozyme and antibiotics. They can also cause infections in immunocompromised humans.
We recently reported the isolation and characterization of the first linear plasmids in different strains of *Micrococcus* from extreme environments in Argentina [1,2]. Among them, linear plasmid pLMA1 (110 kb) proved to be linked to an erythromycin resistance phenotype. In this report we show that this plasmid can be transferred to the type strain of *M. luteus* (NCTC 2665) in a process most closely resembling conjugation. However, clear differences from the classical conjugative plasmid transfer were observed, indicating the existence of a specialized system. We were further able to perform genetic manipulations on the transferred plasmid, which will allow the functional investigation of the elements involved in the transfer process.
In addition, the transfer ability of the linear plasmid pLMA1, together with the rather efficient method for its manipulation, can establish it as an useful tool for biotechnological purposes in the genus *Micrococcus*
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MIP22

Diazotrophic *Beijerinckiaceae* as symbionts of the conifer *Lepidothamus fonkii* (Phil.) in Patagonian peatlands, Chile - a new nitrogen fixing symbiosis in Gymnosperms

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Biological nitrogen fixation is the dominant process for the provision of plant-available nitrogen in many nutrient-limited peatlands. Evidence is building up that methanotroph associated nitrogen fixation is important in such systems. *Lepidothamnus fonkii* (Phil.) is a small conifer of the family Podocarpaceae that thrives in nutrient-limited Patagonian peatlands and has structures similar to root nodules of plants hosting symbiotic diazotrophs. However, evidence for a symbiotic nitrogen fixation associated with *L. fonkii* and its potential importance for the N-cycle in peatlands is lacking to date. Thus, electron microscopy, acetylene reduction assays, $[^{15}N]_2$-tracer studies, *nifH* (encoding nitrogenases) gene and transcript as well as 16S rRNA sequence analyses were applied. *L. fonkii* roots were densely covered by nodules hosting bacteroid like structures. Nitrogen fixation potentials of roots were significantly greater than those of rhizosphere peat. Given a root biomass of 220 g m^{-2}, annual nitrogen fixation rates of up to 2 g m^{-2} were estimated, exceeding background N-deposition by far. Illumina sequencing of RNA derived 16S rRNA gene amplicons indicated an enrichment of active *Acetobacteraceae, Beijerinckiaceae*, *Bradyrhizobiaceae*, and *Planctomycetaceae* associated with roots compared to rhizosphere peat. *Acidobacteria*-related sequences dominated amplicon libraries of rhizosphere peat. Root rather than rhizosphere peat associated transcripts of nifH almost exclusively affiliated with nifH of Beijerinckiaceae. Diversity estimates of root associated nifH genes and transcripts were significantly smaller than those of the rhizosphere peat. The collective data suggest that a novel mutualistic symbiosis of diazotrophic *Beijerinckiaceae* and *L. fonkii* is essential for nitrogen input in Patagonian peatlands.

MIP23

The influence of MyD88 on the microRNA profile of murine macrophages during *Legionella pneumophila* infection

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Introduction: The gram-negative, facultative intracellular bacterium *Legionella pneumophila* (*L.p.*) naturally resides in water-living amoeba. During *Legionella* pneumonia, contracted by inhalation of bacteria-containing water droplets, alveolar macrophages serve as an atypical host for *L.p.* in the lung. Macrophages primarily sense *L.p.* by Toll-like receptors (TLR), which centrally rely on the adaptor protein MyD88 for downstream signaling. TLR activation stimulates macrophages and affects their gene expression profile.
Objectives: The aim of this study was to investigate the impact of the MyD88 knockout on the microRNA (miRNA) profile of murine macrophages during *L.p.* infection.
Materials and Methods: Bone marrow derived monocytes of wild type and $MyD88^{-/-}$ mice were differentiated into macrophages *in vitro*. Their activation status was determined by CD206 and iNOS FACS analysis and by microscopic morphology assessment. A comparison of infected wild type and knockout cells was performed by gene expression analysis and ELISA. Furthermore, the miRNA profile was investigated by Taqman Low Density Array (TLDA), and single miRNAs were validated by qPCR. Finally, synthetics of identified miRNAs were used to evaluate their regulatory capacities on chosen mRNA targets.
Results: Uninfected $MyD88^{-/-}$ cells showed no differences vs. wild type in terms of activation and morphology. After infection, differential regulation of pro-inflammatory cytokines, e.g. KC, IL-1α and TNFα, was observed in $MyD88^{-/-}$ cells. TLDA analysis and subsequent qPCR validation revealed miR-125a-3p to be regulated in a MyD88 dependent way during *L.p.* infection. Additional treatment with Cytochalasin D, Actinomycin D and the IKK XIII inhibitor illustrated an impact of *L.p.* uptake, gene transcription and signal transduction on this miRNA in response to *Legionella*. Analysis of predicted mRNA targets for miR-125a-3p (NTAN1 and GM9705) by synthetic pre-miRNA administration revealed no functional interaction so far.
Conclusion: While the miRNA profile of bone marrow macrophages is at least in part reproducibly changed upon infection with *Legionella pneumophila*, a functional relevance of miR-125a-3p remains to be established. The MyD88-dependency of this miRNA suggests it´s involvement in pro-inflammatory macrophage activation.

MIP24

Genotypic and functional profiling revealed plant-probiotic functions as a key factor that shape endophytic bacterial community in rice (*Oryza sativa* L.)

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Introduction: Endophytic bacterial strains exert several beneficial effects on host plants such as stimulation of plant growth, N_2-fixation, phosphate-solubilization, siderophore-production, synthesis of phytohormone and induction of plant resistance to pathogens. Rice is one of the most important staple foods for the world's population. However, the ecological role played by bacterial rice endophytes and the factors that contribute for their recruitment is not completely understood.
Objectives: The richness, diversity and dynamics in terms of rice endophytic bacteria and their plant-probiotic functions in two soil-types were tested to understand the aspects that shape endophytic community.
Methods: *Oryza sativa* cvs. TCN1, TCS10, TK8 and TN71 were cultivated in greenhouse using non-sterile acidic and near-neutral paddy soils. Seed-borne endophytes were characterized through PCR-DGGE. Root, stem and leaf tissues were screened for culturable endophytes and their plant-probiotic features. The richness, Shannon-Weiner diversity and evenness in terms of endophytic strains and their plant-probiotic features were estimated.
Results: A total of 52 distinct bacterial endophytes affiliated to 20 discrete genera differentially exhibiting plant-probiotic features were isolated, whose distribution fluctuated with soil-type, tissue-type and cultivars. Class *Bacilli* was prevalent in TCS10, TK8 and TN71, whereas *Gamma-proteobacteria* was dominant in TCN1. High strain diversity did not

positively correlate with high functional diversity. The functionality richness was quite balanced in all cultivars irrespective of the soil-type tested. The cultivars recruited relatively high P-solubilizers and siderophore- producers in acidic and near-neutral soils, respectively.
Conclusion: Both rice genotype and edaphic factors influences recruitment of diverse endophytes. Steady richness in functionality and targeted recruitment of strains indicated plant-probiotic functions as a key factor that shape endophytic community in rice.

MIP25
Tracking endofungal bacterial symbionts: establishing a model in *Stachylidium* sp.
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Introduction: Bacteria-fungal symbioses may form enduring associations mutually beneficial for both symbiotic partners and may have played a crucial role in eukaryotic evolution. Symbiotic biology is an emerging field in biodiversity conservation and biotechnology, especially in the discovery of new secondary metabolites with application in the pharma or agro sectors. The marine-derived fungus *Stachylidium* sp. was isolated from the sponge *Callyspongia* cf. *C. flammea* and led to the isolation of more than 25 new compounds, including new cyclic peptides (Steyn *et al.*, 1983)that were also found to be produced by a bacterial endosymbiont in the fungus (Partida-Martinez, *et al.*, 2007).
Objectives: This study has focused on the cultivation of as-yet uncultured intrahyphal symbiotic bacteria and the search for mutualistic fungal-bacterial symbiotic mechanisms that could reveal their widespread presence in ascomycetes, and to establish a new methodology for the study of endofungal bacteria translated to new cases of endobacterial symbionts in MEDINA fungal collection.
Materials and Methods: Cultivation of fungal strains on solid and liquid media was performed using standard media conditiond. 16S rDNA amplification and sequencing were been applied on fungal metagenome to identify symbiotic bacterial species. Fermentations and extractions for determination of biological activity were performed at different periods to follow the production of the compounds of interest by LC-MS and the potential presence of bacterial symbionts.
Results: Two bacterial species have been identified from the analysis of the *Stachylidium* sp fungal metagenome, a *Sphingomonas* sp. already cultivated in axenic conditions and *Acinetobacter* sp. not yet cultivated in axenic conditions that have been studied for the production of the previously described compounds. Addtionaly we have characterized potentially novel cases of endohyphal mutualistic symbiosis involving production of novel endobacterially synthesized secondary metabolites in the Ascomycota.
Conclusions: This research contributes to the expanding field of symbiotic biology by characterizing hypothesized endohyphal symbiotic mutualistic-type relationships between bacteria and fungi, and has developed methods for chemical/biotechnological exploration of symbiotic functions from widespread bacterial-fungal symbioses.

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MIP26
Differentially expressed miRNAs after *Legionella pneumophila* infection of human macrophages
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Introduction: *Legionella pneumophila* (*L. p.*) is a common cause of severe community-acquired pneumonia. This pathogen replicates primarily within alveolar macrophages and manipulates the host reaction by interfering with intracellular signaling pathways and gene transcription to support its own replication. MicroRNAs (miRNAs) have emerged as critical regulators of mRNAs and are also directly involved in the innate immunity and could therefore have an important function in the regulation of the immune response to *Legionella*.
Objectives: The aim of this work was to identify deregulated miRNAs following infection by means of small RNA sequencing experiments and advanced bioinformatics analysis to elucidate miRNA-associated pathomechanisms.
Methods: Primary blood-derived human macrophages of healthy donors were infected *in vitro* using the wild type strain *L. p.* Corby for 24 and 48 hrs, with a multiplicity of infection (MOI) of 0.25. Total RNA was isolated and miRNA libraries were prepared for Illumina small RNA sequencing.
Results: Our analysis revealed infection-specific and statistically significant changes of miRNA expression in human macrophages, such as up-regulation of miR-146a and miR-155, as well as down-regulation of miR-221 and miR-125b. miRNA deregulation seems to be due to transcriptional regulation of miRNA promoters. Overexpression or knock down experiments of miRNAs were performed for functional characterization and showed an influence of selected miRNAs on bacterial replication.
Conclusion: In summary, the results have deepened our insight in the molecular interaction of *L. pneumophila* and its host cells and might help to establish potential new gene candidates for diagnosis and therapy.

MIP27
Effect of the *Salmonella* virulence master regulator HilD on motility
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Introduction: *Salmonella enterica* serovar Typhimurium are Gram-negative enteropathogens, that utilize flagellar motility for a directed movement towards its target cells and translocation of virulence proteins by a "molecular syringe". Assembly and function of the flagellum is regulated on various levels and flagellar motility can be adjusted in response to environmental cues [1]. To invade its host cells, *Salmonella* injects effectors into the eukaryotic cell that trigger an uptake of the bacteria. The effector translocation is mediated by a *Salmonella* Pathogenicity Island 1 (SPI-1) encoded virulence-associated type-III secretion system (vT3SS) [2]. Expression of the *spi-1* genes is regulated by a battery of DNA-binding proteins, including HilD, HilC, RtsB and HilA. In previous experiments it was shown that the SPI-1 transcriptional activator HilD enhances flagellar gene expression by directly binding to the flagellar master operon, *flhDC* [3].
Objectives: In contrast, we found that overexpression of HilD coincides with a motility decrease and results in a non-motile phenotype. Here, we characterized the HilD-mediated motility decrease in detail.
Methods and Results: In agreement with the activation of flagellar gene expression, flagellar assembly and rotation was not impaired by induction of HilD. We found that adhesion was increased upon HilD-induction and that the motility defect was restored by deletion of *spi-1,* suggesting that a *spi-1* encoded component mediates the motility decrease. In order to identify potential HilD-dependent adhesins we performed random transposon mutagenesis revealing an adhesion system ZirST.
Conclusion: We speculate that upon contact with the host cell a SPI-1 mediated adjustment of bacterial motility might be beneficial to ensure an efficient injection of effector proteins and facilitate *Salmonella* invasion.

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MSP01
Responses of *Corynebacterium glutamicum* to iron limitation
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Introduction: Previous studies revealed that iron homeostasis in the Gram-positive *Corynebacterium glutamicum* is ensured at the transcriptional level by a regulatory network controlled by the master regulator DtxR, which represses a large number of iron uptake systems and other regulators under iron excess [1]. One of the regulators repressed by DtxR is RipA, which itself represses synthesis of a number of prominent iron proteins such as succinate dehydrogenase or aconitase under iron starvation [2].
Objectives: Investigation of the influence of iron starvation on growth, by-product formation, and global gene expression of *C. glutamicum*.
Methods: Cells were cultivated in shake flasks with glucose minimal medium. Glucose consumption and organic acid as well as amino acid formation were analyzed by HPLC. DNA microarrays were used to compare the transcriptomes under conditions of iron excess (36 µM) and iron limitation (1 µM).

Results: Cells cultivated with 36 µM Fe transiently acidified the medium to pH 5.9 ± 0.1 due to the formation of lactate and acetate, which were consumed again later on with a concomitant rise of the pH. In contrast, cells cultivated with 1 µM Fe acidified the medium to pH 4.9 ± 0.1, where it remained constant because the cells became metabolically inactive, as indicated by incomplete glucose consumption. Surprisingly, the pH drop was due to secretion of large amounts of pyruvate. As a consequence of excessive pyruvate formation, also L-alanine was secreted, whereas lactate was only produced in low amounts. Transcriptome analyses in the early exponential growth phase revealed only a mild iron starvation response, but astonishingly many CGP3 prophage genes [3] showed elevated expression levels.
Conclusions: Our results indicate that iron limitation strongly influences central carbon metabolism by an inhibition of pyruvate dehydrogenase activity, leading to pyruvate secretion and acidification of the medium. The mechanism causing this inhibition is not yet known.

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MSP02
Adaptation of the marine microorganism *Dinoroseobacter shibae* to iron limitation

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Since iron is crucial for a wide variety of metabolic processes iron is essential for almost all living organisms. In well oxygenated seawater at natural pH the concentration of dissolved ferric iron is rather low leading to iron-limited conditions. The marine bacterium *Dinoroseobacter shibae* belongs to the *Roseobacter* clade, one of the major lineage isolates of near surface waters in the global ocean. We are interested in the iron regulatory network of *D. shibae* to achieve iron homeostasis. In many bacteria iron-dependent gene regulation is mediated by the global ferric-uptake regulator, Fur. Moreover, in alpha-proteobacteria iron-dependent gene regulation is mediated by the iron responsive regulator, Irr, and the rhizobial iron regulator, RirA. Interestingly, *D. shibae* exhibits not only a gene encoding the global regulator Fur, but it also possesses genes encoding Irr and RirA protein homologs. First analyses of *fur* and *irr* mutant strains showed clear growth deficient phenotypes. Furthermore, addition of hemin does not overcome the growth deficiency of the *fur* mutant strain, indicating the presence of a potential Fur mediated hemin uptake system. To define the Fur and Irr regulons we used the corresponding mutant strains for a transcriptomic and proteomic approach. Over 270 genes were differently expressed and 109 proteins differing in more than 2-fold, comparing normal and iron-limited growth conditions. The *hmuSTUV* operon, encoding a potential hemin transport system, showed significantly lower up regulation under iron-limited conditions in the *fur* mutant strain compared to the wild type, supporting the Fur dependent hemin uptake system. Like terrestrial bacteria, marine bacteria often produce siderophores, which are low molecular weight high affinity iron chelating compounds. Genomic analyses of the *D. shibae* genome as well as the used chrome azurol S assay, revealed no evidence for siderophore production. However, the *fhuA* gene encoding a potential Ton B-dependent siderophore receptor was found up regulated under iron-limited growth conditions. Our results indicate a major impact of iron on the physiology of the marine bacterium *D. shibae*. Further studies will give us important insights in the iron regulation of *D. shibae* and may elucidate the environmental success of the *Roseobacter* clade.

MSP03
Physiological role of methionine sulphoxide reductases in *Corynebacterium glutamicum*

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Microorganisms are exposed to oxidative stress in their natural habitat. Continuous formation of reactive oxygen species (ROS) leads to damage of biomolecules, e.g. protein oxidation. As the oxidation of most amino acids is irreversible, methionine sulphoxide reductases (Msr) catalyze the reduction of mono-oxidized methionine. Hence, Msrs are part of the bacterial protection system against ROS, which was shown for *Mycobacterium tuberculosis* [1]. Oxidation of methionine results in the formation of two epimers, methionine-(S)-sulphoxide and methionine-(R)-sulphoxide, resulting in organisms usually having two different stereospecific Msrs, reducing each epimer [1].
To characterize the physiological role of Msr in the amino acid producer *C. glutamicum*, we constructed the single and double deletion mutants lacking *cg3236* as well as *cg2078*, which were identified as genes encoding MsrA and MsrB, and compared them to the wild-type. Surprisingly, the deletion of one *msr* does not result in an increased sensitivity towards ROS in contrast to *Escherichia coli* [2]. However, ROS treatment, e.g. hydrogen peroxide and hypochlorite, led to a significantly reduced growth of the double knockout mutant on agar plates. Subsequently, we used a shotgun proteomic approach applying relative quantification by stable isotope labelling to compare the mutant with the wild-type on the protein level. After the addition of 0.2 mM hypochlorite to liquid cultures, only the wild-type was able to swiftly recover from this treatment. Using the internet application QuPE for data analysis [3], we detected differences in the protein profile as well as in posttranslational modifications (PTM). For example, (transport) proteins of the iron homeostasis and the cysteine/methionine metabolism were up-regulated in the double deletion mutant after stress induction. This up-regulation may well be indicative for depletion of iron as well as sulphur containing amino acids in mutant cultures. This observation, together with a significant increase of methionine oxidized proteins/peptides, may well be the underlying cause of growth inhibition.

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MSP05
Bacillus subtilis spore germination in high-salinity environments – analysis of mechanisms and spore components involved

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Introduction: Upon starvation *Bacillus subtilis* can form dormant spores. When nutrients become available, spores can germinate, thus turning back into vegetative cells. Germination can be affected by various environmental conditions, such as high salinity, which exerts detrimental effects on germination [1].
Objectives: This research topic aims to identify mechanisms and components involved in *B. subtilis* spore germination in high-salinity environments, which is of high significance for astrobiology, soil ecology, food microbiology and basic research.
Materials & Methods: Spores of different *B. subtilis* strains were germinated in the presence of various salts using different triggers. Germination was monitored by several methods including spectrophotometry, fluorometry, microscopy, and plating.
Results: High salinity exerted detrimental effects on *B. subtilis* spore germination, although some germination was still initiated despite very high salt concentrations [1]. The inhibitory strength of different salts varied considerably [2]. While osmotic stress was an important factor, the extent of salt inhibition seemed to be determined by the salts' ionic composition, concentration and chemical properties [2]. With regard to spore components it could be shown that the lack of the spore coat severely impaired germination in the presence of salt and that neither of the two cortex lytic enzymes was particularly sensitive to NaCl. Inhibition of pressure-induced germination indicated that germinant receptor accessibility is not the major inhibition site of NaCl.
Conclusions: The fluxes of ions, Ca^{2+}-DPA and water early in germination seem to be likely general inhibition sites for all types of salts, whereas NaCl-inhibition at the level of germinant receptor accessibility and cortex hydrolysis seems rather unlikely. The exact inhibition mechanism and inhibitory strength of different salts seems to depend on the respective properties of the involved ions.

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MSP06
Improvement of competence in *Bacillus subtilis* 168
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Bacillus subtilis 168 takes up extracellular DNA in a physiological state, called competence. However, less than 10% of the cell population of a wild type strain becomes competent. To facilitate the genome manipulation for strain development, it is important to obtain a higher frequency of competent cells in the bacterial culture. Development of competence depends on ComK, the major transcriptional regulator of DNA-binding, -uptake and -recombination genes (*comG* operon). ComK is protected by ComS from degradation by inhibiting the formation of a stable ternary ComK/MecA/ClpC complex. Besides, expression of *comK* is regulated by additional factors, such as sporulation regulator (Spo0A), nutrition limitation regulator (CodY), transition state regulator AbrB, degradation regulator DegU, and Rok, the ComK repressor. The *comG* operon encodes a pseudopilus structure for binding and uptake of extracellular DNA.[1] After transformation, the assimilated single strand DNA is protected by various single strand binding proteins such as DprA.[2] We constructed a new strain, REG19, which is based on P_{mtlA} controlled expression of *comK* and *comS.* And for this strain we developed a new and more efficient transformation method which is performed in LB. With it, we are able to improve the level of plasmid transformation by 20-fold compared to *B. subtilis* 168 using the established technique in Spizizen's minimal medium.[3] Further, we are independent of various original components which are essential for the competence like the two-component system ComP/ComA and Spo0A. Additionally, with a deletion of the competence inducing factor, DegU, the competence level reached a high value of 6.2 x 10^5 colony forming units per µg plasmid [5.5 kB] DNA [cfu/µg] compared to REG19 of 2.4 x 10^5 cfu/µg.

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MSP07
Economic proteomics mass spectral data usage and its application to staphylococcal proteomics
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Integrated proteomics research on pathogenic microorganisms as *Staphylococcus aureus* has been driven mainly by instrumental and technical advancements the last years [1]. With ever increasing sensitivity and speed of the mass spectrometric instrumentation together with fractional sample preparation it was possible to get access to large fractions of the proteome, mainly by targeting subcellular locations including the cytosol, the membrane, the cell surface and the extracellular space. Currently we are able to get access to about 80% of the proteome by mass spectrometry based proteomics for both identification and quantification [2] by mostly relying on shot- gun proteomics in combination with database searching. Here, we have to reiterate the identification process for each sample repetitively with inherent negative consequences for retrievability of low abundant protein/ peptides in large scale studies. This calls for smarter acquisition of mass spectral data and/ or for sustainable usage of previously acquired mass spectral data which will help to increase the completeness of proteomic data sets as well as the overall sensitivity of proteomics studies on data processing level. In our study we have pursued two main tracks to achieve a more sophisticated way of mass spectrometry analyses: i) we have compiled comprehensive spectral libraries of already published and of newly acquired mass spectrometry based proteomics data for both QqTof and Hybrid Orbitrap based instruments ii) have implemented a workflow for data independent acquisition (DIA) of proteomics data in staphylococcal research. This resulted in acceleration and improvement of our classical shotgun identification based workflows allowing for more complete proteomic datasets that can be used for functional studies.

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MSP08
Osmotic control of the *yqiHIK* operon by the two component DegS-DegU system of *Bacillus subtilis*
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In its main natural habitat, the upper layers of soil, the gram positive bacterium *B. subtilis* has to cope with fluctuations in the external osmolarity. These alternating conditions trigger changes in the gene expression pattern of *B. subtilis* on a global scale (Steil L. *et al*.; 2003; J. Bacteriol. 185:6358-6370) and the *yqiHIK* operon is among those 120 genes, whose expression is enhanced by hyper osmotic stress. It encodes a putative lipoprotein of unknown function (YqiH), an N-acetylmuramoyl-L-alanine amidase (YqiI) and a glycerophosphodiester phosphodiesterase (YqiK).
A previous study uncovered an unusual expression pattern of the *yqiHIK* gene cluster: in cultures growing in minimal medium with increasing salinities *yqiHIK* transcription remained at a basal level until the medium osmolarity reached a concentration of 0.7M NaCl. Above this threshold transcription increased linearly with the increase of NaCl concentration (Fischer KE, Bremer E. 2012; J. Bacteriol. 194:5197-5208).
Here we describe the involvement of the two component system (TCS) DegS-DegU in the osmotic regulation of the *yqiHIK* operon. DegS is one out of two cytoplasmic localized sensor kinase in *B. subtilis* and the cognate response regulator DegU can act as a transcriptional regulator both in its unphosphorylated (DegU) as well as in the phosphorylated form (DegU~P).
We found that the activity of the *yqiHIK* promoter is dependent on DegU~P and on a binding site that is located at a considerable distance from the promoter region. Deletion analysis and mutational studies narrowed the putative binding site for DegU~P to an AT-rich octamer, located160 bp upstream of the σ^A dependent *yqiHIK* promoter. The dependency of the *yqiHIK* promoter on DegU~P is overrun by mutations of the σ^A promoter that increase promoter strength. These promoter mutants remained salt inducible even in the absence of the DegS-DegU TCS.

MSP09
Abiotic stress protection by ecologically abundant DMSP and its natural and synthetic derivatives – insights from *Bacillus subtilis*
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Dimethylsulfoniopropionate (DMSP) is an abundant osmolyte and anti-stress compound produced primarily in marine ecosystems by many groups of marine microorganisms. After its release into the environment, microorganisms can exploit DMSP as a source of sulfur and carbon, or accumulate it as an osmoprotectant. However, import systems for this ecophysiologically important compatible solute and its stress-protective properties for microorganisms that do not produce it are insufficiently understood. Here we address these questions using a well-characterized set of *B. subtilis* mutants to chemically profile the influence of DMSP import on stress resistance, the osmostress-adaptive proline pool and on osmotically controlled gene expression. We included in this study the naturally occurring selenium analogue of DMSP, DMSeP, as well as a set of synthetic DMSP derivatives. We found that DMSP is not a nutrient for *B. subtilis* but it serves as an excellent stress protectant against challenges conferred by sustained high salinity or lasting extremes in both low and high growth temperatures. DMSeP and synthetic DMSP derivatives retain part of these stress protective attributes, but DMSP is clearly the more effective stress protectant. We identified the promiscuous and widely distributed ABC transporter OpuC as a high-affinity uptake system not only for DMSP but also for its natural and synthetic derivatives.

MSP10

Prognostic Evaluation of Antimycotic Status of Human Urban Urogenital *Candida* Multispecies Biotope

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Introduction: The status of antibiotic reactivity of population biotope microcenosis is important to evaluate system risk of infection(s) and plan therapy.
Objective: Development of prognostic approach to evaluation of antimycotic resistance and sensitivity of urogenital *Candida* multispecies microcenosis using functional ranging within antimycotic panel.
Materials & Methods: About 150 urogenital clinical strains of *C.albicans (*I), *C.tropicalis* (II), *C.krusei* (III), *C.glabrata* (IV) from the local clinic diagnostic center were analysed. Standard disc-antibiotic diffusion method of microbial growth inhibition was used. Amphotericin B (Am), Flukonazol (Fl), Itrakonazol (It), Ketokonazol (Ke), Klotrimazol (Kl), Nystatin (Ny) were used**.** Antimycotics effectiveness against I-IV, group-1 (I, II) and group-2 (III, IV) was evaluated as antimycotic codes (I.II.III.IV, I.II or III.IV: as corresponded antimycotic effectiveness ranges in 6 members rows of sensitivity or resistance) and scores (sum of corresponding code ingredients of interest) were calculated and compared.
Results: 1. Biotope resistance scores depending on resistant strains relative contents: both groups: Ke > Fl, Kl, Am > Ny > It; group 1: Ke > Kl, Am > Fl > Ny > It; group 2: Ke > It > Fl > Ny, Kl, Am. **2.** Biotope sensitivity scores: group 1+group 2: Kl, Am > Ke > It > Fl > Ny; group 1**:** Kl > Am > Ke > It > Fl > Ny; group 2: It > Am > Kl, Ke > Fl > Ny. **3.** Characteristic of biotope: Preferential Ke-resistant *Candida* strains (mostly contributed by group 1). Both groups reveal adaptive different ecological niches of antimycotics resistance distribution. Alternatives to biotope treatments with Ke, Kl and/or Ny can be predictably recommended. **4.** Results **1** and **2** support each other and provide additional information.
Conclusions: Results indicate characteristic and prognostic usefulness of system approach for any biotope microcenosis and any antribiotic panel size. Algorithms of code formulas and scores are convenient. Approach is of significance for therapeutic tactics and strategy of choice antimmicrobial panels against urogenital *Candida* multispecies populations.

MSP11

Role of the sRNA RSs2461 in the defense against oxidative stresses in *Rhodobacter sphaeroides*

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Introduction: Singlet oxygen is generated by bacteriochlorophylls when light and oxygen are simultaneously present in *Rhodobacter sphaeroides*. By using the 454 pyrosequencing and Northern blots, a small RNA RSs2461 was identified under singlet oxygen stress. The small RNA has a size of 116nt and is located at the 3'UTR of RSP_0847, for a protein of the OmpR family of response regulators[1]. It was demonstrated by RT-PCR that RSs2461 is cotranscribed with *ompR* from a $RpoH_I/RpoH_{II}$-dependent promoter[2].
Objectives: This research is trying to figure out the target genes regulated by RSs2461 and the function of RSs2461 in the defense against oxidative stresses in *R. sphaeroides*.
Methods or Materials & Methods: Microarray, qRT-PCR, Northern Blot, *In Vivo* Reporting System.
Results: The RSs2461 level is increased under various stress conditions including singlet oxygen, organic peroxide, heat, diamide, SDS+EDTA, and $CdCl_2$. Deletion of RSs2461 leads to a slower growth and higher sensitive to organic peroxide compared to wild type. Microarray analysis revealed that, the gene RSP_6037 and two other small RNAs RSs0680(CcsR1-4) which is cotranscribed with RSP_6037 and RSs1543(SorY) are up-regulated when overexpressing RSs2461. Using an *in vivo* reporter system, we observed overexpressing RSs2461 increases the promoter activity of RSP_6037 and SorY.
Conclusion: It was previously shown that RSP_6037 and CcsR1-4 affect the oxidative stress response of *R. sphaeroides* by regulating the C1 metabolism and affecting the glutathione level(Billenkamp et al., subimited). For SorY, overexpressing strain leads to a higher resistant to organic peroxide compared to wild type. From the results, RSs2461 appears to be involved in defense against oxidative stress by increasing transcription level of CcsR1-4 and SorY. It remains elucidated by which mechanisms RSs2461 affects the promoter activity of RSP_6037 and SorY.

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MSP12

Osmotolerance of the industrially important strain *Gluconobacter oxydans* 621H

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Gluconobacter oxydans 621-H is gram negative, strictly aerobic bacterium, living in sugar rich habitats and belonging to the family *Acetobacteraceae*. It is an industrially important bacterium owing to its regio and enantio-selective incomplete oxidation of various sugars, alcohols and polyols[1]. The complete genome sequence is available but it is still unknown how the organism adapts to highly osmotic sugar rich environment. Therefore, we investigated the mechanisms of osmoprotection in *G. oxydans*. Our main focus was to unreveal and enhance the adaptive strategy for osmotic resistance to optimize the processes of product formation in high concentrated sugar solutions. One common way for osmotic stress response is the intracellular synthesis and accumulation of compatible solutes. To identify such a potential osmoprotective compound, we grew *G.oxydans* on yeast glucose medium (50mM) as carbon and energy source in the presence of 100mM potassium phosphate (pH 7.0) for pH control along with various concentrations of sucrose and analysed the intracellular metabolites by HPLC and ^{13}C NMR spectroscopy. Both of these analytical techniques highlighted the accumulation of mannitol as a potent osmoprotectant[2] inside the stressed cells. This intracellular mannitol accumulation correlated with increased extracellular osmolarity of the medium. For further confirmation, we analyzed the growth behaviour of *G. oxydans* in the presence of small amount of mannitol (2.5 mM and 5 mM) in the YG medium with 300 mM sucrose. As expected, in the presence of extracellular mannitol, the growth rate of the sucrose-stressed cultures was almost identical to control cultures. Thus, mannitol alleviates the osmotic stress of sucrose on cellular growth. Now our objective is to target mannitol producing metabolic pathways and their respective genetic elements to generate *G. oxydans* strains that are more tolerant to high sugar or polyol concentrations.

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MSP13

Base Excision Repair in *Corynebacterium glutamicum*

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Corynebacterium glutamicum plays a principal role in the progress of amino acid fermentation industry due to its unique ability to produce significant amounts of L-amino acids directly from cheap sugar and ammonia. Nevertheless, our understanding of molecular biology and physiology of *C. glutamicum*, such as DNA repair, remained poor compared to other bacteria. DNA repair is a mechanism of great importance in the maintenance of the genomic stability against different sources of potential damage in any organism. In fact, any inefficiency of this system can promote genomic instability which leads to cell death.
Among these mechanisms, Base Excision Repair (BER) is considered the most frequently used DNA repair pathway *in vivo*.[1] BER is initiated by DNA glycosylases that recognize nucleotide lesions and excise the damaged DNA bases by cleavage of the N-glycosylic bond between the deoxyribose and the damaged base.[2] The genome of *C. glutamicum* contains various genes for DNA glycosylases (*ung, fpg/mutM, tagI, alkA, nei/mutY*) and one gene encoding exonuclease Xth.[3] To improve our understanding of this DNA repair mechanism, mutants with deletions of one or more genes in BER pathway were created. Afterwards, the survival capability of those mutants was determined after treatment with different mutagens in comparison to that of the original strain *C. glutamicum* ATCC13032. Alkylation, caused by MNNG (N-methyl-N'-nitro-N-nitrosoguanidine) or Mitomycin C, reduced the survival rate of the Δ*tagI* mutant (- 3-Methyl-adenin-glycosylase) by 50%. Likewise, the endonucleases Nth and Nei turned out to be essential for the repair of

alkylated bases. As the deletion of the Uracil-DNA-glycosylase *ung* has a positive effect on the growth of *C. glutamicum*, the integration of uracil into the DNA, seems to be less dangerous for the cell than repairing the damage. So far, the BER in *C. glutamicum* seems to be very similar to that in *E. coli*.

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MSP14

A single amino acid substitution in the ProB and ProJ γ-glutamyl kinases of *Bacillus subtilis* alters their allosteric properties and affects proline accumulation

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B. subtilis possesses interlinked pathways for the synthesis of proline. The anabolic ProB-A-I route provides proline for protein biosynthesis, whereas the ProJ-A-H route is responsible for the high-level production of proline as an osmoprotectant. The first step of both pathways is catalyzed by the γ-glutamyl kinases (GKs) ProB and ProJ [1]. The transcription of *proB* is controlled in response to intracellular proline levels via a T-box regulatory device, whereas the transcription of *proJ* is up-regulated in response to increases in the external osmolarity. ProB is feedback regulated by proline but such a post-transcriptional regulation is unlikely to control the enzyme activity of ProJ. The enzyme-inhibitor-interaction is modulated by a flexible 16-residue loop in the active center of the GK [2]. We carried out bioinformatic analysis of anabolic and osmoadaptive *Bacillus* GKs and found a striking difference at position 153 in the flexible loop of the enzyme. All ProB type enzymes possess a negatively charged amino acid at this position whereas all ProJ type enzymes possess a positively charged amino acid. We therefore created *B. subtilis* mutants with a substitution of the negatively charged amino acid Glu of the anabolic ProB enzyme against a positively charged amino acid Arg by site directed mutagenesis. Vice versa, we replaced Arg in the osmoadaptive ProJ enzyme by Glu. The amino acid substitution in ProB caused a diminished sensitivity to proline feedback inhibition and resulted in an increased proline accumulation *in vivo*. In contrast, the amino acid substitution in ProJ caused an increased feedback inhibition and led to a decreased proline accumulation *in vivo*. Our findings contribute to a better understanding of the physiological role and post-transcriptional regulation of the two paralogous GKs in *B. subtilis* and provide evidence that the ProJ enzyme [1] has atypical allosteric properties among prokaryotic GKs making it similar to the corresponding plant enzyme [2].

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2 Fujita *et al*., J. Biol. Chem. 278 (2003)

MSP15

Bacilliredoxin-fused redox probes to monitor the bacillithiol redox potential in *Staphylococcus aureus*

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Introduction: Bacillithiol (Cys-GlcN-Malate; BSH) is the glutathione-surrogate and thiol-redox buffer in *Bacillus* and *Staphyloccoccus*. Under oxidative stress, BSH forms mixed disulfides with proteins, termed as S-bacillithiolation which functions as thiol-protection and redox-control mechanism [1, 2]. In *B. subtilis*, the glutaredoxin-like enzymes YphP (BrxA) and YqiW (BrxB) were characterized as bacilliredoxins in the reduction of BSH-mixed protein disulfides [3]. In eukaryotes, glutaredoxin-fused roGFP2 biosensors have been applied for dynamic live-imaging of the glutathione redox potential [4,5]. However, no redox biosensor is available yet to measure the BSH redox potential.
Objectives: Here we have constructed a genetically encoded bacilliredoxin-fused roGFP2 biosensor for dynamic live-imaging of the BSH redox potential in *Staphylococcus aureus*.
Methods: The bacilliredoxin (Brx) homolog 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* cells expressing Brx-roGFP2 fusions were used for live-imaging of the BSH redox potential in vivo using microplate reader measurements and microscopy.
Results: Dynamic live-imaging shows rapid and dynamic responses of Brx-roGFP2 redox biosensors in *S. aureus* COL and USA300 by oxidative stress and selective antibiotics. The in vitro measurements revealed specific oxidation of the biosensor by BSSB but not by GSSG.
Conclusion: The 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 Firmicutes bacteria.

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MSP16

Protein S-mycothiolation in response to oxidative stress in *Mycobacterium smegmatis*

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Introduction: Actinomycetes utilize the thiol-redox buffer mycothiol (AcCys-GlcN-Ins, MSH) for protection against reactive oxygen species (ROS) [1]. We have recently identified 25 *S*-mycothiolated proteins as mixed MSH protein disulfides in response to oxidative stress in *Corynebacterium glutamicum* [2]. Protein *S*-mycothiolation was shown to inhibit the activity of the thiol peroxidase Tpx *in vitro* which could be re-activated by mycoredoxin1.
Objectives: Here, we investigated the level of protein S-mycothiolation in *Mycobacterium smegmatis* under oxidative stress conditions using different thiol-redox proteomics methods.
Methods: We applied fluorescent-label and MS-based thiol-redox proteomics methods (shotgun-LC-MS/MS and OxICAT) for quantification of *S*-mycothiolated proteins in *M. smegmatis* under NaOCl stress. Voronoi Treemaps were used for visualization of the redox changes for mycothiolated proteins.
Results: Mycothiol-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. Mass spectrometry identified 67 S-mycothiolated proteins in the wild-type under NaOCl stress that are involved in energy and fatty acid metabolism, glycerol metabolism, MSH biosynthesis, protein translation, redox regulation and antioxidant functions. The increased level of oxidation could be quantified for many of those mycothiolated proteins using fluorescent-label thiol-redox proteomics and OxICAT.
Conclusions: We demonstrate that protein S-mycothiolation is an important redox modification in *M. smegmatis* under oxidative stress which targets conserved Cys residues of abundant and essential proteins. The regulation of protein functions by S-mycothiolation in Mycobacteria is subject to our current biochemical and physiological studies.

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MSP17

Interdependence between different layers of the cell envelope stress response in *Bacillus subtilis*

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Introduction: The cell envelope is of vital importance for any bacterial cell and serves as a prime target for antimicrobial compounds. Consequently, in *B. subtilis* the response towards cell envelope stress involves several lines of defense: i) the expression of specific transport- and detoxification modules that directly remove or neutralize antimicrobials from their site of action. ii) more unspecific mechanisms stabilize and protect the cell envelope as soon as damage is detected.
Objectives: Here we study the interdependence between these two lines of defense and scrutinize their correlations at the single cell level. To this end we focus on the effect of the ABC transporter BceAB and the UPP

phosphatase BcrC, which are the major resistance determinants against bacitracin, on the expression of the *lia* operon of *B. subtilis*, which protects the cell envelope by a so far unknown mechanism.
Methods: We measured the effect of deletion as well as overexpression of *bceAB*, *bcrC* and *liaIH* on i) susceptibility against bacitracin and ii) their respective regulation. MIC assays were performed with E-Tests; luxABCDE and GFP were used as reporter systems for P_{bceA}, P_{bcrC} as well as P_{liaI}-activity (measured with microtiter plate reader or flow cytometer).
Results: Besides an increased sensitivity of the Lia system towards bacitracin in *bceAB* and *bcrC* mutants, we found that noise levels in the expression of the Lia system differed markedly from that of a wild type population. Specifically, the *bceAB* mutant displayed an almost switch-like response with increasing inducer concentration and had lower noise levels than the wild type. This suggests that stochastic expression of primary resistance determinants might be the origin of the broadly heterogeneous induction of the secondary resistance conferred by the Lia system [1]. In contrast, a deletion of *bcrC* caused bimodal expression patterns.
Conclusion: These observations suggest an intricate connection between primary and secondary resistance determinants and might give hints towards the underlying regulatory hierarchy within the cell envelope stress response of *B. subtilis*.

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MSP18
Global analysis of *Clostridium difficile* stress response to environmental conditions and infection
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The bacterium *Clostridium difficile* is currently a major cause of high morbidity and mortality in Germany with several hundreds if not thousand patients dying per year. *C. difficile* associated diarrhea (CDAD) has caught increasing attention as one of the most deadly, hospital-acquired diseases. Almost nothing is known about the gene regulatory, protein and metabolic networks involved in the host associated life cycle of *C. difficile*. Our work aims to determine the gene regulatory network in response to general and specific stress factors as displayed during the infection cycle. In *Bacillus subtilis* the general stress response is mediated by the alternative sigma factor B (σ^B). However, the role of σ^B in *C. difficile* remains still unclear. To get a deeper insight into the stress response of *C. difficile* we analyzed σ^B, PerR and Fur mutants. We therefore applied a systems biology approach using different omics technologies and infection studies. The changes of interconnected networks in response to different environmental conditions (i.e. heat shock, oxygen exposure and iron limitation) will provide a detailed molecular insight into the *C. difficile* infection process. This constitutes a solid basis for the development of novel prevention, diagnosis and therapeutic strategies.

MSP19
Cannibalism and its connection to the cell envelope stress response network of *Bacillus subtilis*
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Introduction: In the environment, microorganisms constantly compete for nutrients. However, under severe nutrient limiting conditions, *Bacillus subtilis* initiates a strategy called sporulation orchestrated by Spo0A. Sporulation leads to dormant endospores that allow *B. subtilis* to survive long periods of starvation. But since this process is very energy-demanding, *B. subtilis* has evolved another survival strategy, called cannibalism, to delay sporulation. In this state, *B. subtilis* secretes SKF and SDP toxins that lyse sensitive siblings (Spo0A-inactive). As a result, nutrients released from the dead cells slow down Spo0A-active cells from entering sporulation. Importantly, both cannibalism and sporulation are heterogeneous traits, due to cell-to-cell variations in the levels of active Spo0A during the early stages of sporulation.
Objectives: The goal of this study was to elucidate the connection of cannibalism and the cell envelope stress response system of *B. subtilis*.
Methods: We fused a *lux* cassette to target promoters of the cell envelope stress response system and measured luminescence as output for promoter activity over time. Additionally, we performed time-lapse microscopy with promoter-*gfp* and -*mKate2* fusions to follow induction on single cell level.
Results: We recently observed a regulatory link between cell envelope stress-sensing TCS and cannibalism toxin production. The peptide antibiotic-sensing Bce system was found to be intrinsically activated in late stationary phase by SDP and SKF. The Bce system consists of an ABC-transporter, BceAB, a histidine kinase, BceS, and its response regulator, BceR positively regulating *bceAB* transcription upon peptide antibiotic stress signals.
Conclusion: Currently, we are studying the pattern of Bce induction and toxin production at single cell level to gain further insight into the physiological relevance of this process.

MSP20
An essential switch - Dynamic changes of DnaK function as regulator and chaperone
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Introduction: DnaK chaperone homologs are well known for their key roles in protein folding and regulation of the heat shock response (HSR), which is particularly important in thermal stress conditions. In *Caulobacter crescentus* DnaK is required for viability even at normal and low temperatures. Depletion results in a block of DNA replication initiation as well as a growth arrest. Our recent work revealed a mechanism by which DnaK affects DNA replication. However, it remains unknown how this chaperone ensures growth under varying conditions.
Objectives: Here we want to analyze the requirement of DnaK for *C. crescentus* physiology at low (22 °C), normal (30 °C) and high (40 °C) temperatures and how its interaction with the heat shock sigma factor RpoH determines growth and viability.
Results: We hypothesized that loss of DnaK might induce a protein homeostasis collapse even at low and normal temperatures and hence a growth arrest. However, aggregation assays revealed that loss of DnaK does not cause severe protein aggregation in these conditions. We found that at 22 °C deletion of *rpoH* can rescue the viability of DnaK-depleted cells, suggesting that at low temperatures DnaK's main function is the inhibition of the HSR rather than the prevention of protein aggregation. DnaK is also essential to prevent the growth-inhibiting activity of RpoH at 30 °C, however, in the absence of the sigma factor its chaperone activity is required. Heat shock uncouples this functional interdependence and both proteins become essential. Using time-lapse microscopy we found that at 30 °C fluorescently tagged DnaK localizes to two to four dynamic foci and a dispersed fraction. Temperature upshift leads to relocalization to five to eight foci. We hypothesize that this increase in focal localization represents DnaK associated with aggregates. Interestingly, the observed localization pattern contrasts with the known polar localization of DnaK foci in *E. coli*.
Conclusions: Our data show that in *C. crescentus* DnaK's essential tasks switch in a temperature-dependent manner between a mainly regulatory function as an RpoH inhibitor at lower temperatures and a mainly chaperoning function at elevated temperatures. We are currently investigating how the localization pattern correlates with DnaK's dual functions in protein folding and RpoH inhibition.

MSP21
Response of paddy soil microorganisms to salt stress – a meta-transcriptomic approach
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Introduction: The accumulation of salts, particularly sodium salts, is one of the consequences of desiccation and the main physiological threat to ecosystems. However, little is known about how soil microbial communities respond to salt stress at the transcriptome level.
Objective: To understand how paddy soil microbial communities respond and adapt to salt stress.
Materials and methods: Paddy soil slurries amended with rice straw and pre-incubated for 7 days were treated with 0, 300 and 600 mM NaCl over 48 hours. Each treatment condition was analyzed in triplicate. Illumina RNA-seq of total RNA and enriched mRNA was used for metatranscriptomic analysis. Small-subunit (SSU) rRNA and putative mRNA reads were extracted by SortMeRNA and further analyzed using QIIME, USEARCH and MEGAN.

Results: Sequences derived from rRNA dominated the metatranscriptomic libraries of total RNA (95.2-98.7% of all reads). Bacterial rRNA was most dominant (83.5% of total rRNA reads), while archaeal and eukaryal rRNA reads accounted for only 21.4% and 0.3%, respectively. *Clostridiaceae* were the most abundant bacterial group at the family level. Stress had only a minor effect on their relative contribution to the community SSU rRNA pool (mean ± s.d.): 19.7±0.7% (0 mM NaCl), 19.5±0.8% (300 mM NaCl), and 22.3±1.7% (600 mM NaCl). However, the relative abundance of *Clostridiaceae* mRNA transcripts significantly increased from 12.2±2.0% (0 mM NaCl) up to 23.9±4.1% (600 mM NaCl). For the archaeal metatranscriptome, *Methanosarcinaceae* contributed most to the community SSU rRNA pool (12.6±0.6 to 17.5±0.7%). The relative abundance of putative mRNA transcripts associated with *Methanosarcinaceae* increased from 25.4±3.8 (0 mM NaCl) up to 37.2±0.1% (600 mM NaCl). *Methanocellaceae* were the most abundant among the hydrogenotrophic methanogens. However, the relative abundance of their SSU rRNA (from 0.9±0.1 to 0.7±0.0%) and putative mRNA (from 4.9±0.9 to 0.9±0.2%) strongly declined in response to increasing salt stress.
Conclusions: *Clostridiaceae* and *Methanosarcinaceae* were most dominant and showed the most competitive response. By contrast, *Methanocellaceae* were not able to compete under salt stress.

MSP22
The impact of a putative TA-System on the regulation of aconitase biosynthesis in the phytopathogenic bacterium *Xanthomonas campestris* pv. *vesicatoria*
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The plant pathogen *Xanthomonas campestris* pv. *vesicatoria (Xcv)* is an obligately aerobic, oxidase-negative γ-proteobacterium that causes bacterial spot disease on pepper and tomato plants. It grows in the intercellular space between plant cells where it must overcome iron- and oxygen-limitation, as well as cope with reactive oxygen species (ROS), which potentially form during the host defence response. Aconitases (Acn) are FeS-containing enzymes that might be important in sensing the cellular Fe and ROS status. *Xcv* has three aconitases and the genes encoding two of these, AcnA and AcnB, are divergently oriented in the genome of *Xcv*. AcnB has been shown recently to be important for growth of *Xcv* in pepper plants, perhaps suggesting that citrate is an important carbon substrate in the plant apoplast. The *acnB* gene is co-transcribed with two small genes termed *roaX* and *roaY* (regulators of aconitases). RoaX and RoaY show similarity to members of the toxin-antitoxin (TA) family of regulators, whereby RoaX is a putative DNA-binding protein and the RoaY toxin is a predicted ribonuclease. Mutants lacking RoaXY show strong up-regulation of AcnB synthesis. Re-introduction of either *roaX* alone or both the *roaXY* genes results in severe repression of *acnB* transcript levels. In contrast, re-introduction of only *roaY* results in increased transcript levels of *acnB* suggesting that the stability of the transcripts might be enhanced. An imbalance in the copy number of the *roaXY* genes over and above that of genomic levels has a severe impact on growth of *Xcv in vitro* and *in planta*. Notably, however, despite poor growth of the complemented D*roaXY* mutant *in vitro*, growth *in planta* was almost comparable with growth of the wild type. This result suggests that increased gene-dosage of *roaXY in planta* can be tolerated better than *in vitro*. These findings might reflect differences in carbon substrate availability between the two growth regimes.

MSP23
The dynamics of the protein inventory of *S. aureus* in response to an infection related stimulus
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Introduction: *Staphylococcus aureus* causes a broad range of infections ranging from mild skin infections to severe and life threatening infections like endocarditis or sepsis. It is producing a variety of toxins and the treatment is becoming increasingly difficult because of the multiple antibiotic resistant strains.
Objective: Using current proteomics techniques it is possible to identify and quantify almost complete protein inventories of microbial cells. In this study, we used a multi-OMICs approach to analyze the response of the important nosocomial pathogen *S. aureus* to prolonged anaerobic conditions.
Methods: Oxygen starvation is a typical situation in many infection related settings. The data-independent acquisition approach IMS^E in combination with the Hi3 approach was applied to calculate absolute protein concentrations for cytosolic proteins. Furthermore, extra- and intracellular metabolites were analyzed using mass spectrometry and ^{1}H-NMR.
Results: We could provide absolute quantitative data for about 1,300 cytosolic proteins, which corresponds to 90 % of the expressed cytosolic proteins. 78 intra- and 25 extracellular metabolites were quantified for a comprehensive analysis of the response to prolonged anaerobic conditions.
Conclusion: Using the combination of proteome and metabolome data the reorganization of the cellular life during anaerobic growth can be almost completely followed.

MSP24
Small RNAs in the photosynthetic gene cluster of Rhodobacter-sphaeroides
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The facultative phototrophic model bacterium Rhodobacter sphaeroides is known for its metabolic versatility and possesses different ways to generate energy for its growth. Under low oxygen conditions it forms intracytoplasmatic membranes harboring the photosynthetic complexes (PC) needed for anoxygenic photosynthesis. For fast adaption to varying environmental conditions the transcription of genes in the photosynthetic gene clusters is tightly regulated. There are two different gene clusters, the puf and the puc operon, both containing essential genes responsible for the development of the two light harvesting complexes (LHCI and LHCII) and the reaction center (RC). RNAseq and Northern blot analysis of transcripts derived from the puf operon (coding for the LHCI and the RC) revealed that besides normal mRNAs also certain small RNAs (sRNAs) were transcribed. Up to date two different puf operon associated sRNAs were identified. One abundant putative sRNA, RSspufX, was detected downstream of the pufX gene with transcription in the same direction as the puf genes. The putative sRNA was also enriched in an Hfq-immuno precipitation, indicating interaction to this RNA chaperone. Another, less abundant sRNA was detected antisense to the 5´region of the pufL gene (RSaspufL), extending into the pufA-pufL intercistronic region and possibly even further. RNAseq data also indicated that the abundance of the two puf-related sRNAs was strongly dependent on growth conditions. Additionally, in case of RSaspufL growth condition dependant processing was observed. Analysis of the possible function of both sRNAs indicated a clear function in pigment composition control in R. sphaeroides cells. An artificial increase in the amount of these sRNAs by plasmid driven over expression led to a reduced pigmentation and LHC amount in the cells. Taken together, for PC development different signals have to be integrated (e.g. redox state in the cell, light availability, oxygen tension). In addition to the already characterized sRNA PcrZ (photosynthesis control RNA Z; Mank et al., 2012) also other sRNAs seem to play a role in the fine tuning of the photosystem development.

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MSP25

Water structuring induced by Ectoine as a possible explanation for its protection against radiation

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Compatible solutes ectoine and hydroxyectoine are known to be effective protectants of biomolecules and whole cells against heating, freezing, high salinity and radiation damage [1]. It is believed that some of these properties result from water-structuring effects and the influence on hydrogen bonds within water-clusters and biomolecules. Although the beneficial properties of ectoine are already exploited in commercial applications [2] the underlying mechanisms contributing to protection against radiation are still not well understood. We propose an explanation for radiation protection properties of ectoine based on our findings from Raman-spectroscopic measurements. We found out that the vibrational density of states in liquid water changes along with the concentration of ectoine and hydroxyectoine. The data indicate a linear increase in collective behaviour of hydrogen bonds (higher water structuring) with increasing compatible solute concentration. The increased water structuring leads to lower probability for water ionisation and to higher scattering of low energy electrons, which are considered to be one of the major damage species [3, 4].

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MSP26

A spatiotemporal analysis of SOS and prophage dynamics in *Corynebacterium glutamicum* populations

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Introduction: Integrated virus DNA (prophages) represents a common element of bacterial genomes. An important but often unnoted phenomenon is the spontaneous activation of these prophages in single cells of bacterial populations, even in the absence of an external trigger. The largest prophage of *Corynebacterium glutamicum* ATCC 13032, CGP3, comprises 6 % (~190 kb) of the whole genome and was shown to be induced spontaneously in a small subpopulation (
Objectives: The key objective of this study is the quantitative spatiotemporal analysis of CGP3 induction in order to assess the trigger and the physiological consequence of spontaneous prophage induction (SPI) in *C. glutamicum* populations.
Methods: To this end, promoters of phage- and SOS genes were fused to genes encoding autofluorescent proteins (*venus* and *e2-crimson*) to correlate the activation of the response to DNA damage and the spontaneous activation of CGP3 at the single-cell level. Single cell analysis was performed using flow cytometry as well as time-lapse fluorescence microscopy which enables spatiotemporal analysis of cells grown in microfluidic chip devices.
Results: Our studies indicated that the spontaneous induction of the SOS response, which is likely due to spontaneous DNA damage, significantly correlates with the spontaneous induction of the CGP3 prophage [1]. Live cell imaging studies revealed that CGP3 activation is a consequence of SOS induction in about 8 % of SOS^+ cells and exclusively leads to a stop of growth and likely cell death. Furthermore, we could show that external addition of iron triggers CGP3 activation in an SOS-dependent manner. This is in agreement with the finding that SPI is significantly increased in a mutant lacking the iron regulator *dtxR* [2].
Conclusion: Our studies shed new light on the dynamics of bacterial stress responses and its impact on SPI as a general microbiological phenomenon occurring in lysogenic cultures of bacteria [3].

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MSP27

The phage shock protein response system in *Escherichia coli* is essential for bacterial growth under stress conditions

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Many proteobacteria possess a so-called Psp-system that is upregulated under manifold stress conditions that are believed to harm the cytoplasmic membrane. The Psp components are encoded by the *pspABCDE* operon and the monocistronic *pspG* gene. The expression of these genes depends on sigma 54 that is regulated by the PspF component that is divergently encoded upstream of the *pspABCDE* operon. PspF in turn is regulated by PspA, the first product of the *pspABCDE* operon. It is believed that PspA is not only a key regulator but also the active membrane-stabilizing component of the system. However, others reported that the PspB and PspC components are the membrane-active ones, at least in *Yersinia enterocolitica*. In agreement with this, PspA deficient strains of *E. coli* show only very moderate phenotypes that may relate to the dysregulated expression of the whole PspF-regulon. Here we report the analysis of effects of deletions of *pspF* and other *psp* genes on growth and *pspA* promoter activity. Our data reveal that PspA cannot be the key membrane-acting component of the Psp system, albeit the Psp system indeed is essential for bacterial growth under stress conditions.

MSP28

Signal transduction and antibiotic stress response in *Streptomyces venezuelae*

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Introduction: The bacteria belonging to the genus *Streptomyces* show a complex life cycle and produce a range of secondary metabolites making them the most important producers of clinically relevant antibiotics. Most *Streptomyces* species live in the soil, where they share their habitat with bacteria of the phylum Firmicutes, i.e. low G+C Gram-positive bacteria. While Firmicutes bacteria have long been associated with the production of antimicrobial peptides, it has only recently become clear that some Streptomycetes are also able to synthetize such compounds. Despite the resulting likely exposure of *Streptomyces* to peptide antibiotics, both self-produced and derived from competitors, resistance against this class of antibiotics has not been studied in these organisms to date.
Objectives: Therefore, we aimed to discover and characterise determinants of resistance involved in antibiotic resistance in *S. venezuelae*.
Methods: We used random chemical mutagenesis followed by screening for bacitracin sensitive strains to identify the molecular basis of resistance in *S. venezuelae*. Identified mutants will be sequenced to identify the genetic changes leading to sensitivity. In a parallel approach, we are performing transcriptome analyses to study the stress-response of *S. venezuleae* to bacitracin.
Results: Our results show that *Streptomyces venezuelae* is highly resistant to bacitracin. Six bacitracin-sensitive mutants were found in our screening methods. One mutant was also sensitive to the antibiotic penicillin G. Indeed, five bacitracin-sensitive mutants were sensitive to an antibiotic producer strain.
Conclusion: *S. venezuelae* is easy to grow in the laboratory and also is amenable to genetic manipulation, making it an ideal model to study the resistance mechanisms against peptide antibiotics in Actinobacteria.

MSP29

Sucrose causes rapid glucose uptake inhibition and *ptsG*-repression in *Corynebacterium glutamicum* Δ*scrB*

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Corynebacterium glutamicum is a Gram-positive bacterium, used for the industrial production of amino acids and nucleotides. This organism co-utilizes most carbon sources, such as glucose and sucrose. Uptake and phosphorylation of these two sugars are mediated by the phosphotransferase-system (PTS), which has a glucose and sucrose-specific EII permease, encoded by *ptsG* and *ptsS,* respectively.
Deletion of *pgi*, encoding the enzyme phosphoglucoisomerase, blocks the first step of glycolysis and directs the glucose-driven carbon flux towards the pentose phosphate pathway. *C. glutamicum* Δ*pgi* grows poorly with glucose as a sole substrate as the glucose uptake is reduced to avoid glucose-6-phosphate accumulation (1). Growth of the mutant with sucrose

as a sole carbon source is not affected. However, glucose addition to sucrose-cultivated *C. glutamicum* Δ*pgi* cells immediately arrested their growth. In detail, we have shown that in this mutant addition of glucose causes an EII^{Glc}-dependent inhibition of the sucrose uptake within 15 sec and this process is followed by reduced *ptsS*-expression.
On the other hand, deletion of *scrB,* encoding the enzyme sucrose-6-phosphate hydrolase, blocks the first step of sucrose utilization and leads to poor growth of the strain with sucrose, whereas growth with glucose is not affected (2). Here we show that addition of sucrose leads to concentration correlated growth rate reduction of glucose-cultivated *C. glutamicum* Δ*scrB* cells. Furthermore, analogical to *C. glutamicum* Δ*pgi*, in the Δ*scrB*-mutant addition of sucrose caused inhibition of the glucose uptake within 15 sec and led to reduced of *ptsG*-expression. The negative effects caused by the presence of sucrose were abolished in the strain *C. glutamicum* Δ*scrB* Δ*ptsS*. Thus, we show that the glucose specific PTS permease could be both initiator and target of rapid inhibition and conclude that the described in *C. glutamicum* Δ*pgi* rapid EII-dependent response to glucose-6-P accumulation is one manifestation of a more general phosphosugar stress response mechanism in this organism.

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MSP30
Investigations of the Immunonom of *Staphylococcus aureus* under different host relevant growth conditions
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Introduction: The adaptive immune response is considered to be important for limiting *S. aureus* infection in the human host. Human antibodies recognize a large panel of *S. aureus* proteins expressed during infections. We have studied the immunogenic capacity of extracellular and membrane-associated *S. aureus* proteins expressed under three different growth conditions using sera of 22 healthy humans [1].
Objectives: The adaption of *S. aureus* COL *Δspa* to three host relevant conditions (42°C, iron limitation, anaerobiosis) was studied and the corresponding binding of human antibodies characterized by automated 1D Western blots (Simon Simple Western Assays).
Materials & Methods: Preparation of extracellular, membrane and surface-associated protein fractions of *S. aureus* COL *Δspa* were used to quantify total serum IgG binding with Simon Simple Western Assays.
Results: When compared to extracellular proteins, membrane and surface-associated proteins are generally characterized by a lower capacity to bind IgG from human sera. Under all tested conditions extracts of extracellular proteins exhibited comparable IgG binding capacities, albeit anaerobiosis induces a slight decrease in the immunogenicity of extracellular *S. aureus* proteins. Notably, human serum IgG bound to bacterial proteins of different molecular weight in the proteomes elaborated under different infection-relevant conditions. This can be designated as "stimulus-specific immunomes". For instance, specific binding signals can be observed at 32 and 110 kDa with extracellular protein extracts obtained from cultures growing at 42°C and under iron limitation, respectively.
Conclusions: Our results indicate that stimulus-specific gene expression alters the *S. aureus* immunome. 2D immunoblots combined with abundance-based normalization will be now used to identify the immunogenic proteins and their capacity to induce an adaptive immune response in the human host.

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MAP01
Flavin-based electron bifurcation, an Achilles' heel of anaerobes exposed to air
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In flavin-based electron bifurcation (FBEB) the two electrons of a donor are split; one goes to a more positive acceptor and the thereby released energy drives the second electron to a more negative acceptor, in most cases to ferredoxin or flavodoxin (*E*' ca. -500 mV). The reduced ferredoxin is used to form hydrogen, acetate or methane; alternatively it can be re-oxidized by NAD^+, whereby an electrochemical Na^+ gradient is generated(1). Until to date eight FBEB systems from bacteria and archaea have been characterized. The first known FBEB system, widespread in strict anaerobic Firmicutes, comprises an electron transferring flavoprotein (Etf) and a butyryl-CoA dehydrogenase (Bcd), which catalyzes the reduction of thehigh potential crotonyl-CoA to butyryl-CoA by NADH coupled to the reduction of two ferredoxin or flavodoxinby NADH(2). Already in 1964 Baldwin and Milligan (3)reported that Etf and Bcd from the rumen bacterium *Megasphaera elsdenii* mediate the electron transfer from NADH to crotonyl-CoA, but without ferredoxin. Using recombinant Etf and Bcd from *M. elsdenii* under anaerobic conditions we demonstrated that ferredoxin indeed was required as in the other bifurcating systems. Under air, however, oxygen replaced ferredoxin and was reduced to hydrogen peroxide, most likely via superoxide. Furthermore, in the presence of crotonyl-CoA or even butyryl-CoA, Etf + Bcd acts as NADH oxidase. Though for many anaerobes electron bifurcation is a very beneficial bioenergetic tool, upon exposure to air it leads to reactive and destructive oxygen species (ROS).

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MAP02
Establishment of an easy and cost efficient lyophilization protocol for anaerobic bacteria
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Introduction: There are several methods available for strain preservation such as freezing cells or spores. However, some anaerobes can only be kept viable via freeze drying. There are mainly two different forms of freeze drying with certain advantages and disadvantages. (1) If cells are lyophilized in ampoules connected to a manifold, quite good lyophilization results can be achieved, but high acquisition costs for an appropriate manifold, ampoules and a welding set are needed (~3000 €). (2) If cells are lyophilized in glass vials with partially closed rubber stoppers, the method is quite convenient for many samples, but the major disadvantage is a high risk of contamination.
Keeping this in mind, a lyophilization protocol was established with low acquisition and maintenance costs and a minimal risk of contamination.
Material and Methods: The lyophilization was carried out using a "Christ Loc-1M" freeze dryer with a "Pfeifer Balzers Duo 004B" vacuum pump. Prior to the freeze drying process, samples were frozen in liquid nitrogen. The entire sample volume was 400 µl. So far it is possible to lyophilize 12 samples in parallel, but the system can also be easily upscaled.
Results: Several anaerobic wildtype strains of *Acetobacterium woodii*, *Clostridium acetobutylicum*, *C. aceticum*, *C. beijerinckii*, *C. carboxidivorans*, *C. ljungdahlii*, *C.ragsdalei*, *C. sporogenes*, and *Moorella thermoautotrophica* as well as recombinant strains were successfully lyophilized and kept viable for longer than a year using the presented method. Lyophilisates harbouring the dried cells can be easily reactivated by suspending in fresh media and incubating in 5-ml Hungate tubes.
Conclusion: The method can be adapted to different freeze dryers and is therefore a good alternative for lyophilizing anaerobic strains with low costs and minimal risk of contamination.

MAP03
Electron Tomography of *Pyrococcus furiosus*
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Introduction: The hyperthermophilic, strictly anaerobic Euryarchaeum *Pyrococcus furiosus* has emerged as an excellent model system for a number of studies of archaeal cell and molecular biology. The slightly irregular cocci exhibit up to 80 archaella, several µm in length, which have been proven to mediate cell motility. Other functions are adhesion to various surfaces and formation of cell-cell connections, resulting in a network of interconnected *P. furiosus* cells (Näther et al, 2006).
Objectives: As *P. furiosus* can withstand and handle harsh environmental conditions, it`s cellular architecture (e.g. composition of the cell envelope,

organisation of the S-Layer and insertion of the flagella) is of great interest for us.
Methods: We used various electron microscopical methods (Rachel et al, 2010). In particular, TEM-tomography of 300nm thin-sections of high-pressure frozen, freeze-substituted and resin-embedded *P. furiosus* cells was performed. Additionally, STEM-tomography datasets of 600nm sections were recorded, to reach a higher content of information (reduction of focus gradient due to a greater depth of focus in STEM mode), while investigating a bigger volume of the cell (Yakushevska et al, 2007). All datasets were reconstructed using 3Dmod and visualized in AMIRA.
Results: Tomography turned out to be an excellent means for investigating the architecture of a complete *P. furiosus* cell. Several yet undescribed features, like the partial withdraw of the S-Layer in certain areas, or structures associated with the cell wall, could be visualized.
Conclusion: For a proper comparison, the generation of FIB-SEM datasets is planned, in order to obtain an overview of the total architecture of a whole cell. For high-resolution investigation of ultrastructural details, cryo-tomography can also be performed in the future.

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MAP04

Non-acetogenic growth of the acetogen *Acetobacterium woodii* on 1,2-propanediol

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Introduction: Acetogenic bacteria can grow by the oxidation of various substrates coupled to the reduction of CO_2 in the Wood-Ljungdahl pathway. Alcohols can also be used as growth substrate but the metabolism has not been studied in detail.
Objectives: The objective of this work was to unravel the 1,2-propanediol (1,2-PD) metabolisms of the model acetogen *Acetobacterium woodii*.
Methods: We studied the substrate conversion with whole cells by analyzing the product pool with gas chromatography and characterized the individual enzyme activities with spectroscopic methods. Electron microscopy was used to analyze the formation of cell organelles. The genetic organization of the encoding genes and their expression profile was analyzed by RT-PCR.
Results: Growth of *A. woodii* on 1,2-PD as sole carbon and energy source is independent of acetogenesis. Enzymatic measurements and metabolite analysis revealed that 1,2-PD is dehydrated to propionaldehyde, which is further oxidized to propionyl-CoA with concomitant reduction of NAD. NADH is reoxidized by reducing propionaldehyde to propanol. The potential gene cluster coding for the responsible enzymes includes genes coding for shell proteins of bacterial microcompartments. Electron microscopy revealed the presence of microcompartments as well as storage granula in cells grown on 1,2-PD. Gene clusters coding for the 1,2-PD pathway can be found in other acetogens as well but the distribution shows no relation to the phylogeny of the organisms.
Conclusion: The work demonstrates the flexibility of the acetogenic metabolism. Growth on 1,2-PD in *A. woodii* does not involve the Wood-Ljungdahl pathway. Instead, 1,2-PD is disproportionated to propionate and propanol.

MAP05

Crystal structure of a novel membrane-associated octaheme cytochrome *c* nitrite reductase from the Crenarchaeum *Ignicoccus hospitalis*

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The two Archaea *Ignicoccus hospitalis* and *Nanoarchaeum equitans* form a unique association in which the exclusively sulfur-reducing *I. hospitalis* provides amino acids, lipids, and most probably all essential nutrients to the associated *N. equitans* (1, 2). The nature of this intriguing association, i.e., if *N. equitans* is a symbiont or a parasite, remains unclear as large parts of the physiology of the host are still not fully understood. Here, we provide structural and functional data, which shine light on the complex physiology of this intimate association. *I. hospitalis,* although metabolizing solely elementary sulfur, comprises genes that encode for enzymes involved in nitrogen metabolism, e.g., one nitrate reductase and two putative hydroxylamine oxidoreductases (IhHAO1, IhHAO2). In nitrifying Bacteria, such as *Nitrosomonas europaea*, the soluble octaheme enzyme NeHAO oxidizes hydroxylamine (NH_2OH) to nitrite (NO_2^-), by liberating four electrons and five protons (3). Here we present structure-function studies on the most abundant enzyme IhHAO1 using kinetics, EPR spectroscopy, and X-ray crystallography to solve its three dimensional structure at 1.7 Å resolution. The trimeric IhHAO1 exhibits, beside its membrane association, significant differences in secondary structure to previously characterized homologues from nitrifying and anaerobic ammonium-oxidizing Bacteria. Although, having the positions of the eight hemes highly conserved, the heme coordination pattern of the active site is altered in a way that no covalently linked tyrosyl residue to the porphyrin is present. We suggest that by decoupling of this individual heme and the unusual coordination of heme 3, 6 and 7, IhHAO1 efficiently reduces NO_2^- and NH_2OH under sulfur-limited growth conditions.

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MAP06

The 2,3-*seco* pathway for anoxic androgen catabolism widespread in denitrifying bacteria

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Introduction: The biodegradation of steroids is a crucial biochemical process mediated exclusively by bacteria. Prior to our study, the information concerning the anoxic catabolic pathways of androgens is greatly unknown which has prohibited many environmental investigations.
Objectives: Investigations of the androgen catabolic mechanisms operating in the denitrifying sludge.
Materials and Methods: A ^{13}C-metabolomics approach was used to monitoring the appearance of the catabolic intermediates in the bacterial cultures. The androgenic activity of individual catabolic metabolites were assessed using the *lacZ*-based yeast androgen assay. We then applied the UPLC-MS/MS and next generation sequencing approaches to identify the catabolic intermediates, functional genes, and the androgen-degrading bacteria in the denitrifying sludge samples collected from Di-Hua Sewage Treatment Plant located in Taipei, Taiwan.
Results: We first showed that the denitrifying *Steroidobacter denitrificans* DSMZ 18526 and *Sterolibacterium denitrificans* DSMZ 13999 adopt the 2,3-*seco* pathway to degrade testosterone under anoxic conditions. The androgenic activity of the bacterial cultures and the identified intermediates was determined. The androgenic activity in the testosterone-grown cultures decreased significantly over time, indicating its ability to eliminate androgens. The A-ring cleavage intermediate exhibited no

androgenic activity, whereas the sterane-containing intermediates did. The ring-cleavage intermediate, 2,3-*seco*-androstan-3-oic acid, was thus used as a signature intermediate for culture-independent environmental investigations of anaerobic degradation of C_{19} androgens. A novel catabolic enzyme, 1-testoterone hydratase/dehydrogenase, was isolated and characterized from *S. denitrificans* DSMZ 18526. This enzyme is composed of three subunits and contains molybdopetrin, FAD, and the [Fe-S] cluster as the cofactors. The corresponding genes were identified in the *Steroidobacter denitrificans* genome. Several pairs of specific primers were designed from the functional genes corresponding to the large and medium subunits. We then investigate the anoxic androgen catabolism occurring in the denitrifying sewage of Di-Hua Sewage Treatment Plant located in Taipei, Taiwan.
Conclusion: Our data indicate that the bacteria inhabiting the denitrifying sludge adopt the proposed 2,3-*seco*-pathway to degrade testosterone.

MAP07
Anaerobic microbiology in the Soehngen institute
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The Soehngen institute of anaerobic microbiology has recently been funded by a grant from the Netherlands Science Foundation. It is named after Nicolaas Soehngen who was one of the founding fathers of anaerobic microbiology in the Netherlands.
In the Soehngen institute, the complementary expertise centered on anaerobic microbiology will be extended in a synergistic approach. Furthermore this joint effort will be extended in educating a new generation of leading, excellent microbiologists with a balanced gender composition and a large international contribution.
In the soehngen institute for anaerobic microbiology we will engineer microbial ecosystems for our bio economy, improved human health and a better environment in several complementary workpackages.
Human health: The conversion of various polymers into volatile fatty acids (VFA) such as acetate, propionate and butyrate is one of the most important biotransformations catalyzed by anaerobes - not only in natural ecosystems but also in our own intestinal tract! Our intestines are home to trillions of mainly anaerobic bacteria that not only convert our food, but also produce vitamins and stimulate our immune system. Propionate and butyrate are involved in specific signaling. Ultimately, anaerobic bacteria such as Akkermansia, may have potential as therapeutic bacteria that contribute to treating metabolic diseases such as type 2 diabetes and obesitas.
Environment: To better understand the biogeochemical cycles we will have joint expeditions to alkaline soda lakes and marine ecosystems. We will bring the most promising samples to the laboratory to enrich new methane producing and oxidizing anaerobes. We will identify new lipid biomarkers for tracing these organisms. We will use high through put sequencing to unravel their genomic potential. In this way we will discover new enzymes and organisms for application in biofuel production and waste treatment.
Bio-based economy: Finally we will search for the microbial potential to produce valuable chemicals. Non-biodegradable waste can be gasified to syngas (H2/CO) after which anaerobes can produce organic acids. The organic acids can be used to synthesize new building blocks for the biobased society.

MAP08
Production and characterization of an epsilonproteobacterial Hao-type cytochrome *c* nitrite reductase from *Caminibacter mediatlanticus*
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Introduction: Octahaem cytochrome *c* hydroxylamine oxidoreductase (Hao)-type enzymes are predicted from the genomes of several anaerobic Epsilonproteobacteria of the genera *Campylobacter*, *Caminibacter* and *Nautilia*. It was hypothesized recently that these periplasmic enzymes (here named εHao) might catalyze respiratory nitrite reduction yielding either ammonium or hydroxylamine as product [1-4]. Interestingly, εHao enzymes lack the critical tyrosine residue that covalently binds the catalytic haem *c* group within the conventional nitrite-producing Hao homotrimer from typical aerobic ammonia oxidizing bacteria.
Objectives: It was aimed to characterize the prototypic εHao from *Caminibacter mediatlanticus* with respect to its substrate and product range.
Methods: The non-pathogenic Epsilonproteobacterium *Wolinella succinogenes* was chosen as heterologous production host since *W. succinogenes* cells have a high capacity to produce cytochromes *c* using the cytochrome *c* biogenesis system II [5]. Cells were grown by fumarate respiration on supplemented minimal medium. Periplasmic *C. mediatlanticus* εHao was enriched by conventional protein purification methods and enzymatic assays with artificial electron donors and acceptors were performed.
Results: *C. mediatlanticus* εHao was successfully produced in *W. succinogenes*. The enzyme exhibited both nitrite and hydroxylamine reductase activity whereas hydroxylamine oxidation was not observed.
Conclusion: The Hao-type enzyme octahaem cytochrome *c* from *C. mediatlanticus* works as an efficient reductase of hydroxylamine and nitrite. Future determination of the reaction product(s) will help to elucidate the physiological function of the enzyme.

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MAP09
8-Methylmenaquinone-6 is involved in polysulfide and sulfite respiration of *Wolinella succinogenes*
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Introduction: Isoprenoid quinones are widely distributed in bacterial membranes and serve as redox mediators between dehydrogenases and reductases in respiratory electron transport chains. Cells of the Epsilonproteobacterium *Wolinella succinogenes* grow by anaerobic respiration using formate or hydrogen as electron donor and either fumarate, nitrate, nitrite, nitrous oxide, polysulfide or sulfite as electron acceptors [1-3]. *W. succinogenes* synthesizes menaquinone-6 and methylmenaquinone-6 (MMK) and the latter compound was shown previously to be essential for polysulfide respiration [1].
Objectives: This work aimed to determine the position of the additional methyl group in MMK and to elucidate whether MMK is involved in sulfite respiration.
Methods: We recorded quinone profiles of *W. succinogenes* cells grown anaerobically in the presence of various electron acceptors. Quinones were extracted and analyzed by high performance liquid chromatography coupled to spectroscopic detection. MMK was structurally characterized by nuclear magnetic resonance spectroscopy. Chemical shifts were calculated by density functional theory and compared with the experimental data.
Results: The methyl group of MMK is located at position C-8 on the naphthoquinone ring, similar to reports in other bacteria [4]. Characterization of an MMK-deficient *W. succinogenes* mutant indicates that MMK is required for growth by sulfite respiration.
Conclusion: The results imply that 8-MMK is an essential component of electron transport chains that serve in the reduction of polysulfide and sulfite, i.e. electron acceptors with comparatively negative redox potentials.

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MAP10
An Intramolecular Chaperone Coordinates the Synthesis and Assembly of a Modular Membrane-Associated [NiFe]-Hydrogenase
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Membrane-associated [NiFe]-hydrogenase (Hyd) 2 of *E. coli* has four subunits: a large subunit (HybC) that carries a $NiFe(CN)_2CO$-cofactor; a small subunit (HybO) that harbours (iron-sulphur) FeS-clusters; a further FeS subunit (HybA); and a membrane anchor (HybB). During enzyme assembly, a HybC-HybO heterodimer, in which each subunit is fully folded and loaded with its respective cofactor(s), is translocated across the cytoplasmic membrane by the twin-arginine translocon (Tat). HybA and HybB are transported independently of the HybO-HybC heterodimer. We wish to understand how heterodimer assembly is controlled. HybC is

synthesized as an apo-protein with a *C*-terminal extension of 15 amino acids, which is proteolytically removed from the mature enzyme. If this 15-amino acid peptide is not removed from HybC, no heterodimer formation occurs, the precursor of the small subunit, HybO, is degraded and HybC remains in the cytoplasm. HybO, but not HybC, carries a Tat signal peptide, which is required for membrane translocation. The $Fe(CN)_2CO$ moiety of the NiFe-cofactor is synthesized by a complex of Hyp accessory proteins (HypABCDEF). The HybC large subunit is only proteolytically processed at its *C*-terminus after insertion of both the $Fe(CN)_2CO$ cofactor and Ni^{2+}. After NiFe-cofactor insertion and removal of the *C*-terminal peptide, HybO bearing its Tat signal peptide engages with mature HybC and subsequently with the Tat machinery. We show that the *C*-terminal peptide is required for efficient interaction of the HybC apo-protein with the Hyp machinery and efficient delivery of the NiFe-cofactor components. The amino acid sequence, possibly together with the structure of the *C*-terminal peptide governs how the Hyp components associate with the apo-protein. Moreover, we show that it is possible to dupe the Tat machinery into translocating a HybC-HybO heterodimer lacking the NiFe-cofactor by synthetically removing the 15-amino acid *C*-terminal peptide from HybC. Thus, this *C*-terminal peptide functions as an intramolecular chaperone coordinating the temporal assembly of the HybO-HybC heterodimer.

MAP11

Enzymes involved in anaerobic *ortho*-phthalate degradation

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Phthalic acids are massively used for polyester resins, plastic bottles, plasticizers and other petroleum based products. While the aerobic degradation by microorganisms is well established, the enzymology involved in the anaerobic degradation is largely unknown. Though a decarboxylation of phthalic acids to benzoate has been proposed, *in vitro* evidence for such a reaction is missing (1, 2). We demonstrate the complete degradation of *o*-phthalate in various denitrifying pure cultures. In "*Aromatoleum aromaticum*", phthalate-induced proteins and the encoding genes were identified. They comprised enzyme homologous to a dicarboxylic acid transporter, UbiD- and UbiX-like decarboxylases and a CoA transferase. Results from *in vitro* assays suggest the following enzyme activities involved in anaerobic *o*-phthalate degradation: (i) activation of phthalate to phthaloyl-CoA by a succinyl-CoA-dependent CoA-transferase, followed by (ii) the decarboxylation of phthaloyl-CoA to benzoyl-CoA. The latter activity was oxygen-sensitive and K^+-dependent.

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MAP12

Altering the substrate specificity of an acyl-CoA synthetase by reversible lysine acetylation

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Aromatic compounds can be fully mineralized by bacteria, archaea and fungi under aerobic or anaerobic conditions. In anaerobic bacteria monoaromatic carboxylic acid growth substrates such as benzoate or *p*-hydroxybenzoate are initially activated to the corresponding CoA-esters. The strictly anaerobic Fe(III)-respiring bacterium *Geobacter metallireducens* uses an AMP-forming benzoate-CoA ligase (BamY) (2) or a succinyl-CoA:benzoate CoA transferase (3) for benzoyl-CoA formation. However, heterologously produced BamY merely activated *p*-hydroxybenzoate. As no additional gene encoding a *p*-hydroxybenzoyl-CoA synthetase is present in the genome, the enzyme involved in *p*-hydroxybenzoyl-CoA formation was at issue.
Surprisingly, *bamY* knockout-mutants lost the ability to grow with *p*-hydroxybenzoate, suggesting that BamY is involved in both benzoate and *p*-hydroxybenzoate activation. Using heterologously produced BamY, an acetyl-CoA- and p-hydroxybenzoate-grown cell extract-dependent 6-fold increase of *p*-hydroxybenzoyl-CoA forming activity of BamY was observed. Three lysines were identified as putative targets for lysine acetylation. In addition to this posttranslational modification, *bamY* transcription was 5-fold upregulated during growth with *p*-hydroxybenzoate compared to benzoate. Both the transcriptional and posttranscriptional effects resulted in a 30-fold stimulation of *p*-hydroxybenzoyl-CoA synthetase activity that was sufficient to meet the requirements for the observed growth rate. The observed acetylation-dependent activation of an enzyme is the first report on an alteration of an enzyme activity which differs from the previously reported on/off-regulation of acyl-CoA synthetases (4).

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MAP13

Elucidating Maturation and Regulatory Events during Synthesis of the [NiFe]-Hydrogenase Electron-Transfer Subunits

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The model organism *Escherichia coli* encodes 4 different [NiFe]-hydrogenases (Hyd). Each Hyd comprises at least one large subunit that carries a $NiFe(CN)_2CO$-cofactor and a small subunit, which harbors a dedicated set of (iron-sulphur) FeS-clusters. Despite biosynthesis of Hyd having been studied intensively over many decades, there are nevertheless proteins encoded in the hydrogenase structural operons for which no function has yet been assigned, e.g. HyaE, HyaF and HybE. While the maturation of the large subunits has been studied intensively, the maturation of the small subunits has been largely neglected in *E. coli*.
The routes of delivery of the iron ions to the small and large subunits of the Hyds are unknown. Recent studies demonstrated that the Isc FeS-cluster machinery is essential for the maturation of the small but not for the large subunits of all three characterized Hyds in *E. coli*[1]. It has been proposed that the two A-type carrier (ATC) proteins ErpA and IscA deliver at least 3 different FeS-cluster types to the small subunits. The factors that confer upon these ATC [FeS]-cluster-delivery proteins specificity for a particular apo-protein substrate, direct the different [FeS]-cluster types to their correct respective targets or possibly modify these [FeS]-clusters in situ are still unknown.
Furthermore HyaE- and HybE-like proteins are predicted to fulfill roles in the guidance of their respective redox enzymes to or across the cytoplasmic membrane, depending on their degree of maturation. Furthermore, they have been suggested to mediate the crosstalk between different redox enzymes, allowing adaptation to environmental conditions[2].
To address this question we are currently investigating the biological and biochemical properties of the putative recruitment or cofactor-inserting chaperones HyaE, HyaF and HybE by characterizing the phenotypes of the respective deletion mutants and performing protein interaction studies with ATC proteins. The findings of these studies will be reported.

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MAP14

A unique heterodimeric cytochrome c complex from the anammox bacterium *Kuenenia stuttgartiensis*

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Anaerobic ammonium oxidizing (anammox) bacteria are major contributors to the earth's nitrogen cycle and have been successfully applied in novel waste water treatment procedures. The metabolism of these microorganisms is based on ammonium oxidation coupled to nitrite reduction which results in the production of dinitrogen gas, a process involving the unusual intermediate hydrazine [1]. A complex of the two monoheme c-type cytochromes kustA0087 and kustA0088 has been recently purified from the anammox bacterium *Kuenenia stuttgartiensis.* This complex is homologous to the NaxLS complex from the anammox *Planctomycete* KSU-1, which was previously reported to possess a very low redox potential [2]. Biophysical investigations of kustA0087/A0088 using analytical ultracentrifugation (AUC) as well as size-exclusion chromatography combined with multi-angle static light scattering (SEC/MALS) resulted in a molecular mass of approx. 24 kDa, consistent with a heterodimeric complex in solution. Moreover, the complex could be reconstituted *in vitro* from its individual components that were

heterologously expressed in *Shewanella oneidensis* MR-1. We determined the structure of the heterodimer at 1.7 Å resolution using X-ray crystallography. In the complex, kustA0087 shows a four-helix bundle fold typical for class II cytochromes c, whereas kustA0088 displays a typical class I cytochrome c structure. The heme iron in each subunit is coordinated by a rare cysteine ligand at its distal side. UV-Vis spectroscopy revealed that the complex and its components possess a Soret band maximum at around 420 nm showing a unique blue shift upon reduction. Finally, the UV-Vis spectra indicate binding of nitric oxide and carbon monoxide to the hemes in both kustA0087 and kustA0088, individually and in complex. The obtained spectroscopic features match with cytochrome c' [3] and CO-sensing hemoproteins [4], suggesting a role in gas sensing or -scavenging.

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MAP15

High-level production of cytochromes *c* in *Escherichia coli*: optimization of the heterologous production of pentaheme cytochrome *c* nitrite reductase from *Campylobacter jejuni*

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Introduction: Multiheme cytochromes *c* are essential components in processes forming the earth's nutrient cycles, including the nitrogen cycle, and catalyze electron transport as well as various oxidation and reduction reactions [1]. Although widely distributed in Bacteria and Archaea, a thorough biochemical characterization of cytochromes *c* is available for only few enzymes purified from a small number of model organisms. Due to easy handling and genetic accessibility, recombinant cytochrome *c* production is often carried out using *Escherichia coli* [2]. However, existing protocols often failed to overproduce multiheme cytochromes at sufficient quantity and/or quality.
Objectives: This work aimed to modify a basic protocol to optimize the heterologous production of tagged *Campylobacter jejuni* pentaheme cytochrome *c* nitrite reductase of (*Cj*NrfA) in *E. coli*.
Methods: We constructed a modified pET vector to produce TwinStrep-tagged *Cj*NrfA under the control of a T7 promoter using *E. coli* BL21 (DE3) as host strain and pEC86 [3] as helper plasmid. To optimize the production level of *Cj*NrfA several cultivation parameters (oxygen availability, production time span and growth medium composition) were varied and the protein production was judged by Coomassie and heme staining.
Results: Highly active *Cj*NrfA was purified from *E. coli* to apparent homogeneity. Compared to standard conditions the optimized production and purification protocol resulted in a tenfold increased *Cj*NrfA yield.
Conclusion: The production and purification of multiheme cytochrome *c* enzymes is challenging and many cultivation parameters influence the production yield. We identified four essential parameters and optimized these for the production of cytochromes *c* in *E. coli*, exemplified by the production of *Cj*NrfA.

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MAP16

A novel thioredoxin like protein (Tlp) in *Clostridium acetobutylicum*

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Thioredoxins are ubiquitous and antioxidant enzymes which fulfil a number of different cellular functions for example in the oxidative stress response. They are characterized by a highly conserved C-X-X-C-motif [1]. In thioredoxins and similar proteins, this motif acts as an active site and confers thiol-disulfide oxidoreductase activity [2]. Here we present a thioredoxin like protein (Tlp) of *C. acetobutylicum* that lacks one of the highly conserved cysteine residues in its active site motif, but is still able to reduce insulin disulphides. A feature of this Tlp is a kind of multimerization which was verified by SDS-PAGE. The protein size was further investigated using FPLC (fast pressure liquid chromatography) with mercaptoethanol and DTT. The results indicated that even under these denaturing conditions the multimeric structure was stable. Quantitative assays were carried out and disclosed that the protein is able to catalyse the reduction of insulin with only one cysteine in the active site due to the formation of a disulphide between two protein molecules of Tlp. To further investigate the reducing properties, the cysteine was exchanged towards an alanine by site-directed mutagenesis and the reducing properties were tested as well. The results lead to the assumption that the described protein is a novel Tlp of *C. acetobutylicum*.

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MAP17

Annotation vs. Reality – Is Cap0025 from *Clostridium acetobutylicum* really a pyruvate decarboxylase?

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Clostridium acetobutylicum belongs to the group of solventogenic clostridia, which have the natural capability to produce *n*-butanol as a next generation biofuel. Moreover, the solvents acetone and ethanol are also produced. Due to this, *C. acetobutylicum* was used for decades in industrial acetone-butanol-ethanol (ABE) fermentation [1]. In this context, there were many efforts to increase the product yield, especially for *n*-butanol which leads to *C. acetobutylicum* moved into the focus of many researches [2]. The genome of *C. acetobutylicum* was completely sequenced in 2001 [3]. As part of this, *cap0025* was annotated as pyruvate decarboxylase (PDC). This enzyme enables a previously unconsidered branch in the metabolism of *C. acetobutylicum* which might affect the ethanol biosynthesis. Corresponding measurements *in vitro* did not show any activity of the recombinant Cap0025. So it is doubtful whether Cap0025 is actually a pyruvate decarboxylase. For that reason, additional analyses have been carried out: I. *in situ* analyses in comparison to other PDCs, II. an amino acid exchange in the catalytic center of the enzyme and III. the influence of the gene knock out on the physiology of the organism.

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MAP18

Hydrogen metabolism and hydrogen production of *Sulfurospirillum* spp. (epsilonproteobacteria)

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Introduction: Little is known about the hydrogen metabolism of free-living ε-proteobacteria, despite their abundance in a variety of ecosystems. *Sulfurospirillum multivorans*, an organohalide-respiring ε-proteobacterium, harbours genes coding for four NiFe-hydrogenases: two putative hydrogen uptake hydrogenases (MBH, Hup) and two putative hydrogen evolving hydrogenases (Ech, Hyf) [1], the MBH and the latter two being encoded on most *Sulfurospirillum* spp. genomes. When growing *S. multivorans* and other *Sulfurospirillum* spp. on fermentable substrates like pyruvate in the absence of an electron acceptor, hydrogen is produced.
Objectives: This study focusses on the hydrogen metabolism of *S. multivorans* and the production of hydrogen during pyruvate fermentation of various *Sulfurospirillum* spp.
Methods: *S. multivorans* was grown under different conditions and the mRNA levels of hydrogenase genes were quantified via quantitative reverse transcriptase (qRT) PCR. The hydrogenase responsible for hydrogen oxidation was enriched and a first characterization was done using photometric assays and activity-stained native PAGE. Hydrogen production was quantified using gas chromatography.
Results: Transcripts of only one uptake (MBH) and one H_2-producing hydrogenase (Hyf) were detected in qRT PCR of *S. multivorans*. Transcript levels were similar under all tested growth conditions, pointing toward a constitutive expression of both hydrogenases and no substrate-dependent regulation. A high H_2-oxidizing activity was measured for the MBH and a size of around 270 kDa for the native complex was determined. H_2 production during pyruvate fermentation was measured for all tested *Sulfurospirillum* spp. Two non-dechlorinating species produced the highest amount of hydrogen (~ 7% of the gas phase).
Conclusion: Here we report first insights into the hydrogen metabolism of *S. multivorans* and hydrogen production capability of *Sulfurospirillum* spp. The involvement of one of the uptake hydrogenases (MBH) in organohalide respiration and of the Hyf hydrogenase in H_2 production is

discussed. Considering the importance of hydrogen in anaerobic ecosystems, *Sulfurospirillum* spp. may be an overlooked hydrogen producer in syntrophic relations.

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MAP19

Regulation of anaerobic respiratory pathways in *Dinoroseobacter shibae*

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Within the great family of *Rhodobacteriaceae* one major subdivision is the marine Roseobacter clade. Its globally abundant members are involved in nitrogen, sulfur and carbon cycles. Based on their planktonic lifestyle they have to face great changes in living conditions including anaerobiosis. Our model organism is *Dinoroseobacter shibae* is capable of performing nitrate respiration and subsequent denitrification under anaerobic conditions. In the annotated genome clustered genes were found which are involved in anaerobic respiratory energy generation. Beside the periplasmic nitrate reductase Nap, it features nir, nor and nos operons. Little is known so far about the regulatory network regarding the nitrate utilisation in this clade. In *D. shibae* an unusual high number of Crp/Fnr-like regulators were found. Beside one FnrL-homologue with a proposed $[4Fe\text{-}4S]^{2+}$-cluster, six Dnr-like regulators can be found. Cluster analyses classified DnrD, DnrE and DnrF within DNR group of the great Crp/Fnr family. To study the role of the different Dnr regulators for anaerobic adaptation we decided to create knock-out mutants. Transcriptome analysis of a Δ*dnrF* mutant strain in comparison to the wild type strain revealed potential targets of DnrF. Interestingly; we observed a regulatory effect of DnrF on *dnrE* transcription. To confirm our findings we combine transcriptome data with deep sequencing results to identify the transcriptional start sites. In combination with virtual footprint we were able to identify a conserved binding motive within several promoters. These binding sequence exhibits a high similarity to the Fnr-box of *Pseudomonas aeruginosa*. In order to analyse binding of DnrF to the promoters we produced DnrF heterologously in *Escherichia coli* and established electro-mobility shift assays (EMSA).

MAP20

Butanediol metabolism in *Acetobacterium woodii*

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Introduction: Acetogenic bacteria can reduce CO_2 with electrons coming from molecular hydrogen in a pathway called the Wood-Ljungdahl pathway. The end product of this pathway is acetate. Additionally, the acetogenic bacterium *Acetobacterium woodii* can grow on a variety of other substrates such as fructose, betaine or lactate, but also on alcohols such as methanol and butanediol, respectively [1]. The metabolism of the latter one intrigued us most, as it may lead to the production of ethanol as known for other microorganisms [2].
Objectives: Unraveling the pathway of 2,3-butanediol degradation in the model acetogen *A. woodii*.
Methods: We performed growth experiments and analyzed the degradation of 2,3-butanediol using whole cells and crude extracts. Substrates as well as products were detected by gas chromotography and corresponding enzyme activities were measured using spectroscopic methods.
Results: *A. woodii* oxidizes 2,3-butanediol first to acetoine, which is then oxidized by an acetoin dehydrogenase to acetyl-CoA and acetaldehyde. Both intermediates are converted to acetate in an ATP-forming pathway. Reducing equivalents generated are reoxidized in the Wood-Ljungdahl pathway thus forming additional ATP by a chemiosmotic mechanism.
Conclusion: In contrast to other microorganisms, *A. woodii* degrades 2,3-butenadiol to acetate exclusively, producing neither ethanol nor any other products. Instead, reducing equivalents are channeled into the Wood-Ljungdahl pathway.

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2. B. Schink *Arch Microbiol* **137** (1984), p. 33-41

MAP21

Methanogenic hydrocarbon biodegradation by indigneous microbiota from a water-flooded oil reservoir

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Introduction: Microbial transformation of hydrocarbons to methane is an environmentally relevant process taking place in many subsurface environments, including oil reservoirs, and it could constitute a recovery method of carbon from exhausted reservoirs.
Objectives: The aim of the study was to assess the ability of indigenous microbiota from the Dagang oil reservoir (China) to transform hydrocarbons to methane.
Methods: Chemical and isotopic analyses of injection and production fluids of the oil field were performed. Microbial abundances were assessed by qPCR, and clone libraries were performed to study the diversity. In addition, microcosms with either oil or ^{13}C-labelled hydrocarbons were inoculated with injection or production waters to characterize microbial processes *in vitro*.
Results: Geochemical and isotopic data from reservoir fluids were consistent with in situ biogenic methane production linked to oil degradation. Laboratory degradation experiments revealed that autochthonous microbiota are capable of significantly degrading oil within several months, while producing methane, and of producing heavy methane from 13C-labelled n-hexadecane or 2-methylnaphthalene. These results suggest that in situ methanogenesis may occur from the aliphatic and polyaromatic fractions of Dagang reservoir fluids. In addition, methane-producing Archaea (hydrogenotrophic, methylotrophic and acetoclastic) and hydrocarbon-degrading Bacteria were abundant in produced oil-water samples. Syntrophic Bacteria and methanogenic Archaea were predominant in oil and 2-methylnaphthalene-degrading enrichment cultures, as well.
Conclusion: The studied areas of the Dagang oilfield may have a significant potential for the in situ conversion of oil into methane as a possible way to increase total hydrocarbon recovery.

MAP22

A novel heterotrimeric NADH-dependent methylenetetrahydrofolate reductase in a catabolic C1 pathway

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Introduction: The methylene-tetrahydrofolate (THF) reductase (MTHFR) is a central enzyme in the pathway of carbon dioxide reduction to acetate as carried out by acetogenic bacteria. Since the reduction of methylene-THF with NADH as electron donor is exergonic (ΔE_0'= -120 mV), it was assumed to be involved in energy conservation [1].
Objective: Purification and biochemical characterization of the MTHFR from the model acetogen *Acetobacterium woodii*.
Methods: The MTHFR was purified by chromatographic steps from fructose-grown cells of *A. woodii.*
Results: The MTHFR forms a stable and yet unprecedented heteromeric complex consisting of MetF and the subunits MetV and RnfC2. The complex contains 23.5 ± 1.2 mol iron and 24.5 ± 1.5 mol sulfur per mol enzyme, which fits the predicted two [4Fe4S] clusters in MetV and four [4Fe4S] clusters in RnfC2. One mol of the purified complex contains two mol FMN. The complex reduced methylene-THF with NADH as electron donor. The purified enzyme did not catalyze the reverse reaction and ferredoxin was neither required nor reduced.
Conclusion: The MTHFR from *A. woodii* consists of three subunits MetF, MetV and the unique subunit RnfC2. In contrast to most known MTHFRs the enzyme from *A. woodii* contains FMN instead of FAD as a cofactor. The enzyme is not involved in energy conservation in *A. woodii.*

1. K. Schuchmann and V. Müller *Nat Rev Microbiol* **12** (2014), p. 809-821

MAP23
Isolation of methanogenic consortia from a thermophilic biogas plant
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Introduction: The production of biogas from renewable resources and agricultural wastes aiming a sustainable and CO_2-neutral energy generation has increased over the last years. In biogas plants a complex and dynamic process causes the anaerobic degradation of organic biomass to biogas.
Objectives: In order to understand and improve the process of thermophilic biogas production methanogenic archaea were isolated and characterized.
Material & Methods: From samples of a North Rhine-Westphalian thermophilic biogas plant (54 °C), which was fed with renewable substrates (e.g. maize) and swine manure, methanogens were isolated. The techniques for cultivating strict anaerobes described by Balch et al. [1] were used throughout the study. Strains were isolated and grown on a basal medium.
Results: Up to now two different species of hydrogen-consuming methanogens were isolated from a thermophilic biogas plant. Comparative 16S rRNA gene sequence analysis revealed 100 % sequence identity with *Methanothermobacter wolfeii* and *Methanoculleus thermophilus*.
Conclusion: Further studies are necessary to characterize all bacterial and archaeal species involved in the last step of anaerobic degradation of renewable plant material.

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MAP24
Microbial community shift during establishment of steady-state biogas fermentation of grass silage
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Introduction: Biogas production is an important contributor to renewable energy supply for sustainable living society. The fermentation is accomplished by a complex community of microorganisms, being in ecological relation to each other. Several studies indicate that microbial community changes affect important process parameters such as biogas production and formation of organic acids. Therefore, molecular marker systems for specific microbial groups need to be designed to describe, monitor and evaluate the microbiological counterpart of the biogas process.
Objectives: In this study, the qualitative and quantitative occurrence of microorganisms during grass silage fermentation was analyzed to reveal shifts in the community composition, which are accompanied by changing process conditions.
Materials & Methods: Thermophilic (55 °C) and mesophilic (38 °C) two-stage two-phase biogas fermenters were fed with grass silage batch wise every 28 days. Successive increase of substrate was applied in a start-up procedure for five batch fermentations to ensure adaptation of the microbial community. 16S rRNA gene sequence libraries were constructed and analyzed before reactor start and after 310 days during steady process conditions. Additionally, bacterial and archaeal qPCR assays were performed quantifying the 16S rRNA gene.
Results: The steady-state fermentation yielded in stable biogas formation. Here, an increase of the total concentration of 16S rRNA gene copies was detected compared to the reactor start. The structure of the bacterial community shifted in both, the thermophilic and mesophilic reactors. The sequence library derived from the reactor start consisted to 59 % of microorganisms assigned to the phylum *Firmicutes*. During on-going fermentation, this partition increased to 90 % in the thermophilic and decreased to 51 % in the mesophilic habitat. The archaeal community shifted in the same period and showed varied compositions in the thermophilic and mesophilic process.
Conclusions: The on-going biogas fermentation effects a proliferation of distinct microbial groups well adapted to the apparent process conditions. The identification of such groups is an indispensable pre-requisite for the development of qPCR-based molecular markers for cultivation-independent process monitoring.

MAP25
Anaerobic Degradation of Coumarin under Methanogenic Conditions
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The plant secondary metabolite coumarin is naturally present in plants like sweet clover (*Melilotus* spp.) and sweet vernal grass (*Anthoxanthum odoratum*). Both plants are potential alternative substrates for biogas production. Common energy crops like maize could be at least partly replaced and thereby, their adverse effects on food prices and biodiversity could be reduced. During the anaerobic digestion of coumarin-rich substrates coumarin causes an inhibition noticeable as decreased biogas production. However, anaerobic degradation of coumarin was observed and associated with the recovery of the biogas process from the inhibition.
Anaerobic enrichment cultures were established to study the degradation of coumarin in detail. A modified complex medium DSM 120 was inoculated with sludge from a biogas reactor continuously exposed to coumarin and capable of degrading it. The degradation process in these enrichment cultures was monitored by determining the concentrations of coumarin and potential intermediates, gas production as well as gas composition.
Results from the fifth transfer showed that within the first ten days after inoculation coumarin caused an inhibition of gas production. Until day 10 only 38% of the gas volume of a coumarin-free control was produced and the gas had a low methane content of 6%. In contrast, the produced gas of the coumarin-free control contained 33% methane. The initial coumarin concentration of 1.0 g L^{-1} decreased slowly but steadily until day 10 to a concentration of 0.7 g L^{-1}. Afterwards the coumarin concentration decreased considerably faster to almost 0 g L^{-1} on day 20 testifying efficient degradation. At the same time the gas production increased. On day 27 there was as much gas produced as in the coumarin-free control. However, the carbon dioxide content was higher and the methane content was lower than in the coumarin-free control. Dihydrocoumarin was identified as intermediate which probably resulted from hydrogenation of coumarin in a first degradation step. To the best of our knowledge, this conversion reaction has not been described yet under anaerobic conditions.

MAP26
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.

MAP27

***Aromatoleum aromaticum* maintains anaerobic growth on Phe in the absence of the metabolic enzyme(s) for phenylacetaldehyde oxidation**

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We study the anaerobic degradation of phenylalanine (Phe) and related compounds in the denitrifying betaproteobacterium *Aromatoleum aromaticum* which degrades many refractory aromatic compounds and hydrocarbons under anoxic conditions. Phenylalanine degradation proceeds via phenylacetate to benzoyl-CoA - the common intermediate in anaerobic metabolism of most aromatic compounds.
When *A. aromaticum* was growing on Phe two distinct enzymes were found to catalyse the oxidation of the intermediate phenylacetaldehyde (PAAld) to phenylacetate. We identified a NAD/NADP-dependent, highly specific phenylacetaldehyde dehydrogenase (PDH) as the main metabolic enzyme. Additionally, a tungstate-dependent aldehyde:ferredoxin oxidoreductase (AOR) was found to catalyse this step, albeit AOR also oxidises other aldehydes with similar rates suggesting a more general function for aldehyde detoxification (1). In tungstate-free medium AOR activity was absent, but growth of *A. aromaticum* on Phe was not affected.
To investigate the necessity of PDH for Phe metabolism, a *pdh*-deletion strain was constructed (*A. aromaticum*_Δ*pdh*). This strain was still growing on Phe without any detectable NAD/NADP-coupled PAAld-oxidising activity, but with increased AOR activity. Our data indicate that AOR can serve as a backup enzyme when PDH activity is absent. In tungstate-free medium with Phe as sole carbon source *A. aromaticum*_Δ*pdh* still was able to grow without any detectable PDH- or AOR-activities, albeit with a significantly lower rate. These cells contained a new NAD-dependent PAAld-oxidising activity which was apparently only induced during growth on Phe in the absence of PDH and AOR.
In conclusion, *A. aromaticum* exclusively uses PDH as main enzyme and AOR as backup enzyme to oxidise PAAld, but can yet employ at least one additional aldehyde-oxidising enzyme activity to ensure that the cell survives on Phe when PDH and AOR are missing. We are currently working on the identification and characterisation of this enzyme and the underlying regulatory mechanisms.

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MAP28

Structure and function of tungsten-containing class II benzoyl-CoA reductase

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The Birch-reduction of aromatic rings is a basic reaction in synthetic organic chemistry and requires alkali metals dissolved in ammonia as reductants. In nature a similar reaction is catalysed by cyclohexa-1,5-diene-1-carbonyl-CoA (dienoyl-CoA) forming class I and II benzoyl-CoA reductases (BCR). The ATP-dependent class II BCR is present in obligately anaerobic bacteria such as the Fe(III)-respiring *Geobacter metallireducens*; it has a $[(BamBC)_2DEFGHI]_2$ subunit architecture [1] with an active site W-factor in the BamB subunit [2]. The $(BamBC)_2$ sub-complex of *G. metallireducens* was crystallized in the presence of the substrate, product, the substrate-analogue cyclohex-1-ene-1-carbonyl-CoA and in the CoA-ester-free, Zn^+-bound form; it was further analyzed by EXAFS, FT-IR and EPR spectroscopy. The results obtained provide initial insights into the structure-/function relationship of an enzymatic electron-transfer reaction at an extremely-low redox potential [3]. The results are in favour for a radical-based mechanism in an essentially aprotic reaction cavity.

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2. J.W. Kung *et al. Proc Natl Acac Sci U.S.A.* **106** (2009), p. 17687-92.
3. J.W. Kung *et al. J. Am. Chem. Soc.* **132** (2010), p. 9850-56.

PFP01

Structural and functional diversity of the *Candida glabrata* epithelial adhesin family

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Introduction: Host colonization by the human pathogenic fungus *Candida glabrata* is known to utilize a large family of highly related surface-exposed cell wall proteins, the lectin-like epithelial adhesins (Epas), which share a modular domain structure. Our previous detailed analysis of one family member, Epa1, has revealed that its adhesion (A) domain functions by recognizing specific host glycan structures and provided first high-resolution insights into the structural features required for ligand binding and discrimination. The precise ligand binding patterns and/or precise binding pocket structures of other family members, however, has remained largely unknown.
Objectives: In this study, we aimed at revealing the structure-function relationships within the entire Epa family, in order to obtain a better understanding of the cell-surface interactions of *C. glabrata* with its host.
Methods: We performed a large scale *in vitro* and *in vivo* functional analysis of the A domains of 17 Epa paralogs from *C. glabrata* by glycan array screening, fluorescence titration analysis and epithelial cell adhesion assays in combination with 3D-structural studies of selected EpaA domains.
Results: We find that most EpaA domains exert a lectin-like function and together recognize a wide variety of galactosidic glycan ligands for conferring epithelial cell adhesion. We further identify several conserved and variable structural features within the diverse Epa ligand binding pockets, which appear to be crucial for affinity and specificity. These features rationalize why phylogenetic relationships within the Epa family are often independent of the functional classification and explain how Epa-like adhesins have evolved in *C. glabrata* and related fungal species.
Conclusion: Our study indicates that *C. glabrata* has developed an extensive array of functionally diverse lectin-like adhesins that might be crucial as a whole for efficient host invasion and dissemination. Our study further permits to assess the potential host-ligand binding capacity of *C. glabrata* and together with our precise characterization of ligand binding sites might also contribute to the development of novel antimycotics.

SMeP01

Cyclic di-GMP comprises versatile cellular functions in the symbiotic α-proteobacterium *Sinorhizobium meliloti*

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Introduction: Cyclic di-GMP (cdG) is a common second messenger used in the bacterial kingdom to switch between different lifestyles. The α-proteobacterium *S. meliloti* exists either as free-living soil bacterium or as nitrogen-fixing plant symbiont. Its envelope composition and cell motility must be tightly regulated for optimal fitness upon continuous environmental changes in both the free-living state and *in planta*.
Objectives: Elucidation of the role of cdG in free-living and plant-associated *S. meliloti*.
Methods: Fluorescence microscopy; Bacterial Adenylate Cyclase Two-Hybrid system (BACTH); Co-Immunoprecipitation; cdG quantifications; promoter-reporter fusions; microarray; Electrophoretic Mobility Shift Assay (EMSA); assays for estimation of Congo red binding, Calcofluor brightness, motility, attachment and formation of symbiosis.
Results: The *S. meliloti* genome encodes 20 putative cdG-metabolizing enzymes (diguanylate cyclases/DGCs or phosphodiesterases/PDEs) as well as two putative cdG receptors (PilZ proteins). Single mutants were generated for each of these genes except for *cddF* identified as essential. None of them showed distinct alterations in exopolysaccharide production, motility, biofilm formation or symbiosis. Strains lacking all deletable DGCs exhibited only moderate phenotypic changes. In contrast, raising intracellular cdG levels by overexpressing different DGCs resulted in decreased mucoidity and reduced motility on semi-solid agar, whereas Congo red staining, Calcofluor brightness, biofilm formation and cell aggregation increased. New effectors for cdG were identified: a homeodomain-like transcriptional regulator was shown to activate the production of a Congo red-binding polysaccharide and one PilZ protein was involved in cdG-dependent motility control. Moreover, CddF

appeared to play an important role at late stages of the cell division process.
Conclusion: cdG had significant influence on different aspects of the *S. meliloti* physiology. In the free-living state it promoted the transition from a motile planktonic lifestyle to a biofilm-associated sessile lifestyle. There are also strong hints for cdG to be involved in cell proliferation of *S. meliloti*.

SMeP02
One protein, two functions; the case of the diguanylate cyclase DgcZ in *Escherichia coli*
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Introduction: Cyclic dimeric GMP (c-di-GMP) is a common second messenger in the bacterial world. It controls the transition between motile and sessile life-styles, but it can also be involved in regulation of numerous other bacterial functions, such as virulence and cell cycle progression. Diguanylate cyclases (DGCs) are enzymes responsible for c-di-GMP production. DgcZ is the main diguanylate cyclase involved in poly-GlcNAc (PGA) production in *Escherichia coli* (1). PGA is an exopolysaccharide produced by *E. coli* and other bacteria and it promotes bacterial attachment to abiotic surfaces (2).
Objectives: We investigated DgcZ localization to test whether it had a role in the attachment process.
Methods: Fluorescence microscopy, microfluidic, Co-IP, bacterial two-hybrid.
Results: We applied fluorescence microscopy to assay protein localization in single bacterial cells from liquid cultures or in bacteria growing in microfluidic devices that allow time lapse studies. DgcZ showed a complex localization behavior regulated by nutrient availability and by other external conditions. Co-Immunoprecipitation studies and a bacterial two-hybrid assay revealed potential DgcZ interaction partners.
Conclusion: DgcZ differential localization under different conditions suggests a new additional physiological function for this protein. We are currently testing the hypothesis of an involvement of the diguanylate cyclase DgcZ in regulation of flagellar activity in surface-attached bacteria.

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SMeP03
Local c-di-GMP signalling by a GGDEF and EAL domain protein module in *E. coli* biofilm control
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Introduction: C-di-GMP—which is produced by diguanylate cyclases (DGC) and degraded by specific phosphodiesterases (PDEs)—is a ubiquitous second messenger in bacterial biofilm formation. In *Escherichia coli*, several DGCs (YegE, YdaM) and PDEs (YhjH, YciR) and the MerR-like transcription factor MlrA regulate the transcription of *csgD*, which encodes a biofilm regulator essential for producing amyloid curli fibres of the biofilm matrix. Since the c-di-GMP controlling module of YdaM/YciR is highly specific for regulation of *csgD* transcription, we investigated a potential local signalling by the global second messenger.
Methods: For determination of *csgD* transcription regulation, we performed genetic epistasis experiments by measuring gene expression using *lacZ* reporter gene fusions in various mutation backgrounds. Protein interaction studies *in vitro* and *in vivo* show detailed protein domain contacts. Furthermore, we confirmed altered protein activity by complex formation using enzymatic and DNA binding assays.
Results: Here, we demonstrate that this system operates as a signalling cascade, in which c-di-GMP controlled by the DGC/PDE pair YegE/YhjH (module I) regulates the activity of the YdaM/YciR pair (module II). Via multiple direct interactions, the two module II proteins form a signalling complex with MlrA. YciR acts as a connector between modules I and II and functions as a trigger enzyme: its direct inhibition of the DGC YdaM is relieved when it binds and degrades c-di-GMP generated by module I. As a consequence, YdaM then generates c-di-GMP and—by direct and specific interaction—activates MlrA by altering its DNA binding which leads to stimulation of *csgD* transcription.
Conclusion: Altogether, we present a novel concept of local c-di-GMP signalling where not only an EAL domain protein acts as a trigger enzyme but where also a GGDEF domain protein acts as a co-activator for transcription and as a DGC. Bifunctionality, i.e. the combination of DGC/PDE activity with highly specific protein-protein interactions, may represent a general principle in local c-di-GMP signalling.

SMeP04
SiaABCD interconnects c-di-GMP and RsmA signalling during surfactant stress in *Pseudomonas aeruginosa*
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Introduction: The presence of macroscopic cell aggregates in response to surfactant exposure has recently been shown to increase the fitness of *Pseudomonas aeruginosa* in the presence of additional stressors[1]. Further studies identified the c-di-GMP dependent signaling pathway SiaABCD as a central hub for phenotype expression by regulating several target genes, including those encoding for the adhesive fimbriae CupA[2].
Objectives: This study was conducted to gain a deeper understanding of the underlying molecular mechanisms involved in macroscopic aggregate formation.
Methods: Mutagenesis and phenotypic assays in combination with transcriptional reporter, qPCR and surface plasmon resonance was used to investigate the SiaABCD dependent regulation of the adhesive fimbriae CupA.
Results: We demonstrate that the RsmA/Y/Z system is involved in cellular aggregation during surfactant exposure and identify the *cupA* mRNA as a novel target for RsmA regulation. We further provide strong evidence that the SiaABCD pathway itself is under posttranscriptional control, which directly links RsmA and c-di-GMP signaling *via* the mRNA stability of the di-guanylate cyclase encoded by *siaD*.
Conclusion: From our data we suggest that the SiaABCD- and RsmA/Y/Z pathways operate in a dynamic equilibrium *in vivo*. This would allow for a rapid and energy-efficient response to external stimuli by shifting the equilibrium in favor of either component, resulting in cellular aggregation trough SiaD dependent biosynthesis of c-di-GMP or a freely suspended lifestyle of the cell by - at least in part - the RsmA dependent regulation of the SiaABCD pathway.

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SMeP05
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 secondary messenger found in Gram-positive bacteria, which is involved in many diverse aspects of bacterial physiology such as cell wall metabolism, sporulation, detection of DNA-damage, potassium uptake, virulence, and osmotic stress response [1]. For the non-pathogenic *Corynebacterium glutamicum* that, besides its high relevance in biotechnology, serves as a model organism for pathogenic species such as *M. tuberculosis*, neither enzymes for synthesis and degradation of c-di-AMP have been identified nor has a function of c-di-AMP hitherto been established.
We here show the identification and characterization of the diadenylate cyclase (DAC) homolog DisA of *C. glutamicum* that produces c-di-AMP from two molecules of ATP but lacks its annotated function of DNA integrity scanning. Besides we identified and characterized the *cg2174* encoded phosphodiesterase (PDE^{Cgl}), which hydrolyzes c-di-AMP. Reportergene assay using the c-di-AMP sensing *yadO* riboswitch from *Bacillus subtilis* [2] as sensor showed, that in *C. glutamicum* c-di-AMP levels decreased in response to potassium limitation, whereas no changes

in reporter gene activity were observed in response to the DNA damage caused by mitomycin addition. Overexpression of *disA* as well as inactivation of *cg2174* 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 [3], which consist of the full-length CglK protein and a separate soluble protein harboring only the RCK domain, and both are essential for full CglK functionality. 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.
From these data 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.

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SMaP01
Title: Investigating the molecular basis of substrate specificity in reductive carboxylases: Towards engineering extender units of polyketides
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Introduction: The recently discovered crotonyl-CoA carboxylase/reductase (Ccr) is a highly efficient CO_2-fixing enzyme that catalyzes the NADPH-dependent carboxylation of crotonyl-CoA to ethylmalonyl-CoA. Ccr is a key enzmye in the ethylmalonyl-CoA pathway, a central metabolic pathway used for acetate assimilation[1]. Close homologs of Ccr were also found in bacterial secondary metabolism where they catalyze the activation of extender units in the biosynthesis of polyketides, an extremely diverse and often bioactive class of molecules. Interestingly these homologs do not only carboxylate the "standard" substrate crotonyl-CoA, but also more exotic CoA thioesters like octenoyl-CoA, isobutenoyl-CoA, or chloro-crotonyl-CoA [2].
Objective: To establish a systematic screen for assessing substrate promiscuity of selected Ccrs and homologs in high throughput fashion. To identify and change specificity determining amino acids at the active site of selected candidates to engineer substrate promiscuity.
Methods: A library of potential substrates was synthesized and purified. In parallel a library of selected Ccrs and homologs from different host organisms were expressed in *E.coli.* Enzyme assays of all possible combinations were conducted and analyzed by mass spectrometry. Mutants of model Ccr and homologs were produced and screened against the substrate library.
Results: Screening revealed a greater substrate promiscuity of some homologs than previously expected. This promiscuity was successfully introduced into a non-promiscuous Ccr by site directed mutagenesis of the active center.
Conclusion: Substrate promiscuity is determined by three amino acids. Promiscuity of novel Ccrs and homologs can be predicted by sequence analysis and changed by active site mutagenesis.

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SMaP02
***In-vitro* antitumor activity and metabolic fingerprinting of the actinomycetes isolated from various ecological niches in Pakistan**
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Actinomycetes are Gram positive bacteria with high G+C content in their DNA and are capable of producing variety of secondary metabolites. Many of these metabolites possess different biological activities and have the potential to be developed as therapeutic agents. The aim of the present study was to screen actinomycetes of various ecological niches in Pakistan, for cytotoxic, anti-proliferative and antitumor compounds. In this screening program, several water and soil samples were collected from areas in Pakistan and more than 500 actinomycete isolates were recovered by selective isolation techniques. Among them 120 isolates were selected for identification and nonspecific cytotoxicity. The strains were identified on the basis of their morphological, biochemical, physiological and genetic characterization. The morphological characteristics of mycelium and spores were studied by scanning electron microscopy (SEM). In a biological screening the crude extracts obtained from the culture broth of selected strains were analyzed for their cytotoxic activity by brine shrimp microwell cytotoxicity assay against *Artimia salina* larvae. The isolates such as MKA 17, SSA 13 and KML 2 showed highest larvicidal activity with having 92%, 84% and 84% larval mortality respectively. Theses potent isolates were selected and were tested for anti-tumor or anti-proliferative activity against various proliferative cell lines including Hela, MD-BK, Vero etc., by methyl thiazolyl tetrazolium (MTT) bioassay method. 10 isolates were selected with high inhibition rate against proliferative cell lines among which KML 2 and SSA 13 showed highest activity with having IC_{50} values of 12.17µg/ml and 16.4 µg/ml against hela cell line respectively. Chemical screening profile (the so-called metabolic finger printing) by TLC and UPLC-MS, showed very distinctive chemical diversity in the crude extracts of the selected isolates. The genetic characterization by 16S rRNA gene sequencing exhibited maximum genetic similarity upto 100% with different species of the genus *Streptomyces*.

SMaP03
Transcriptomic profiling of *Pseudozyma aphidis* during mannosylerythritol lipid synthesis
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Question: An increasing number of fungi are described that produce the promising biosurfactant mannosylerythritollipid (MEL) [1]. But still the regulation mechanisms of MEL-biosynthesis and its natural role remain unknown. *Pseudozyma aphidis* is able to convert vegetable oils to abundant amounts of MEL-A, -B, -C and -D, a product pattern which is unique among all observed *Pseudozyma* strains. To investigate the metabolism of MEL production, we analyzed the transcriptome of *P. aphidis* under MEL-inducing and non-inducing conditions.We expected to find gene clusters and regulatory networks relevant for the production of MELs which may offer a starting point for metabolic engineering.
Methods: After annotating all gene models manually using transcriptome data, MEL-synthesis was induced in dependence of vegetable oil. The transcriptional profile was analysed by RNAseq.
Results: The experimentally verified genome annotation led to a number of 6,347 genes. Using this database, our expression analysis revealed a vegetable oil dependent upregulation of the MEL-Cluster with exception of the acetyltransferase *PaGMAT1*. This may explain the secretion of MEL with different degrees of acetylation. We further observed morphological changes in parallel to MEL secretion, accompanied by expression of genes responsible for cell development. Beside further metabolic pathways a set of transcription factors was identified which may be responsible for regulation of MEL-synthesis and cell development.
Conclusion: The collected data reflect a specific transcriptomic profile of *P. aphidis* during MEL production. Especially cell expansion and nitrogen metabolism seem to be further morphological and metabolic processes which are co-regulated with glycolipid synthesis.

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SMaP04
Antithelmintic and cell death inducing effect of tropodithietic acid
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Marine organisms produce a plethora of bioactive substances, which are of high interest in drug discovery programs. Latest research showed their potential to alter neurological activity (1). While crude extracts of *Sulfitobacter*, member of the marine Roseobacter group, showed a growth inhibiting and apoptotic effect on different cancer cell lines (2), only little is known about the therapeutic potential of the antibiotic tropodithietic acid (TDA) produced by the marine bacterium *Phaeobacter inhibens* and related species (3,4). In this study, we analyzed cytotoxic and neurological effects of TDA by testing for antinematode and cell death inducing effects using the multicellular model organism *Caenorhabditis elegans* and two representative cell lines of the Central Nervous System (CNS). The used model systems include both established multicellular organism and non-main stream cell lines such as the permanent oligodendroglial cell line OLN93 and mouse derived Neuroblastoma 2A cells (N2A). Initial results indicated a cytotoxic effect on *C. elegans* as well as cell death inducing properties on these cell lines.

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SMaP05
Genome Analysis of Secondary Metabolism in *Ustilago maydis*
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Introduction: Secondary metabolites (SM) have roles in a range of cellular processes such as transcription, development and intercellular communication. In fungi, the genes required for the biosynthesis of SM are clustered. Despite the large number of known bioactive compounds produced by fungi, their biosynthetic potential is underestimated due to many of these SM gene clusters are silent under standard laboratory conditions.
Objectives: For having better understanding of the biosynthetic potential of *U. maydis*, three main objectives have been proposed: identification of potential SM gene clusters, identification of novel metabolites by forced expression of silent gene clusters and elucidation of global and pathway-specific regulators of secondary metabolism.
Methods: For the identification of potential SM gene clusters two strategies were followed: the first one consisted on the search of central biosynthesis genes by taking advantage of bioinformatics tools (SMURF and antiSMASH) and the well annotated *U. maydis* genome sequence. The second strategy was to seek groups of co-regulated gene clusters in the microarrays publicly available of *U. maydis.*
Results: The overexpression of the transcription factors associated with the potential biosynthetic gene clusters identified in both strategies allowed the identification of one gene cluster that triggers the production of a green-brownish pigment. Single knockout mutants of the cluster genes were generated in order to determine the role of each gene in the production of the pigment by LC-MS.
Conclusions: Genome wide-analysis of *U. maydis* allowed the identification of one SM gene cluster which upon its activation triggers the production of a green-brownish pigment. Further experiments need to be performed in order to determine the its biological role in *U. maydis*.

SMaP06
A novel two-stage fermentation system to produce polyunsaturated fatty acids and xanthophylls from brown seaweed extracts
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Introduction: Seaweeds are an attractive, sustainable feedstock for fermentation due mainly to their uncompetitiveness with the food supply in terms of land use and their availability in the open ocean and along shorelines [1]. However, they cannot be readily assimilable by widely used fermentative strains due to their unusual carbohydrate composition. This, therefore, emphasizes the need for the development of pre-treatment steps before they can be utilized. In this study, a two-stage fermentation system that will use brown seaweed extracts to produce polyunsaturated fatty acids (PUFA) and xanthophylls was developed.
Methods: Fermentation was divided to two stages; the first stage is the mannitol to fructose conversion by *Gluconobacter oxydans* while the second is the fructose consumption by the protist *Aurantiochytrium* sp. KH105 to produce the desired products. Sea salts solution was introduced between the stages to shift the productivity from the first organism to the second. Finally, aqueous extracts of brown seaweed was used as culture feed for a bench-top fermenter scale application of the system.
Results: Bench-top fermenter culture using brown seaweed extract medium resulted to efficient mannitol to fructose conversion by *G. oxydans*. After its bacteriostasis by the addition of sea salts solution, the *Aurantiochytrium* sp. KH105 strain yielded substantial amounts of the PUFA docosahexaenoic acid and the xanthophyll astaxanthin.
Conclusion: This study further contributes to the increase in the number and variety of value-added compounds that can be sourced from seaweed feedstock. Also, the simple modification of the productive strain in this study to other strains can lead to the production of a wider range of metabolites from brown seaweeds.

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SMaP07
Investigating the potential of Hülle cells in *Aspergillus nidulans*
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Question: Filamentous fungi have an enormous potential to secrete proteins and are therefore widely used in biotechnology, whereas the secretion potential of yeasts as single cell fungi is often limited. It is our aim to explore whether we can use specialized cells of constitutively filamentous fungi as single cell tools for biotechnology. We started to address this issue by using Hülle cells. Hülle cells are a specialized and unique cell type that is specific to the genus *Aspergillus.* They are globose single cells that contain several nuclei. In the model organism *Aspergillus nidulans* Hülle cells emerge from hyphal tips after entering sexual development and form an envelope around the closed developing fruiting body (cleistothecium). In the absence of Hülle cells cleistothecia do not reach maturity suggesting that they act as auxiliary nursing cells. Hülle cells express numerous hydrolyses which might produce building material for the fruiting body. They might also contribute to the defence against fungivore attack by secreting secondary metabolites.
Methods: We are investigating (i) whether we can construct Hülle cells with different nuclei to expand the genetic potential, (ii) whether we can enrich Hülle cells i.e. obtain cultures with Hülle cells as the major growth form, (iii) whether we can use Hülle cells as tools for biotechnology to specifically produce secondary metabolite. We address these questions by investigating the role of Hülle cells in the development of the sexual fruiting body and their ability to germinate, to nurse and/or protect the cleistothecium. We are combining the analysis of deletion strains with altered Hülle cell formation with global systems biology analyses to obtain a comprehensive understanding of the biological role of Hülle cells.
Results: *Results have not been obtained yet.*
Conclusion: These findings should help answering the question whether and how Hülle cells as single cells of filamentous fungi can be used for biotechnological applications.

SMaP08
Acetylcholinesterase Inhibitors from a Marine Fungus *Talaromyces* sp. strain LF458
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Introduction: There is a strong demand to discover new molecules which could be potential drugs for treatment of Alzheimer`s disease. Worldwide, nearly 36 million people have Alzheimer`s or related dementia. It is estimated, that by 2050 the prevalence will be tripled. Most of the drugs currently available are inhibitors of, such as tacrine, donezepil, rivastimine, and galanthmaine.
Objectives: Fungi from marine habitats are a rich source of new natural products which might be promising drug candidates for the treatment of Alzheimer`s disease.
Methods: Microbial fermentation, analytical reversed phase HPLC-DAD(UV)-MS, preparative HPLC, NMR analyses, and bioactivity assays were applied.
Results: *Talaromyces* sp. strain LF458, a fungus associated with the marine sponge *A. verrucosa*, produces two new oxaphenalenone dimers, talaromycesone A (**1**) and talaromycesone B (**2**), and a new isopentenyl xanthenone, talaroxanthenone (**3**), together with six known diphenyl ether derivatives, e.g., $\Delta^{1',3'}$,-1'-dehydroxypenicillide (**4**), 1',2'-dehydropenicillide (**5**), vermixocin A (**6**), vermixocin B (**7**), 3'-methoxy-1'2'-dehydropenicillide (**8**), and AS-186c (**9**). Compound **2** represents the first example of 1-nor oxaphenalenone dimer carbon skeleton. Compounds **1**, **2**, and **9** exhibited potent antibacterial activities with IC_{50} 3.70, 17.36, and 1.34µM, respectively, against human pathogenic *Staphylococcus* strains. Compounds **1**, **3**, and **9** displayed potent acetylcholinesterase inhibitory activities with IC_{50} 7.49, 1.61, and 2.60µM, respectively. Interestingly, phosphodiesterase PDE-4B2 was inhibited by compounds **3** (IC_{50} = 7.25µM) and **9** (IC_{50} = 2.63µM).
Conclusion: Talaromycesone B (**2**) is the first 1-nor oxaphenalenone dimer skeleton isolated from a natural source. The two new oxaphenalenones **1** and **2** possess similar antibacterial activities, despite their different carbon skeleton. The new compounds **1** and **3** as well as the known AS-186c (**9**) inhibit the activity of acetylcholinesterase. To our best knowledge, this is the first report on an oxaphenalenone acting as acetylcholinesterase inhibitor.

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SMaP09
Structural diversity of ergot alkaloids and genetic potential for their biosynthesis
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Introduction: Ergot alkaloids are important mycotoxins and meanwhile drugs. Naturally occurring ergot alkaloids as well as their semisynthetic derivatives are used as pharmaceuticals in modern medicine [1].
Objectives: To investigate the distribution of ergot alkaloids and the diversity of their biosynthesis, we searched for putative ergot alkaloid genes in the genome sequences of ascomycetous fungi. In total, 207 putative ergot alkaloid biosynthetic genes were analyzed and compared.
Methods: To identify putative ergot alkaloid gene clusters in various fungi, the sequences of the seven homologous genes from *Aspergillus fumigatus* and *Claviceps purpurea* were obtained from the NCBI database and used for screening the available fungal genomes by using the program "BLAST". The sequence identities of the homologues were calculated by using the tool "EMBOSS Needle". Multiple sequence alignments with all identified homologous proteins were carried out by using the program "MUSCLE" and visualized with "ESPript 2.2" to identify the strictly conserved amino acid residues. The phylogenetic trees were created with the help of the tool "Phylogeny.fr".
Results: We identified ergot alkaloid gene clusters in 33 fungi, belonging to the families of Aspergillaceae, Clavicipitaceae, Arthrodermataceae, Helotiaceae and Thermoascaceae. Detailed analysis of the genes showed that fungi with putative ergot alkaloid gene clusters were widely distributed in the mentioned phylum. Within the identified families, they are however only present in a small taxa.
Conclusion: Literature search revealed a great diversity of ergot alkaloid structures in different fungi [2]. This diversity was not limited to the same genera, also fungi of the same species showed differences on ergot alkaloids. In addition to the diversity of chemical structures, investigations on the stereochemistry of ergot alkaloids were made in the last years. From these results, it could be speculated that many new substances will be isolated in future.

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SMaP10
Heterologous expression of biosynthetic gene clusters in *Bacillus subtilis*
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Introduction: *Bacillus subtilis* belongs to the most frequently used cell factories for the industrial production of recombinant proteins. This is mainly due to its growth properties, effective secretion machinery and the lack of toxic by-products.
Objectives: For the optimization of this expression host and for the stable and functional establishment of biosynthetic gene clusters either gene knock-outs or knock-ins are required.
Methods: To achieve this, an optimized genome editing protocol for *B. subtilis* using transformation-enhancing sequences in combination with the Cre-*lox*-system for marker gene removal has been developed [1].
Results: With 6-deoxyerythronolide B, the macrolide core of erythromycin, and enniatin B, a fungal cyclohexadepsipeptide, as two examples the expression of a heterologous polyketide synthase (PKS) and a non-ribosomal peptide synthetase (NRPS) was investigated in *B. subtilis*. This big two heterologous gene clusters could be functionally established in the genome and on a multi-copy-vector in this bacterial host. Modifications in the cultivation conditions combined with the deletion of selected target genes or gene clusters were addressed to optimize the heterologous expression of these model PKS and NRPS clusters [2].
Conclusion: It could be shown that *B. subtilis* is a suitable heterologous host for the secretory production of complex polyketides or non-ribosomal peptides

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SMaP11
Co-cultivation of *Serratia plymuthica* and *Bacillus subtilis* leads to interaction zones
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Introduction: Rhizobacteria live associated with plant roots. Because the plant releases spare nutrients into the soil, this habitat is tremendously populated by rhizobacteria. The rhizobacterial survival in this interactive zone depends on one hand on their ability to compete for water and nutrients, but on the other hand on the production of secondary metabolites. Up to now, research has mainly focused on secondary metabolites released by individually cultured bacteria. Recently, it was shown that various secondary metabolites are only produced due to microbial interaction [1].
Objectives: We question, how the co-cultivation of bacteria alters the rhizobacterial secondary metabolite profile and further whether there is an effect of this altered metabolites on other bacteria. Here, we will present the initial screening to find interactive potential between *Serratia plymuthica* species and *Bacillus subtilis*.
Methods: To initially evaluate the interactive potential of different rhizobacterial isolates, co-cultivations of different rhizobacteria on agar plates were performed. The growth of co-cultivated rhizobacteria was compared with the growth of self-paired rhizobacteria. Since, the interaction might be influenced by nutrients, complex medium and soil simulating medium (SSM) were used.
Results: Co-cultivation of different *S. plymuthica* sp. with one *B. subtilis* strain revealed species-specific effects on the growth of *B. subtilis*. While

the co-cultivation of *S. plymuthica* 4Rx13 with *B. subtilis* led to a strong interaction zone, this distinct interaction zone wasn't present in co-cultivations between *S. plymuthica* AS9 and *B. subtilis*. The strong interaction zone was appearing on complex medium and SSM. One difference between the two *Serratia* strains is the production of the fungicide oocydin A. By testing oocydin A knock-out mutants we couldn't observe an effect of oocydin A on *B. subtilis*.
Conclusion: Co-cultivation studies revealed a strong interaction between *S. plymuthica* 4Rx13 against *B. subtilis*. The active agents are unknown so far, and the isolation and identification is the focus of our current research.
1. Schroeckh et al. (2009) Intimate bacterial-fungal interaction triggers biosynthesis of archetypal polyketides in *Aspergillus nidulans*. PNAS

SMaP12
The role of histone acetyltransferases in the secondary metabolism of *Aspergillus fumigatus*
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Introduction: Genome analyses of the human pathogenic fungus *Aspergillus fumigatus* have revealed a large number of putative secondary metabolite (SM) biosynthesis genes commonly found in specific SM gene clusters. However, only a few SM gene clusters are activated under standard laboratory conditions, making it difficult to assign SMs to their corresponding gene clusters. One approach to identify silent gene clusters uses the regulation of gene expression via histone modifying enzymes. Especially, modification of histone acetylation can be achieved by deletion of histone acetyltransferases (HATs), which are generally involved in global gene activation. Previous studies in the model organism *Aspergillus nidulans* have demonstrated that HATs play an important role in secondary metabolism, strikingly during the interaction with the bacteria *Streptomyces rapamycinicus* (1). In the pathogenic fungus *A. fumigatus* approximately 50 putative acetyltransferase-encoding genes were identified. By creating a complete HAT deletion library we aim to broaden the understanding for their global function and in particular their role in microbial interaction, regulation of SM gene clusters and pathogenicity of the fungus.
Methods: Therefore, all target genes were deleted by homologous integration of a selection marker. Targets with lethal deletions were covered using an inducible promoter system. Confirmed mutants were extensively examined on different stress conditions. Co-cultures of deletion mutants with *S. rapamycinicus* were conducted to study the HAT's role in the microbial interaction and SM production. Moreover, mutants were analyzed for new and known activated SMs in monoculture by an LC-MS approach.
Results: By screening the complete HATs library we identified several mutants with growth defects under different conditions. LC-MS analysis revealed also mutants with altered SM production in monoculture. Further, data on the involvement of HATs in microbial interaction will be also presented.
Conclusion: The continuing work on the HATs in *A. fumigatus* will help identifying their exact functions and hopefully help in understanding their roles in both secondary metabolite production and virulence.

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SMaP13
Identification and characterisation of new biosurfactants from basidiomycetes
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Many fungi are able to synthezise complex secondary metabolites. These are not essential for viability but procure the fungi advantages over other microorganisms.
One important group of secondary metabolites are glycolipids. These amphipathic, surface-active compounds act as biosurfactants. In general they increase the availability of hydrophobic nutrients and enhance attachment to nonpolar surfaces. In some cases they also display antimicrobial actvity and play an important role in biofilm formation.
The basidomycetous fungus *Ustilago maydis* is known to produce large amounts of two structurally different extracellular glycolipids under nitrogen starvation. Ustilagic acid (UA), which is a cellobiose lipid with antimicrobial activity and the extracellular oil mannosylerythritol lipid (MEL). The genes, which are essential for the synthesis of both biosurfactants were identified, and showed clustered formation in the genome.
In our project we want to identify new biosurfactants first from basidiomycetes and later from other fungi, which are collected by our research partners from the Integrative Fungal Research Group (Frankfurt/Main). We will sequence the genomes and identify the gene clusters of the biosurfactants. Then the new biosurfactants will be characterised concerning their biotechnological applications.

SMaP14
Production of new secondary metabolites upon addition of small-molecule epigenetic elicitors to fungal endophytes fermentations
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Introduction: Small molecule Histone Deacetylase (HDAC) and DNA methyltransferase (DNMT) inhibitors are being used to perturb the production of fungal metabolites, leading to the induction of the expression of silent biosynthetic pathways. Several reports have described the variable effects observed in natural product profiles in fungi treated with HDAC and DNMT inhibitors, with enhanced chemical diversity or new molecules previously unknown to be produced by the organism. Fungal endophytes are known to produce a wide variety of secondary metabolites (SMs) involved in their adaptation and survival within higher plants. The plant-microbe interaction may influence the expression of some biosynthetic pathways, otherwise cryptic in these fungi when grown in vitro.
Objectives: The aim of the study was to identify the effect of several HDAC and DNMT inhibitors on the metabolic profile of selected fungal endophytes and the chemical characterization of the new SMs produced.
Material and Methods: Seven small molecule elicitors with different chemical structure and targets were added to the production medium of eight fungal endophytes that were cultivated for 7 and 14 days. Ultra-HPLC chemical profiles of extracts were analyzed to evaluate the general influence of these compounds in generating new chemical entities or in over-expressing the production of specific SMs by fungal endophytes in submerged cultivation conditions.
Results: We have observed three different behaviors within the group of endophytes tested: 3 strains show few changes in metabolic profile, another 3 species produce higher amounts of previously observed SMs and two species show new SMs that were not observed in non-elicited fermentations.
Conclusions: These preliminary results confirm the differential expression of silent pathways within this group of fungi in the presence of specific elicitors

SMaP15
In vitro* reconstitution of the alkylquinolone biosynthesis pathway of *Pseudomonas aeruginosa
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Introduction: *Pseudomonas aeruginosa* synthesizes a set of alkylquinolone-type secondary metabolites (AQs) with antibacterial activity. 2-Heptyl-3-hydroxy-4(1*H*)-quinolone (PQS) and its biosynthetic precursor 2-heptyl-4(1*H*)-quinolone (HHQ) also act as signal molecules in quorum sensing [1]. 2,4-Dihydroxyquinoline (DHQ), a metabolite of unknown function, and 2-aminoacetophenone (2-AA), which triggers chronic infection phenotypes, also branch off the AQ biosynthetic pathway [2]. Based on gene knockout and heterologous expression experiments, it was proposed that HHQ biosynthesis depends on the genes *pqsABCD* [2-4].
Objectives: To gain a detailed understanding of the reactions involved in AQ biosynthesis, we attempted to reconstitute HHQ synthesis *in vitro*.
Methods: Products formed in enzyme assays containing (*i*) individual Pqs proteins, (*ii*) combinations of Pqs proteins and (*iii*) Pqs proteins supplemented with soluble cellular proteins were analyzed using HPLC/MS and thin layer chromatography. Substrate analogs were used to detect analogs of unstable intermediates.

Results: While the current model of the AQ biosynthesis pathway suggests that PqsD condenses anthraniloyl-CoA and malonyl-CoA, and PqsBC introduces an octanoyl moiety into the intermediate 2-aminobenzoylacetate (2-ABA) to form HHQ [2], our *in vitro* assays with PqsD and PqsBC failed to produce HHQ, but exclusively yielded DHQ. However, addition of cell extracts led to HHQ formation, suggesting yet unknown factors to be involved. It turned out that, besides PqsD and PqsBC, a thioesterase cleaving 2-ABA-CoA, whose existence was indirectly proven with analogs, is required for HHQ formation.
Conclusion: The PqsABCD proteins of *P. aeruginosa* do not suffice for biosynthesis of HHQ. The pathway involves a thioesterase that releases 2-ABA from 2-ABA-CoA. Housekeeping thioesterases support this reaction to some extent, explaining why *pqsABCD* enable HHQ synthesis in a heterologous host. In *P. aeruginosa,* the activity of the thioesterase may affect the ratio of the secondary metabolites HHQ, DHQ and 2-AA.

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SAP01
Dual (activation/inhibition) interplay of the PtsN protein of *Pseudomonas putida* with its target sensor kinase KdpD
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The nitrogen branch of the phosphotransferase system (PTS^{Ntr}) of *Pseudomonas putida* is a multicomponent regulatory device that participates in controlling a variety of physiological processes in a post-translational fashion. *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}.
A general survey of the genes regulated by PtsN in *P. putida* revealed that transcription of the entire *kdpFABC* operon is influenced by this protein. In order to investigate this in detail, we constructed a *kdpF*-promoter (*kdpFp)* reporter plasmid by fusing the promoter region of the *kdpFABC* operon to the *lux* gene reporter system. This allowed us to thoroughly analyse the activity of this promoter in various strains and conditions in a highly time resolved manner. Therefore, we chose otherwise isogenic mutants of *P. putida* KT2440 with a defined deletion of either *ptsP*, *ptsO*, or *ptsN* and a strain expressing a PtsN variant that is fixed in the non-phosphorylated form (*ptsNHA*). This unambiguously showed that PtsN is responsible for both, repression and activation of the transcription of the *kdpFABC* genes, and that regulation depends on the phosphorylation state of PtsN, in a way that non-phsophorylated PtsN stimulates transcription whereas PtsN~P seems to have the opposite effect. Bacterial two-hybrid assays were employed to give insight into the mechanism of regulation. They clearly demonstrated that the regulation is implemented through direct interaction of the PtsN protein with the sensor kinase KdpD of the KdpD/KdpE two-component system. Interaction between KdpD and PtsN was detectable with a PtsN variant that imitates the non-phosphorylated form as well as with a PtsN type mimicking the phosphorylated form of PtsN.
These results raise a regulatory scenario that bear a resemblance to -but is ultimately different of the state of affairs in *E. coli,* where only non-phosphorylated PtsN interacts with KdpD. Thus, in *P. putida* the same regulatory parts are involved in controlling transcription of the *kdpFABC* genes, but the mechanism has diversified in a way that the system can both be induced and turned off by the alternative forms of the PtsN protein.

SAP02
Investigation of the phospho-protein Saci_1079 from *Sulfolobus acidocaldarius* DSM639
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Posttranslational modifications are of major interest for the regulation of cellular processes. Reversible protein phosphorylation is one of the main mechanisms to control the functional properties of proteins in response to environmental stimuli [1]. In the 80's protein phosphorylation was demonstrated in the third domain of life, the Archaea and the knowledge about archaeal protein phosphorylation is very limited. Recently, we analyzed the phosphoproteomes from two thermoacidophilic Crenarchaea *S. solfataricus* P2 [2] and *S. acidocaldarius* DSM639 [3]. In both species a very high no. of phosphoproteins were identified, revealing that proteins involved in all cellular processes are targeted via protein phosphorylation. Especially, proteins of the central carbohydrate metabolism were found to be phosphorylated. Among the identified phosphoproteins was the putative sugar dehydrogenase Saci_1079 from *S. acidocaldarius*, which is the first enzyme of the branched Entner-Doudoroff pathway (glycolysis).
Her we present the detailed biochemical investigation of Saci_1079. The enzyme was overexpressed in the bacterium *E. coli*, to investigate the un-phosphorylated enzyme, and also in *S. acidocaldarius*, to investigate the phosphorylated version. Furthermore, the identified phosphorylation sites (T328, S329 and T344) were changed to the negative amino acid Asp, to imitates the negative influence of the phosphorylation site [4], in order to analyze the impact of the phosphorylation on the biochemical properties of Saci_1079. In addition, a deletion mutant of this gene was constructed and his growth phenotype analyzed to investigate how protein phosphorylation regulates the metabolism.

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SAP03
Signal processing and transmission to the chemotaxis pathway by the phosphotransferase system in *E.coli*
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Introduction: The phosphotransferase system (PTS) of *E. coli* plays a pivotal role in coordinated uptake of sugars in *E. coli* and other bacteria. It also transmits signals to the chemotaxis pathway, mediating tactic responses to multiple sugars (1,2). Although several examples of sugar uptake regulation by the PTS have been extensively studied, the overall signal processing by the PTS network and transmission to the chemotaxis system remain poorly understood.
Objectives: We aimed to quantify cellular processing of signals from different PTS sugars as well as to understand the molecular mechanisms of PTS signal transmission to the chemotaxis pathway in *E. coli*.
Methods: Using previously known interactions we constructed FRET reporters to quantitate PTS signaling parameters. We used FRET to map and characterize interactions between the PTS components and between the PTS and the chemotaxis proteins.
Results: Our FRET reporters reliably sensed concentration-dependent changes at several levels of phosphotransferase cascade. Dose-response curves for PTS sugars could be measured with PTS- and chemotaxis-specific FRET reporters. We observed that all curves aligned with approximately equal apparent EC50 values, suggesting that that there is no amplification or attenuation of the PTS signals within the cascade. We further observed inhibition of interactions between the EI and $EIIA^{Glc}$ PTS components with CheA and CheW subunits of the chemosensory complex upon stimulation with PTS sugars, suggesting a mechanism of the signal transmission.
Conclusions: Our results indicate that PTS processes signals in narrow micromolar range and simultaneously performs regulatory roles such as inducer exclusion and chemotactic signaling, which primarily depend on phosphorylation state of the PTS components. PTS signal transmission to the chemotaxis pathway occurs by inhibition in interaction of phosphorylated EI and $EIIA^{Glc}$ with CheA and CheW, which inhibits CheA kinase activity. Intracellular PTS signals are transmitted linearly to the chemosensory complex and in contrast to periplasmic signals do not undergo modulation on processing by the chemotaxis system.

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SAP04

New insights into a nutrient-sensing network in *Escherichia coli*

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Histidine kinase / response regulator (HK/RR) systems represent a prevalent bacterial mechanism to respond to changing environmental conditions. Upon nutrient limitation, *E. coli* activates a network of two HK/RR systems, YehU/YehT and YpdA/YpdB, to facilitate optimal carbon scavenging [1].
The network becomes active at the transition from mid- to late-exponential growth phase and regulates resource selection upon carbon limitation. External pyruvate stimulates the YpdA/YpdB system, which results in expression of *yhjX,* encoding a putative carboxylate transport protein. The YehU/YehT system is stimulated in media containing peptides or amino acids and regulates the expression of *yjiY,* encoding a putative carbon starvation transport protein. Moreover, interconnectivity between the transporters and both HK/RR systems was observed, suggesting formation of a large signalling unit.
In order to get further insights into the regulatory dynamics within this nutrient-sensing network, the ligands and substrates of the HKs and transporters need to be verified by biochemical methods. Furthermore, the effect of different environmental cues, e.g. pyruvate, lactate and serine, on signal transduction is studied.

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SAP05

A novel type of signal transduction cascade in *Corynebacterium glutamicum*

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Introduction: We previously identified a novel regulatory mechanism for the TCA cycle enzyme 2-oxoglutarate dehydrogenase (ODH) in *Corynebacterium glutamicum*, a Gram-positive soil bacterium used for the production of amino acids and proteins. The key protein is OdhI, which in its unphosphorylated state binds to the OdhA subunit of the ODH complex and inhibits its activity. Phosphorylation of OdhI by the Ser/Thr protein kinase PknG abolishes binding to OdhA and inhibition of ODH activity [1-4]. ODH inhibition by OdhI was shown to be crucial for glutamate production [5]. The *pknG* gene is located downstream of *glnX* and *glnH*, which code for a membrane-integral protein and a putative glutamine-binding lipoprotein, respectively. Like a Δ*pknG* mutant, Δ*glnX* and Δ*glnH* mutants showed a growth defect on glutamine, indicating a functional association of GlnH, GlnX, and PknG [1].
Objectives: Gaining further evidence for the proposed signal transduction cascade by analysing the properties, localisation and interaction of the proteins GlnH, GlnX, and PknG.
Methods: Various biochemical assays for analysing protein properties, localisation, and protein-protein interaction.
Results:
1. Globomycin, a lipoprotein signal peptidase inhibitor, as well as a Cys27Ala exchange in GlnH, prevented processing of a 50-kDa form of GlnH to the 45 kDa mature form.
2. GlnH could be co-purified with *Strep*-tagged GlnX.
3. GlnX could be co-purifed with His-tagged GlnH.
4. GlnH was predominantly detected in the membrane fraction.
5. GlnX was exclusively detected in the membrane fraction.
6. PknG, which lacks transmembrane helices, was predominantly found membrane associated in glucose-grown cells, whereas an equal distribution between soluble and membrane fraction was found in glutamine-grown cells.
Conclusions: Our results indicated that GlnH is in fact a lipoprotein and support the proposed signal transduction cascade by providing evidence for the interaction of GlnH and GlnX and by showing that the membrane-association of PknG can be varied by the carbon source.

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SAP06

Biochemical and functional analysis of 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]. In order to investigate 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, with only the second GAF domain (sGAF2), 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 wild type MA0863-sGAF2 were determined to investigate the redox sensory function. Also, the redox potential of protein variants lacking the cysteine residue for heme binding was determined to examine whether the covalent linkage has an influence on the oxidation state of sGAF2. 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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SAP07

Heterogenous activation of *Vibrio harveyi* cascade is determined by its phosphorylation flow

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Bacterial populations are able to communicate via autoinducers in a process called quorum sensing enabling the population to coordinate and synchronize specific behaviors. The bioluminescent bacterium *Vibrio harveyi* integrates three autoinducers (AIs) signals into one quorum sensing cascade. The autoinducers (AI-2, HAI-1, CAI-1) are produced by three synthases (LuxS, LuxM, CqsA) and sensed by their cognate sensors, LuxQ, LuxN and CqsS, respectively. The information is transduced via phosphorelay and a Hfq/small RNAs switch to the master regulator LuxR, which controls several AIs-dependent genes and phenotypes.
It was shown, that the combinations of AIs available within different growth phases and media, rather than the cell density *per se* are crucial for the timing of various AIs-dependent phenotypes (1).
Using a new set of *V. harveyi* mutants lacking genes of the synthases and/or the sensors, we monitored bioluminescence as a direct read-out for quorum sensing. The autoinducer concentration and composition as well as phosphatase and kinase activities of the receptors fine-tune the signal transduction and tightly control the copy number of LuxR. Furthermore, quorum sensing regulated promoters were fused to fluorescent reporters to determine quorum sensing activation at the single cell level in different genetic backgrounds, with a specific focus on signal integration and noise levels. These studies revealed that the strength of the phosphorylation flow in the cascade drives the level of heterogeneity in the quorum sensing response. Under conditions where the quorum sensing cascade is not fully activated, single cells show increased noise in quorum sensing activation. We conclude that the ability to drive heterogenous expression with respect to quorum sensing regulated genes is an inherent feature of the architecture of the cascade. In a natural habitat where nutrients are hardly abundant, this behavior could be beneficial for the population by expressing both, quorum sensing negative traits (e.g., siderophore

production) as well as quorum sensing positive traits (e.g., proteolytic activity).

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SAP08

Specificity determinants of *bceA*-like promoters in *Bacillus subtilis*

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Introduction: Two component systems (TCSs) are comprised of a membrane-anchored histidine kinase (HK) that senses the stimulus and a cognate response regulator (RR), which can bind to specific promoter regions to trigger a cellular response. The genome of *B. subtilis* encodes three Bce-like TCS: BceRS, PsdRS and YxdJK [1], which share significant similarities and are predicted to have considerable cross-talk [2]. But instead they are insulated quite well from one another, with only minor cross-talk occurring between BceS and PsdR [3].
Objectives: Understand the mechanism ensuring the specific binding of BceR-like RRs to their cognate target promoters.
Methods: *In vivo* ß-galactosidase assay, *in vitro* electrophoretic mobility shift assay (EMSA) and surface plasmon resonance (SPR).
Results: Exchanging the currently known binding sites of BceR and PsdR did not result in any change of specificity. A detailed analysis of series of chimeric promoters identified the specificity determinants directly downstream of the known binding site. *In vivo* and *in vitro* assays of randomized promoters deconvoluted this region into a linker region and a second binding site. Subsequent *in vitro* assays demonstrated that the first binding site has much higher binding affinity compared to the second binding site.
Conclusion: The regulatory region within *bceA*-like promoters consist of a high affinity but low specificity main binding site, which enables the tight binding of a response regulator dimer, a non-conserved linker region, and a second low affinity/high specificity binding site directly downstream that allows discrimination between the highly paralogous BceR-like RRs, thereby determining specificity.

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SAP09

Unraveling the regulatory network of archaellum assembly in the crenarchaeon *Sulfolobus acidocaldarius*

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Introduction: The ability to move is an enormous fitness advantage for living organisms, as it allows them to leave inhospitable environments and to conquer more prosperous habitats. The organisms sense environmental stimuli and transmit this integrated information to the motility machineries in order to direct migration. The motility organelles are complex surface appendages that require a tight, precise and hierarchical regulation. *S. acidocaldarius* is able to induce archaellum biosynthesis in response to starvation[1]. In this organism we have identified archaellar regulatory network proteins that differentially regulate archaellum subunit expression in a phosphorylation dependent manner[2,3].
Objective: Our goal is to identify and characterize the different players of this reversible phosphorylation cascade.
Methods: In close proximity to the archaellum operon we have identified a putative membrane-bound S/T/Y kinase (Saci_1181). A deletion strain of this gene was generated and several assays were performed to analyze the archaellum biosynthesis at different levels such as signal sensing, expression, assembly and functionality.
Results: The Saci_1181 transcript was already induced shortly after starvation indicating a possible role in regulation of archaellum expression. Several archaellum components were expressed early at higher levels in the deletion mutant strain of this kinase suggesting that the tightly controlled response is impaired. Interestingly, although the archaellum is assembled in Δ*saci_1181*, this strain is less motile than the wild type strain as the result of a decreased number of swimming cells and reduced average speed.
Conclusion: Archaellum biosynthesis is the result of a precise and complex process, where each component has to be expressed at a specific time and defined level. Our results indicate that the kinase Saci_1181 would be responsible of ensuring that the archaellum works as a precision machinery.

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SAP10

Detailed protein-protein interaction studies between the PtsN protein and the sensor kinase KdpD of *Pseudomonas putida*

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The nitrogen branch of the phosphotransferase system (PTS^{Ntr}) of *Pseudomonas putida* is a multicomponent system that participates in the regulation of a variety of physiological processes. It consists of three proteins PtsP, PtsO, and PtsN, which form a phosphate transfer chain.
Recently, we could add a new role to the portfolio of actions of the PTS^{Ntr}: the regulation of transcription of the *kdpFABC* genes. This gene cluster encodes the high affinity KdpFABC potassium transporter and its transcription is regulated by the activity of the KdpD/KdpE two component system (TCS). From *kdpFp* promoter activity studies we had strong indications that the non-phosphorylated as well as the phosphorylated form of PtsN exert a regulatory function.
In this work we aimed to unravel the molecular mechanism of the regulation of transcription of the *kdpFABC* genes by the PtsN protein. To this end, we analysed in detail the protein-protein interaction between PtsN and its possible targets by means of bacterial two hybrid (BACTH) assays and with purified proteins. We could unambiguously show that the regulation is executed by direct physical interaction of the PtsN protein with the sensor kinase KdpD. Furthermore, the interaction between KdpD and PtsN was detectable with a PtsN variant mimicking the non-phosphorylated form as well as with a PtsN variant mimicking the phosphorylated form of PtsN.
Together with other findings these results raise a regulatory scenario that is comparable -but by no means identical to what is known in *E. coli,* where only non-phosphorylated PtsN interacts with KdpD. Thus, in *P. putida* the same regulatory parts participate in controlling transcription from *kdpFp*, but the mechanism has changed in a way that that the system can be both induced and turned off by the alternative forms of the PtsN protein.

SAP11

Polar localization of a tripartite complex of the two-component system DcuS/DcuR and the transporter DctA in *E. coli* depends on the sensor DcuS

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Although bacteria do not possess cell compartments some proteins exhibit a distinct localization within the membrane or the cytoplasm. The membrane-embedded C_4-dicarboxylate responsive sensor kinase DcuS of the DcuSR two-component system of *E. coli* displays a polar localization (1) and forms a functional sensor unit with the aerobic C_4-dicarboxylate transporter and co-regulator DctA (2).
Fluorescence microscopy studies demonstrate a dynamic and preferential polar localization of DcuS-YFP, even at chromosomal expression levels. The DcuS clusters showed high mobility in fast time lapse acquisitions as well as fast recovery in FRAP experiments, eliminating cluster formation as the result of DcuS-YFP aggregation. Cellular factors such as cardiolipin, the high curvature of the cell poles, and the cytoskeletal protein MreB were not required for polar accumulation of DcuS. However the cytoplasmic PAS_C and kinase domains of DcuS were necessary for its localization. In contrast, YFP fusions of the cognate response regulator DcuR and the co-regulator DctA were randomly distributed throughout the cytoplasm or the membrane, respectively. Presence of sufficient amounts

of DcuS however led to the co-localization of the DcuR and DctA fusions with sensor kinase.
Accordingly, DcuS is crucial for the formation of the tripartite DctA/DcuS/DcuR sensor/regulator complexes and their polar localization by trapping DctA and DcuR at the cell poles.

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SAP12
TM2-TM2 association is essential for DcuS homodimerization and functionality
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Membrane proteins play important roles in biological processes such as metabolism signal recognition, transport of ions and substrates as well as cell-cell communication. The oligomerization of transmembrane helices (TMs) is essential for the accurate membrane assembly and function of α-helical membrane proteins (1). These TM-TM interactions are influenced by sequence context as well as properties of the biomembranes. The C_4-dicarboxylate responsive sensor kinase DcuS of the DcuSR two-component system forms a functional dimer in the membrane of *E. coli* (2).
GxxxG-like sequence motifs, which form the framework for TM-TM interactions, were identified in TM1 and TM2 of DcuS and the homodimerization of both TMs was verified by the GALLEX (3) two-hybrid system. Site-directed mutagenesis of the residues of the tandem ^{186}Sxxx^{190}Gxxx^{194}G motif of TM2 in combination with reporter gene expression experiments and BACTH interaction studies demonstrate the importance of TM2-TM2 interaction for the formation of the functional DcuS dimer.
Thus, TM2 represents central interaction site for the DcuS-DcuS dimerization, which is essential for the function of the sensor kinase.

SAP13
In-vivo* Analysis of cAMP Signaling in *Escherichia coli
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Introduction: Cyclic adenosine monophosphate (cAMP) is a universal second messenger. Located at the center of a multi-input, multi-output network, in *Escherichia coli* it coordinates nutrient assimilation, metabolism, motility, stress response, etc.
Objectives: Although cAMP-dependent lactose operon regulation was the first described example of second-messenger based regulation, much remains unknown about the cAMP signaling itself. Here we investigate the intracellular cAMP signaling using FRET-based reporter and also explore intercellular signaling mediated by cAMP.
Materials & Methods: FRET reported based on CRP is fused to CFP and YFP (*cfp*-CRP-*yfp*) was used for cAMP detection in *E. coli* (MG1655) and its mutants. FRET measurements were performed using custom-made microscopy system where the signal of a cell population is detected using photon-counting photomultipliers.
Results: *In vitro* assay showed that the cAMP FRET reporter can accurately reflect changes in cAMP concentration. Response to different carbon sources observed *in vivo* allowed us to measure changes in the cAMP (i.e., in the activity of adenylate cyclase) induced by catebolite repression. We could further monitor responses to extracellular cAMP, which was itself dependent on the growth conditions.
Conclusion: We suggest that cAMP can mediate cell-cell communication and that different carbon sources affect not only intracellular cAMP concentration, but also cAMP secretion and sensitivity to extracellular cAMP. We present a model of bacterial growth regulation that combines the intracellular and extracellular cAMP signaling.

SAP14
Cross-talk among *Escherichia coli* copper-responsive two-component systems
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Introduction: In microorganisms two-component system signaling constitutes the predominant way for sensing of environmental conditions. Orthodox two-component systems are comprised of a membrane-bound sensory histidine kinase (HK) and a cytoplasmic response regulator (RR) which gets phosphorylated by the HK and serves as a transcription factor. Two-component proteins of different systems exhibit structural and functional conservation. The signaling specificity of cognate HK/ RR pairs is determined by variable regions close to the contact site of HK and RR. Nevertheless the results of several studies indicate that functional complementation, cross-interaction and -regulation among non-cognate components is possible.
Objectives: Here we focused on the interaction and cross-regulation of CusSR and YedVW two-component systems, both of which have been shown to respond to copper.
Methods: In our prior experiments using homo-FRET, we observed that homo-dimer arrangement of both CusS and YedV are affected by stimulation with copper. We thus characterized physical interactions of the two systems *in vivo* using FRET and investigated their cross-regulation using promoter activation assays and RNA deep sequencing.
Results: We observed that the YedVW system is at least partially controlled by the CusR and in turn interacts with and activates the CusS kinase. We further observed cross-interactions at the kinase-response regulator level, altogether suggesting *in-vivo* allosteric cross-regulation between the two systems. Such cross-regulation is supported by preliminary results of the promoter activation assay.
Conclusion: Our results indicate that the activation of YedV system can serve as the positive feedback loop for the CusSR system. The physiological significance of such regulation is currently being investigated.

SAP15
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 and 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 ΔcryB (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 photolyse-typical histidine was exchanged to alanine which reduced the survival rate to half of the complementation strain. In the future, complementations with characterized photolyases of other organisms will be performed. This might reveal details about a possible photolyase activity.
[1] Geisselbrecht, Y. et al. (2012) CryB from *Rhodobacter sphaeroides:* A unique class of cryptochromes with novel cofactors. **EMBO *rep.*** 13: 223-229

SAP16

Novel Flp pilus biogenesis-dependent natural transformation

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Natural transformation has been described in bacterial species from a broad variety of taxonomic groups. However, the current understanding of the structural components and the regulation of competence development is derived from only a few model organisms. Generally, proteins building special transport systems and cell appendages have been implicated in the uptake of external DNA into the cell.
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. The genome of the actinobacterial species *Micrococcus luteus* was found to contain only two typical competence genes, *comA* and *comC*, but lacks the other genes involved in natural transformation or competence regulation known from model transformable bacteria belonging to the genera *Bacillus*, *Streptococcus*, *Haemophilus*, *Neisseria*, *Vibrio* or *Thermus*. We show that *tad*-like genes are required for genetic transformation in *M. luteus*. We analyzed individual knockout mutants for every open reading frame of the two predicted *tad* gene clusters as well as for a potential prepilin processing peptidase. By expressing a tagged variant of the putative major prepilin subunit and immunofluorescence microscopy, filamentous structures extending from the cell surface could be visualized. Our data indicates that the two *tad* gene islands complementarily contribute to the formation of a functional competence pilus in this organism. We corroborate that in *M. luteus* competence is a transient property whose development depends on the growth phase and nutrient availability. Finally, we show that regulation of competence gene expression in *M. luteus* underlies a stochastic differentiation process, which under inducing conditions leads to *com* gene expression in only a fraction of the cells of an isogenic population.
In conclusion, this is the first report about the transformation machinery of a representative of the phylum of *Actinobacteria*. Our finding that a distinct type of pilus biogenesis genes, which so far have been connected only with biofilm formation, adherence and virulence traits, is involved in natural DNA exchange broadens the spectrum of macromolecular transport systems associated with natural transformation

SAP17

Improving Differentiation of *Bacillus anthracis* from Closely Related Species in the *Bacillus cereus sensu lato* Group via MALDI-TOF MS

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Introduction: Fast and reliable differentiation of *Bacillus anthracis* from closely related species of the *B. cereus sensu lato* group is important. Especially, differentiation of *B. anthracis* and *B. cereus* is crucial as severities of infections caused by the two species differ remarkably. While *B. anthracis* causes often fatal anthrax disease (inhalational-, injectional-, cutaneous- and gastrointestinal anthrax), *B. cereus* is mainly associated with non-lethal foodborne illnesses. Thus, corresponding treatment and disease management differ significantly. Species identification based on diagnostic methods querying the genome, such as 16S rRNA gene sequencing, are not sufficient as the various *Bacillus* species exhibit very high sequence similarities. Nowadays, identification of many bacteria is facilitated by quantifying and matching specific protein profiles using Matrix Assisted Laser Desorption/Ionization-Time of Flight mass spectrometry (MALDI-TOF MS). However, the state of commercial databases is especially poor for unambiguous identification of highly pathogenic bacteria.
Methods: A verified collection of 189 strains from the *B. cereus s. l.* group was analysed with the aim to elucidate unique characteristics for unequivocal identification of *B. anthracis* via MALDI-TOF MS. Unsupervised and supervised statistical methods were compiled to identify putative protein biomarkers in the corresponding mass spectra.
Results: Several putative biomarkers for a diverse set of *B. anthracis* strains were determined in comparison to *B. cereus, B. mycoides, B. thuringiensis,* or *B. weihenstephanensis*, consecutively. For example, in comparison to *B. cereus*, seven *B. anthracis*-specific biomarkers were identified and the most abundant of those was found in 54 % of the tested strains.
Conclusion: We succeeded in identifying putative biomarkers for *B. anthracis* that improve differentiation of *B. anthracis* from related species in the *B. cereus s. l.* group by MALDI-TOF MS on the basis of a dedicated database.

SAP18

Construction of a GFP-based reporter system to characterize the transcriptional differentiation of haloarchaeal biofilms

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Introduction: Biofilms are adherent communities of microorganisms within a matrix of extracellular polymeric substance. The biofilm formation is a complex process that induce a strong transcriptional differentiation of the planktonic cells. Initial proteome analyses of *Hbt. salinarum* strain R1 indicate transcriptional differentiation of more than 60% of the encoding genes (G. Losensky *et al*., unpublished results). To study the development and differentiation of biofilms *in vivo* and *in vitro* fluorescence based reporter systems are desirable.
Objective: The goal of this study was the construction of a GFP-based reporter system to investigate the transcriptional differentiation and the development of *Hbt. salinarum* biofilms.
Methods: The reporter system based on the high salt tolerant GFP [1] and the *E. coli / Hfx. volcanii* shuttle plasmid pWL102 [2]. The GFP signals obtained from planktonic and biofilm-forming cells were quantified by phosphorimager analyses. The biofilm structure formed by the *gfp*-expressing *Hbt. salinarum* R1 cells was visualised by confocal laser scanning microscopy (CLSM).
Results: To validate the reporter system two constructs were tested. The constitutive strong rRNA P2 promoter of *Halobacterium* fused to *gfp* (positive control) and one promoter deficient *gfp* (negative control). Fluorescence microscopy of the transformants with the P2-*gfp* control showed an evenly distributed GFP fluorescence in all cells. The strongest GFP signals of planktonic P2-*gfp* cells were obtained in the early stationary growth phase. A significant GFP fluorescence signal of surface attached biofilm-forming P2-*gfp* cells was detected after two days of incubation. The biofilm development investigated by CLSM showed the formation of the primary surface attached cell and growth of bulky tower-like microcolonies.
Conclusion: The results of the validation showed that the GFP-based reporter system is suitable to study transcriptional differentiation of surface attached haloarchaea.

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SAP19

***Psilocybe cyanescens* Wakef. in Germany**

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Introduction: *Psilocybe cyanescens* is a rarely studied species of the *Strophariaceae* known from North America. The species seems to be an invasive neomycete in Europe, anthropogenicly distributed by mulch and garden waste. Since the morphological description by Wakefield (1946, Trans. Br. Mycol. Soc. 29:141-142) of the holotypus found in Kew Gardens (UK) there is an ongoing taxonomical discussion concerning the "*Psilocybe cyanescens*-complex" identity in Europe.
Objective: The origin and the degree of distribution of this potent psychoactive fungus in Germany are currently not known. This study targets at collecting, describing and comparing isolates from locations throughout Germany.
Methods: It was first necessary to gather as many reports as possible on verified locations recognized by mycologists during the last decades. In October-November 2013, *Psilocybe cyanescens sensu stricto s*pore prints of 17 collections from different regions all over Germany have been collected, and also samples of related species like *P. serbica* and *P. semilanceata* for comparison. Mono- and dikaryotic isolates have been grown on artificial media for observations on fungal growth and morphologies and for mating tests.
Results: Most of the detected *Psilocybe cyanescens* colonies were located in urban parks and garden habitats, connected to a new type of habitat based on extensive mulch usage. Importantly, but so far largely unnoticed by the society, this psychoactive mushroom is found in large colonies on

playgrounds as well as in zoological gardens and other public park areas. Mycelial features and the conidiogenesis of different *P. cyanescens* isolates were recorded. Mating tests showed that the species is heterothallic with a tetrapolar mating system. In comparison with other *Psilocybe* species, *P. cyanescens* grow faster with a fluffier aerial mycelium. *P. cyanescens* isolates can be distinguished from strains of related species by mating tests. However, isolates of all *Psilocybe* appear to produce psilocin as indicated by a blue coloration of aerial and mechanically damaged mycelia.
Conclusion: Comparison of basic aspects such as environmental requirements, morphology and mating compatibility between collections, followed by genetic observations on isolated strains, will contribute to the taxonomical discussion on the *Psilocybe cyanescens*-complex and hopefully clarify further biological aspects in genetics and development.

SAP21
Towards a characterization of the developmental transcriptomes of *Myxococcus xanthus* and *Myxococcus stipitatus*
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Introduction: Cystobacterineae are a suborder of gram-negative bacteria that under starvation conditions form organized, multicellular fruiting bodies. Within these structures, the rod-shaped vegetative cells differentiate into dormant spores. Different species within this group form fruiting bodies with diverse forms, but each species follows an ordered set of morphological processes controlled by a complex pattern of gene expression.
Objectives: We hope to use transcriptomic analysis to identify core groups of genes involved in development in two closely related Cystobacterineae, *Myxococcus xanthus*, and *Myxococcus stipitatus*, and to identify species-specific developmental mechanisms and pathways as well.
Methods: We are carrying out an ongoing analysis of the developmental transcriptome and have carried out RNAseq at discrete developmental timepoints of the model organism Myxococcus xanthus and related species Myxococcus stipitatus.
Results: In addition to identifying groups of genes that share developmental expression patterns in the two species, we are mining the RNAseq data to identify previously unannotated transcripts. These transcripts include short secreted peptides and putative riboswitches, as well as putative non-coding RNA.
Conclusions: These findings will provide insights into development in the Cystobacterineae, and will also provide broader insights into the evolution of bacterial multicellular behaviors.

SAP22
Functional *MAT1-2* mating-type locus characterization of the penicillin producer *Penicillium chrysogenum*
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Introduction: In heterothallic ascomycetes, mating is controlled by two non-allelic idiomorphs that determine the "sex" of the corresponding strains. The two mating-type idiomorphs typically carry one to several mating-type genes [1, 2]. All idiomorphs encode conserved TFs, which act as major regulators of sexual communication and mating [3, 4]. We recently discovered mating-type loci and a sexual life cycle in the penicillin-producing fungus, *Penicillium chrysogenum*. All industrial penicillin-production strains worldwide are derived from a *MAT1-1* isolate. No *MAT1-2* strain has been investigated in detail until now.
Objectives: Here, we provide the first functional analysis of a *MAT1-2* locus from a wild-type strain.
Methods: The functional characterization of *MAT1-2* mutant strains included the following methods: spore quantification assays, a semi-automatic pellet-quantification system, light-microscopy, qRT-PCR and crossing experiments.
Results: Here, we functionally characterized a *MAT1-2* locus from a wild-type strain that like other wild-type isolates produces a rather low penicillin titer compared to recently characterized industrial *P. chrysogenum* strains [5]. Similar to *MAT1-1*, the *MAT1-2* locus has functions beyond sexual development. Unlike *MAT1-1*, the *MAT1-2* locus affects germination and surface properties of conidiospores and controls light-dependent asexual sporulation. Mating of the *MAT1-2* wild type with a *MAT1-1* high penicillin-producer generated sexual spores. Molecular characterization provides evidence for genome-wide recombination.
Conclusion: Our results point to a strategy combining the use of mating-type genes and sexual recombination to optimize conventional strain improvement methods.

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SAP23
ROS1, A KEY REGULATOR OF SPOROGENESIS IN USTILAGO MAYDIS
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Ustilago maydis is a biotrophic pathogen of maize. Its life cycle begins with the mating of two compatible haploid cells to form a dikaryotic infectious filament. After penetration, the dikaryon spreads inside the plant tissues and induces the formation of tumors. At a defined time of development, the sporogenesis program begins: nuclei fuse, hyphae fragment into individual diploid cells and these differentiate into mature teliospores.
We have identified Ros1, a key regulator for this program in *U. maydis*. Ros1 belongs to a family of transcriptional regulators binding DNA via a WOPR domain. Members of the WOPR family regulate virulence in *Fusarium oxysporum*, *F. graminearum*, *Botrytis cinerea* and *Zymoseptoria tritici.*
ros1 deletion mutants were able to mate and induce tumors on maize; however they failed to produce teliospores. Microscopy revealed that karyogamy, hyphal fragmentation and consequently spore maturation were abolished. This developmental block coincided with an upregulation of *ros1* in wild type infections.
To study the function of *ros1*, we expressed the gene in a haploid strain where the filamentous program can be induced via expression of the bE/bW heterodimer. Ros1-expressing cells failed to switch to filaments and showed unusual septation. This suggests that Ros1 counteracts the function of the bE/bW heterodimer and triggers fragmentation. To elucidate how Ros1 controls spore formation, we are currently identifying Ros1 target genes through RNAseq and ChIP-Seq approaches.

SAP24
Yeast as a tool to identify infection-relevant genes in phytopathogenic fungi
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Introduction: Fungal plant pathogens penetrate host cell walls as an initial step for infection. Attachment of fungal pathogens to their hosts is mediated by adhesive proteins and is crucial during the host-parasite interaction. Hyphae of the ascomycete *Verticillium* attach directly to plant roots and enter the plants with the help of hyphopodia. The control mechanisms and the adhesive proteins involved in the initial infection process are not yet known.
Objectives: We aimed to identify infection-relevant genes in the plant pathogen *Verticillium.* Genes involved in adhesion to the host surface were addressed in this study.
Methods: We used a non-adherent mutant strain of the model yeast *S. cerevisae* to isolate genes required for *Verticillium longisporum* or *V. dahliae* adhesion and pathogenicity. Candiate genes were phenotypically analyzed by using genetics, cell biology, transcriptomics, secretome proteomics and plant pathogenicity assays.
Results: Control elements for early plant infection were identified in a genetic screen and resulted in the discovery of six transcription regulatory genes of *Verticillium* (Vta1-Vta6) [1, 2]. All candidate genes were shown to reprogram non-adherent budding yeasts for adhesion. Vta2 is conserved in filamentous fungi, is required for fungal growth and is mandatory for accurate timing and suppression of resting structures (microsclerotia). *Verticillium* impaired in Vta2 is unable to colonize plants and induce disease symptoms. Vta2 controls expression of 270 transcripts and is a major regulator of fungal pathogenesis and controls H_2O_2 detoxification. Vta2 represents an interesting target to control growth and development of these vascular pathogens. Additional candidates of the screen are the focus

of our current research and we will include data about these genes in our poster.
Conclusion: We could show that yeast is a suitable tool to screen for infection-relevant genes of *Verticillium* and our current work is to investigate further candidates of the screen.

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SAP25

Analysis of mutants of the major gas vesicle protein GvpA

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The haloarchaeon *Halobacterium salinarum* produces spindle-shaped gas-filled, nanostructures. Fourteen *gvp* genes are involved in the formation of the gas vesicle wall and are arranged in two oppositely oriented clusters, *gvpACNO* and *gvpDEFGHIJKLM* [1]. The gas vesicle wall is mainly composed of GvpA (95%), an 8 kDa hydrophobic protein forming a single-layer. The wall consists of 4.6 nm wide ribs representing a helix running perpendicular to the long axis of the gas vesicle [2]. The secondary structure predictions suggests an α-β-β-α structure, and a *de novo* structural model was determined by high performance modelling [3].
Objectives: Single amino acid mutants of GvpA were tested for their ability to complement a ΔA construct to yield gas vesicles in *Haloferax volcanii* transformants.
Methods: The $gvpA_{mut}$ constructs were prepared by site-directed mutagenesis and used to transform *Haloferax volcanii* ΔA transformants. The presence of the $gvpA_{mut}$ construct was confirmed by Southern analysis and the presence of $GvpA_{mut}$ by Western analysis. Colonies were inspected for gas vesicles, and cells or isolated gas vesicles were analyzed by TEM to determine the gas vesicle shape.
Results: Vac^- transformants and also Vac^+ $\Delta A+A_{mut}$ transformants were obtained using different $GvpA_{mut}$ for the complementation studies. $GvpA_{mut}$ leading to Vac^- transformants pinpoint positions in GvpA required for gas vesicle assembly. Vac^+ cells often contained gas vesicles with altered sizes or shapes. For example short, spherical, or long and thin cylinder shaped gas vesicles were detected. These $GvpA_{mut}$ proteins influence the gas vesicle architecture and are important for their assembly. Some mutations had no effect and gas vesicles similar to wild type were formed.
Conclusion: Point mutations in GvpA strongly affect gas vesicle formation, or lead to alterations in gas vesicle shape. Altered gas vesicles are useful for further applications such as cryoelectronmicroscopy.

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SAP26

***Agrocybe aegerita* as a new model organism for the genetics of basidiocarp development**

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Introduction: The research on model organisms is important for the optimization of strains and breeding strategies to increase fruiting body yield and quality in edible mushroom production [1]. Still, scientific findings with single models are of limited representativeness, especially for commercially interesting mushrooms, and with some of the few current models molecular studies are difficult [2].
Objectives: Here, we propose the industrially cultivated basidiomycete *Agrocybe aegerita* as a promising candidate for a new model agaricomycete largely combining advantageous features from established models.
Materials & Methods: A suitable dikaryotic strain was selected by the criteria of being easy to cultivate, exhibiting a short life cycle including fruiting and asexual sporulation under axenic conditions, being sensitive to dominant selection markers plus being generally accessible for transformation, an acceptable genome size for whole genome sequencing and a good yield of aromatic fruiting bodies on different mushroom spawn substrates.
Results: The selected strain, *A. aegerita* AAE-3, complies with these criteria. AAE-3 exhibits a short life cycle of 21 days on agar media. At the same time, AAE-3 is sensitive to widely-used dominant selection markers. Forty monokaryotic strains were generated from AAE-3. Among them, all published monokaryotic fruiting types [3] could be observed. One mating compatible pair of monokarya was selected for their abundant oidia formation potentially useful for transformation. The whole genome of AAE-3 has been sequenced is in the process of being annotated.
Conclusion: These findings together with the general accessibility of *A. aegerita* to transformation [4], and its excellent basidiocarp aroma profile [5] provide a promising basis for the establishment of *A. aegerita* as a new model agaricomycete.

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SAP27

Molecular Analysis of OriC1 and OriC2 in *Haloferax volcanii*

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Haloferax volcanii H26 is polyploid and contains about 25 copies of its main chromosome, which contains four origins of replication (ori) [1,2]. To enable the molecular characterization of copy number regulation, fragments of various sizes around oriC1 and ociC2 were cloned into a suicide vector to generate **H**aloarchaeal **A**rtificial **C**hromosomes (HACs). Deletion mutants of OriC1 and OriC2 and were constructed and complemented with the cognate HACs. Real Time PCR analysis was used to quantify the copy numbers of the main chromosome and the HACs. Deletion of OriC1 resulted in slightly lower copy numbers of the main chromosome, which increased to wildtype level after complementation with the oriC1-HAC, while the HAC containing OriC1 showed very high copy numbers. Deletion of OriC2 resulted in higher copy numbers of the main chromosome, which decreased after complementation with the oriC2-HAC, while the HAC containing OriC2 showed very low copy numbers. In addition, the numbers of repeats of OriC1 were doubled on the HAC and repeats of OriC1 were placed in front of the *orc5* of OriC2. Doubling the repeats of OriC1 on the HAC had very little impact on the genome copy number of the main chromosome, while the copy number of the HAC rose significantly. Placing repeats of OriC1 in front of *orc5* of OriC2 resulted in slightly higher copy numbers of the main chromosome in stationary growth phase. Copy number of the HAC was lower than that of the one with doubled repeats or that of the HAC containing native OriC1. Furthermore, in comparative growth experiments the fitness of the wild-type is higher than that of the oriC1 deletion mutant, but lower than that of the oriC2 deletion mutant. Taken together, the results show that 1) oriC1 is much more effective than oriC2, 2) that the copy number is influenced by the numbers or origin repeats, the cis-encoded Cdc6 protein, and additional sequences surrounding the core origin, and 3) by genes encoded in trans on other replicons. Application of the HACs in further experiments will shed light on the molecular details of replicon copy number control in haloarchaea.

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SCP01
Investigation of Hülle cells from the filamentous fungus Aspergillus nidulans
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Hülle cells are unique cell types of the Aspergillus genus and have crucial functions during sexual development. These globose thick- walled cells develop from hyphae and associate with the developing closed sexual fruiting bodies named cleistothecia. Hülle cells have different nuclei and they can fuse to form a macronucleus. The absence of Hülle cells results in the formation of significantly smaller cleistothecia. This fact provides a possible auxiliary and a nursing function of Hülle cells during the development of the cleistothecium. To explore the molecular switches from filamentous growth to single Hülle cells we are in the process of analyzing the transcriptome, proteome and metabolome of these cells and therefore we have developed methods to isolate them from the remaining fungal structures and we have compared these results to other fungal structures such as the hyphae.

SCP02
Extracting quantitative microbiological data from single-molecule localisation microscopy
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Question: The development of various concepts that demonstrated "super-resolution" microscopy started a new era of fluorescence microscopy. It has therefore become possible to address a great deal of microbiological questions on organisation, interaction and dynamics of individual proteins in a cellular context in a new quantitative manner.
This methodological toolbox allows addressing questions like e.g. how are biomolecules clustered, and what is the molecular composition of these clusters? Here, we will present our recent findings on work in *E. coli*.
Methods: Single-molecule localisation microscopy (SMLM) is particular as it relies on the determination of the centre of mass of individual fluorophores. In most applications so far, these coordinates have been used to reconstruct an intensity-like image with superior spatial resolution reaching 20 nm or less. However, more valuable information is available: single-molecule registration allows for molecular counting, as well as for discovering sub-populations and heterogeneities which are otherwise hidden through ensemble averaging. This provides, next to super-resolution images, quantitative, single-molecule resolved information.[1]
Results: We quantify the distribution of active RNA polymerases (RNAPs) in *E. coli* under different environments which are organised in chromosomal bands and investigate substructures within the banding using single-molecule cluster analysis (DBScan).[2] Further, we map the organisation of replication and chromosome segregation and spot highly-defined cell-cycle dependent hetero-structures, which contain discrete DNA fibres with diameters far below the diffraction limit.[3] Finally, we introduce correlative PALM, PAINT and *d*STORM imaging of the RNAP, membrane and chromosomal DNA for structural fixed studies as well as live RNAP tracking and subsequent structural imaging of the respective nucleoids.[4]
Conclusion: We demonstrate easily applicable protocols for SMLM and correlative imaging of protein distributions and dynamics, membrane and chromosome structure. These results show that it is strikingly interesting to apply similar protocols e.g. to other nucleoid-associated proteins in order to elucidate interactions occurring at such a small scale.

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[2]Endesfelder et al. (2013), Biophys Journal, 105(1), 172-181.
[3]Spahn et al. (2014), Journal of Structural Biology, 185 (3), 243-249.
[4]Spahn et al., accepted in Methods and Applications in Fluorescence

SCP03
Deciphering the regulatory networks involved in phenotypic heterogeneity in *Sinorhizobium fredii* NGR234
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We are interested in analyzing molecular switches involved in the regulation of phenotypic heterogeneity in *Sinorhizobium fredii* NGR234. NGR234 is a broad host range and nitrogen fixing rhizobium that shows high levels of phenotypic heterogeneity with respect to the expression of autoinducer (AI)-dependent and AI-independent genes.[1] To analyze the complex regulatory networks involved in switching from heterogeneous to homogeneous gene expression, we have analyzed the expression profiles of the AI-synthase genes *traI* and *ngrI* in this model organism in the background of the parent strains as well as in the background of *traI*, *ngrI* deletion mutants and in the background of an antiactivator deletion mutant *traM*. These tests plus tests with exogenously added *N*-3-oxooctanoyl-L-homoserine lactone indicated that phenotypic heterogeneity strongly depends on the level of synthesized AI and that TraM plays a pivotal role in regulation of AI-dependent heterogeneity. Further experiments with promoter fusions of two of the quorum quenching (QQ) genes, *dlhR* and *qsdR1*, showed, that these genes are heterogeneously expressed as well. Interestingly, the heterogeneity of either the quorum sensing or the quorum quenching genes could be quenched by the addition of plant root exudates. Especially plant-released octopine had a strong influence on the level of phenotypic heterogeneity suggesting a wider regulatory role of this molecule in NGR234.

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SCP04
Direct geneFISH – Introducing simultaneous gene - and 16S ribosomal RNA - hybridization using dye - labeled probes and super-resolution structural illumination microscopy (SR-SIM)
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GeneFISH is a method based on Fluorescence *In Situ* Hybridization (FISH) to detect specific genes in microorganisms *in situ*. Previous GeneFISH protocols were based on enzyme labeled oligonucleotide probes and were comprised of several consecutive FISH steps. First, the taxonomic identification of the microbial cells was achieved by Catalyzed Amplification Reporter Deposition (CARD)-FISH, using oligonucleotide probes targeting the ribosomal RNA. Subsequent, the gene detection was executed using polynucleotide probes multiple labeled with digoxigenin and a combined antibody-CARD signal amplification system. The detection efficiency of the method was up to 94% using three polynucleotide probes targeting consecutive locations on the same gene. The high sensitivity was largely achieved due to the signal amplification by the CARD steps. Nevertheless, the CARD amplification steps make the protocol long and labor intensive. The whole protocol takes on average four days. Furthermore, many steps and long period of incubations increased the damage of DNA and cell morphology. Additionally, the signal amplification decreased also the spatial resolution of the geneFISH signal.
Our aim was to considerably shorten the geneFISH protocol and to detect the genes at a high spatial resolution. For optimization *Escherichia coli* clones with different copy numbers of a test target gene (*unk* gene) were used. We used eight specific probes for different regions of the gene simultaneously and multiple labeled with dye molecules and increased the gene probe concentrations six times compared to the original protocol. After testing different dyes we yielded a high detection efficiency of geneFISH signals (80%). Since the amplification steps were omitted the 16S rRNA FISH probes could be simultaneously hybridized together with the geneFISH probes reducing the overall handling time down to four hours. Furthermore the visualization with super-resolution structural illumination microscopy (SR-SIM) allowed us to localize the geneFISH signals within individual *E. coli* cells. Future optimizations will allow for the simultaneous detection of genes and identity of environmental microorganisms in high spatial resolution and in less than a day.

SCP05

Single Cell and Meta-Genomic study of *Dehalococcoidetes* species from Deep-sea Sediments of the Peruvian Margin

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Introduction: The phylum *Chloroflexi* is one of the most frequently detected phyla in the subseafloor of the Pacific Ocean margins. Dehalogenating *Chloroflexi* (*Dehalococcoidetes, Dhc*) were originally discovered as the key microorganisms mediating reductive dehalogenation via their key enzymes reductive dehalogenases (Rdh) as sole mode of energy conservation in terrestrial environments. The frequent detection of *Dhc*-related 16*S* rRNA and *rdh* genes in marine subsurfaces implied a role for dissimilatory dehalorespiration in this environment, however the two genes have never been linked to each other.
Objectives: In order to provide fundamental insights into the metabolism and genomic population structure of marine subsurface *Dhc sp*., we analyzed a non-contaminated deep-sea sediment core sample from a Peruvian Margin Ocean Drilling Program site by single cell and meta-genomic approaches.
Methods: DNA was isolated and sequenced on an Illumina® HiSeq platform. Physical isolation of the single cells was performed by Fluorescent Activated Cell Sorting. The single cells were then lysed and DNA was amplified by Multiple Displacement Amplification (MDA). The MDA products were sequenced separately on Illumina® HiSeq and PacBio® *RS* platforms. Bioinformatics tools were used to conduct assembly, orf calling and annotation.
Results: We present for the first time genomic data of four deep-sea *Chloroflexi* (Dsc) single cells from a marine sub-surface environment. Surprisingly, the assembled genomes revealed no *rdh* but the presence of multiple haloacid dehalogenase (HAD) genes. This class of enzymes catalyzes the hydrolytic dehalogenation of halogenated organic acids. The HAD and Rdh families are non-homologous, mechanistically different, and have evolved independently. In addition, multiple genes forming the Wood-Ljungdahl pathway of acetogens, including an Rnf/Etf enzyme complex, were found.
Conclusion: Despite a high degree of sequence-level similarity between the shared proteins in the Dsc and terrestrial *Dhc,* no evidence for catabolic reductive dehalogenation was found in Dsc. The genome content is, however, consistent with a strictly anaerobic organotrophic or lithotrophic lifestyle.

1. Kaster et al., The ISME Journal, 2014; 1-12

SCP06

Epigenetic vs. Genetic Heterogeneity with Respect to bla_{L1} and bla_{L2} Gene Expression in *Stenotrophomonas maltophilia*

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Stenotrophomonas maltophilia is a Gram-negative, non-fermentative bacterium, which is associated with the rhizosphere in the environment but can also cause opportunistic infections in immunocompromised patients such as those of the respiratory tract. It is highly resistant to many antibiotics and its genome encodes for an arsenal of antibiotic resistance mechanisms. The high resistance of *S. maltophilia* to ß-lactam antibiotics is primarily due to two functional ß-lactam resistance genes designated bla_{L1} and bla_{L2}. The strain K279a forms colonies of different size and appearance in presence of sub-inhibitory concentration of ampicillin in the LB agar plates. Analysis of single cells studies by fluorescence microscopy indicated that bla_{L1} and bla_{L2} were subject to a heterogeneous and bistable expression under laboratory conditions. This was independent from the presence of ampicillin and other ß-lactams and diffusible signal factors in both, the parent and *smlt0387* knock-out strain. Interestingly, the addition of sterile-filtered culture supernatants obtained from stationary phase cells altered strongly the levels of phenotypic heterogeneity in exponential cultures. This response could be quenched by heat treatment (10 min > 90°C) of cell supernatants, suggesting the involvement of a heat-labile but not yet identified factor involved in regulation of ß-lactamase gene expression in K279a at the single cell level. While this high level of heterogeneity was perhaps partly caused by non-genetic alterations, next generation sequencing of large and small colony variants indicated that single nucleotide polymorphisms (SNPs) existed within K279a population. We are therefore interested to identify genetic and non-genetic mechanisms involved in the bistable phenotypes observed with respect to colony heterogeneity and expression of the bla_{L1} and bla_{L2} genes.

SYP01

Going through walls – active invasion of bacteria into living fungal cells

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Introduction: The phytopathogenic fungus *Rhizopus microsporus* harbors a bacterial endosymbiont, *Burkholderia rhizoxinica*, and together they produce the highly potent antimitotic phytotoxin, rhizoxin [1]. In this obligate endosymbiotic interaction, the fungus fully depends on the bacterial presence to sporulate, and the bacteria are transmitted vertically by fungal spores. In contrast to many known endosymbiotic fungal-bacterial interactions, both *R. microsporus* and *B. rhizoxinica* can be maintained in pure cultures. Reestablishment of endosymbiosis is possible in co-culture of sterile counter parts that makes *Rhizopus-Burkholderia* symbiosis a good model system to study endosymbiotic interactions [2].
Objectives: We focused on understanding the molecular mechanisms for colonisation of fungal hyphae by endobacteria and the maintenance of the endosymbiotic interaction.
Methods: In order to understand the molecular factors involved in the invasion process genomic and proteomic analyses were conducted. The bacterial entry into fungal hypha was investigated by cryo-electron microscopy.
Results: *In silico* predictions revealed a type 2 secretion system (T2SS) and a set of chitinolytic proteins secreted by the T2SS to be key factors. Gene deletion and *in vitro* enzymatic studies showed that the T2SS and chitinase mutants could not have fungus sporulate and lost endosymbiosis. Microscopic snapshots of the bacterial entry into fungal hypha showed an active local fusion of bacteria.
Conclusion: The molecular mechanism of the endobacterial invasion of its fungal host has been clarified. Partial lysis of the fungal cell wall is a prerequisite to the entrance for the bacteria, after which the fungus survives without any cytosolic leakage [3].

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SYP02

Microbial abundances and metabolic functions of sponge microbiomes

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In spite of considerable insights into the microbial diversity of marine sponges, quantitative information on microbial abundances and community composition as well as the functional gene repertoire remain scarce. Firstly, we established qPCR assays for the specific quantification of four bacterial phyla of representative sponge symbionts as well as the kingdoms *Eubacteria* and *Archaea*. We showed that the 16S rRNA gene numbers of *Archaea*, *Chloroflexi*, and the candidate phylum *Poribacteria* were 4-6 orders of magnitude higher in HMA than in LMA sponges, that actinobacterial 16S rRNA gene numbers were 1-2 orders higher in HMA over LMA sponges, while those for *Cyanobacteria* were stable between HMA and LMA sponges. Fluorescence *in-situ* hybridization (FISH) of *A. aerophoba* tissue sections confirmed the numerical dominance of *Chloroflexi*, which was followed by *Poribacteria*. Archaeal and actinobacterial cells were detected in much lower numbers. By use of fluorescence activated cell sorting (FACS) and whole genome amplification (WGA) as a primer- and probe-independent approach, the dominance of *Chloroflexi*, *Proteobacteria*, and *Poribacteria* in *A. aerophoba* was confirmed. Secondly, the GeoChip 4 gene array was employed to interrogate the microbial functional gene repertoire of sponges and seawater collected from the Red Sea and the Mediterranean. Altogether 20,273 probes encoding for 627 functional genes and representing 16 gene categories were positively identified. Minimum curvilinear embedding (MCE) analyses revealed a clear separation between the samples. Except for few documented specific differences, the

functional gene repertoire between the different sources appeared largely similar. Our studies contribute to a better understanding of the HMA/LMA dichotomy, provide new quantitative insights into sponge microbiology, and suggest that sponge-associated and seawater microorganisms may have most of their functional gene repertoire in common.

SYP03

'*Candidatus* Adiutrix intracellularis', a homoacetogenic deltaproteobacterium colonizing the cytoplasm of termite gut flagellates

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Introduction: The cellulolytic termite gut flagellates are typically colonized by multiple bacterial symbionts specific for their respective host. In a recent study with capillary-picked cells of *Trichonympha* flagellates from the dampwood termite *Zootermopsis nevadensis*, we had discovered a symbiont that belongs to a novel lineage of uncultured bacteria distantly related to sulfate-reducing *Deltaproteobacteria* [1].
Objectives: The aim of this study was a detailed characterization of this symbiont, including its phylogeny, subcellular location, ultrastructure, and metabolic potential.
Methods: We combined a 16S rRNA-based approach with fluorescent in situ hybridization (FISH) and transmission electron microscopy (TEM). The draft genome of the endosymbiont was reconstructed from a metagenomic library obtained from an intracellular fraction of the host flagellate.
Results: '*Candidatus* Adiutrix intracellularis' belongs to a clade of uncultured *Deltaproteobacteria* (between family and order level) that is widely distributed in the intestinal tract of termites and cockroaches. Fluorescence in situ hybridization and transmission electron microscopy showed that the symbiont densely colonizes the inner anterior part of the host cell and situated close to a second deltaproteobacterial symbiont, '*Desulfovibrio trichonymphae*'. The draft genome of '*A. intracellularis*' (~2 Mbp) contained near-complete set (> 92%) of essential genes and 48 tRNAs. It is not a sulfate reducer but possesses a complete set of genes for reductive acetogenesis from CO_2 and H_2 via the Wood-Ljungdahl pathway, including gene modules encoding the hydrogen-dependent carbon dioxide reductase (HDCR) producing formate. The presence of genes for an alternative nitrogen reductase (AnfHDK) indicates the capacity for dinitrogen fixation.
Conclusion: '*Adiutrix intracellularis*' is an endosymbiont with the capacity for reductive acetogenesis and dinitrogen-fixation. The presence of relatives also in flagellate-free hosts indicates that the endosymbionts may have originated from free-living gut bacteria, possibly after acquiring essential symbiotic functions by lateral gene transfer from other gut microbiota.

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SYP04

Finding the "brain bugs" - Identification of sialic acid-utilising bacteria in a piglet caecal community by RNA-based stable isotope probing

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Introduction: Sialic acids are acid monosaccharides typically found on oligosaccharide chain termini expressed on cell surfaces or attached to soluble proteins, or as essential components of ganglioside structures that play a critical role in brain development and neural transmission [1]. Human milk contains high concentrations of sialic acid conjugated to oligosaccharides, glycolipids, and glycoproteins. These nutrients are able to reach the large intestine where they can be metabolised by the resident microbiota [2]. However, very little is known about the intestinal microbiota involved in sialic acid metabolism. Clearly, identification of sialic-acid utilizing bacteria is just a first, but pivotal step to unravel whether intestinal microorganisms might affect brain function via intestinal sialic acid metabolism, such as through competition with the host for this nutrient.
Objectives: The aim of this study was to identify bacteria that utilise sialic acid and sialic acid-containing nutrients in the large intestine.
Materials & Methods: We cultured the caecal microbial community from piglets in the presence of GD3 ganglioside, the most abundant ganglioside in human colostrum, or ^{13}C-labelled sialic acid. Using an established RNA-based stable isotope probing approach [3] in combination with 454-amplicon sequencing, we were able to identify bacteria that consumed ^{13}C-sialic acid by isopycnic buoyant density gradient fractionation of total RNA and subsequent 16S rRNA gene analysis.
Results: Sialic acid was consumed by a wide range of bacteria, with the most prolific users being bacteria affiliated with the genera *Prevotella* and *Lactobacillus*. The higher representation of RNA sequences affiliated with *Prevotella* and *Lactobacillus* among the isotopically labelled RNA was accompanied by a corresponding reduction in sequences representing *Escherichia/Shigella*, *Ruminococcus* and *Eubacterium* species. While sialic acid was utilised by many members of the cultured microbial community, GD3 was not readily consumed.
Conclusion: This pilot study data will help to better understand the microbial transformation of sialic acids and their conjugates in the human intestinal tract.

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SYP05

Investigation of the endobacterium *Rhizobium radiobacter* F4 from beneficial fungus *Piriformospora indica*

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Rhizobium radiobacter F4 (*Rr*F4) was isolated from *Piriformospora indica*, a fungus in the order *Sebacinales* which possess beneficial activity towards a broad range of host plants [1]. While *Rr*F4 can be grown in pure culture, all attempts to cure *P. indica* from this endobacterium failed. Thus, the role of *Rr*F4 in the tripartite Sebacinalean symbiosis with *P. indica* and its host-plants remains unresolved. Interestingly, like *P. indica*, *Rr*F4 can induce growth promotion and systemic resistance in barley [2]. Here we present new information on *Rr*F4's colonization pattern in barley and Arabidopsis roots.
Inoculation of barley and Arabidopsis with *Rr*F4 were performed under sterile conditions using ½ MS medium. Plant seedlings were dip-inoculated with *Rr*F4 and root-colonization was investigated 5, 7, 14 and 21 days after inoculation (dpi). The proliferation of *Rr*F4 was determined by q-PCR analysis targeting the ribosomal RNA ITS-1. Localization studies were performed with GUS and GFP-tagged *Rr*F4 using light and epifluorescence microscopy as well as confocal laser scanning and transmission electron microscopy.
The proliferation of *Rr*F4 was clearly shown in both barley and Arabidopsis. Colonization started at the maturation zone close to the root cap of the primary root and spread over the root hair zone. The protrusions of lateral root and root hair zone also were heavily colonized. In clear contrast to *P. indica*, *Rr*F4 also colonized the inner root tissue beyond the endodermis, including the central cylinder. Further studies need to elucidate *Rr*F4`s critical role in the Sebacinalean symbiosis.

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2. Sharma *et al.*, Cellular Microbiology **10** (2008) 2235-2246

SYP06

Culturable endophytic fungi dominate the roots of two selected plants in permanent grassland of GiFACE

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Plants in natural ecosystems living in symbiosis with fungal endophytes that can have impacts on plants through increasing fitness by conferring abiotic and biotic stress tolerance, increasing biomass and decreasing water consumption, without causing disease symptoms.

The diversity and composition of the fungal communities colonizing both monocotyledonous (*Arrhenatherum eletius*) and dicotyledonouse (*Gallium mollugo*) selected plants in the permanent grassland of the Giessen Free Air Carbon Dioxide Enrichment (GiFACE) system was evaluated by microscopy and DNA sequencing of the 18S rRNA-ITS seqeunce.

Preliminarily results show that some of the culturable endophyte fungi isolated from surface sterilized plant roots on MYP medium belong to dark septate fungi. Phylogenetic identification of 22 fungal isolates obtained from the two plant species suggests that 90% of the isolated fungi representing the phylum Ascomycota (mostly *Pleosporales*), the others being from Basidiomycota (*Polyporales* and *Russulales*). The most common potentially endophytic fungi within the grasses are related to *Paraphaeospheria* sp. and *Pleospora* sp. (6 and 3 of 22 sequences, respectively). These results raise the possibility that at least some of these common endophytic members of the fungal community form cordial relationships between plants in one ecosystem.

SYP07

Ectomycorrhizal symbiosis: inter-kingdom signaling

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With respect to the ectomycorrhizal symbiosis, *Tricholoma vaccinum* shows strong host selectivity. Using mycorrhiza specific gene expression studies, an aldehyde dehydrogenase has been identified that was shown to be inducible by indole-acetic aldehyde. Hence, we investigated the biosynthetic pathway of indole-3-acetic acid in the mycorrhizal fungus and compared this feature to other mycorrhizal as well as saprobic basidiomycetes. IAA export from the hyphae could be shown by heterologous expression and functional analysis of a transporter of the MATE family. On the fungal side, specific host interaction had been coupled to expression of hydrophobin genes. The hydrophobins of *T. vaccinum* were analyzed with respect to potential functions in different life stages of the fungus, with specific regard to host interaction. The predicted secretome of the fungus was analyzed to identify enzymes specific for host interaction.

SYP08

Phenotypic divergence of gastrointestinal microbes can be explained by their metabolic content

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Introduction: The human gastrointestinal tract harbours a diverse microbial community, in which metabolic phenotypes play important roles in health. Recent developments in meta-omics try to unravel individual metabolic roles of microbes by linking the genotype with phenotype [1]. This connection, however, remains poorly understood with respect to its evolutionary and ecological context.

Objectives: Here, we aim to analyse the metabolism of various intestinal microbes with respect to their evolutionary and ecological relationship within the human gut.

Methods: We reconstructed genome scale metabolic models of 301 representative intestinal microbes. We applied a combination of data mining and systems biology techniques [2] to study individual and global differences on the genomic and metabolic level.

Results: Based on the global metabolic differences, we found that energy metabolism and membrane synthesis play important roles in discriminating different taxonomic groups. Furthermore, we found an exponential relationship between these metabolic differences and the phylogeny, meaning that closely related microbes can be very different in their metabolic traits while at a certain genetic distance no additional metabolic diversity was observed. This finding was further substantiated by the metabolic divergence within different genera. In particular, we could distinguish three sub-type clusters within the *Lactobacillus* as well as two clusters within the *Bifidobacteria* and *Bacteroides*. The differences between those sub-types can be explained by differentiated pathways for either energy metabolism or membrane synthesis.

Conclusion: Based on our results, we could show that phenotypic divergence within closely related intestinal strains and species can be explained by the metabolic content of those microbes rather than their phylogenetic relationships. This has important implications in understanding the ecological and evolutionary complexity of the human gastrointestinal tract.

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SYP09

Radical host defense erodes symbiont genome

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Introduction: The European beewolf engages since 68 million years in a defensive symbiosis with *Streptomyces philanthi* to protect its offspring against mold fungi. Additionally, the wasp egg releases high concentrations of the strong antimicrobial radical nitric oxide (NO) into the brood cell. This gas - in the presence of the *Streptomyces* bacteria - prevents microbial growth long before the antibiotic mediated protection of the *Streptomyces* bacteria comes into effect. The repeated NO exposure and strong transmission bottlenecks during the *Streptomyces'* life cycle poses probably a strong selection pressure.

Objectives: We hypothesized that despite possible influence of NO on genome erosion, the beewolf symbiont is especially adapted to cope with the exposition to nitric oxide.

Methods: We sequenced the genome of the *Streptomyces* symbiont and analyzed the proteome in the beewolf brood cell, with a particular focus on defensive and oxido-regulating enzymes. Furthermore, using *in vitro* assays and transcriptomics analyses, we tested for the ability of the beewolf symbiont as well as a free-living relative to cope with high NO levels.

Results: Genome sequencing of the symbionts revealed an accumulation frameshifts, hypothetical and pseudogenes and a severely impaired DNA repair machinery. The proteome analysis revealed a high number of enzymes involved in oxidative stress response, including radical scavengers, superoxide/peroxide detoxifying enzymes, and high levels of chaperones and other general stress response proteins. Upon *in vitro* exposure, *S. philanthi* proved to be much more resistant to NO than the free-living *S. coelicolor*, through the upregulation of the above mentioned protective genes.

Conclusion: Our results indicate a high degree of resistance to the host-produced NO in *S. philanthi*. Interestingly, despite the strong protection against nitrosative stress, the fingerprint of DNA suggests that NO exposure might play an important role in accelerating genomic decay. The partial impairment of the DNA repair machinery led to a cycle of increased accumulation of DNA damage, indicating ongoing genome erosion. Thus, the beewolf-*Streptomyces* symbiosis represents a unique system to study actual progression of genome decay and the role of host and symbiont defenses in the evolution of their symbiosis.

SYP10

Symbiosis *in Bathymodiolus azoricus* – the physiological perspective

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Introduction: *Bathymodiolus azoricus* mussels dominate the hydrothermal vents of the Mid-Atlantic ridge. These deep-sea mussels largely depend on chemosynthetic sulfur-oxidizing (thiotrophic) and methane-oxidizing (methanotrophic) γ-proteobacteria that they host in their gills to fulfill their carbon and energy requirements. So far, the exact dynamics of this host-symbiont interdependence and the metabolic niche of each partner is unknown. This is partly due to the inability to culture these bacteria under laboratory conditions for extended time periods.
Objective: In our study we examined the interactions between *Bathymodiolus azoricus* and its symbionts by reconstructing their physiology using a proteogenomic approach. We analyzed the cystosolic and membrane proteome of enriched symbiont and host cellular fractions and complemented the resulting expression data with the available genomic data to construct metabolic maps.
Results: Protein abundance indicated intensive carbon fixation and assimilation by the symbionts facilitated by active carbon concentration of the host. The thiotrophs appear to have an incomplete TCA cycle and are also lacking proteins involved in other anaplerotic pathways. On the other hand, they have a nearly complete amino acid and cofactor biosynthetic machinery, which is absent in the host. The methanotroph proteome was dominated by proteins involved in cellular carbon and energy biosynthesis.
Conclusion: An obligate dependence of the thiotrophs on their host for carbon intermediates is evident from our data. The host might receive amino acids and cofactors essential for cellular biosynthesis from the thiotrophs. This indicates an obligate association between both partners. Finally, we also report a repertoire of "symbiosis" proteins with varied putative functions that might shed light on host-symbiont interactions, nutrition acquisition of the host by digestion of symbionts, pathogen resistance and the role of innate immunity in the host.

SYP11

Kinetics of syntrophic propionate degrading bacteria in defined mixed-cultures

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Introduction: To obtain energy for their metabolism sythrophic propionate oxidizing bacteria (SPOB) need either a chemical hydrogen-acceptor like sulfate or a hydrogen using partner like methane producing *Archaea*. However, the energy output is still very low (-25.19 kJ per mole propionic acid) which is resulting in relatively slow growth rates of these organisms. Known mesophilic SPOBs are species of the genera *Syntrophobacter* and *Pelotomaculum* and the species *Smithella propionica*.
Objectives: For kinetic models of syntrophic propionic acid degradation, parameters like K_M and V_{max} or yield factors (g cells per mol propionate) must be estimated [1].
Materials & Methods: Michaelis-Menten-kinetics for propionate degradation of SPOB were performed by measuring the oxidation of propionate and the production of methane and acetate using different GC-systems. *S. sulfatireducens*, *P. propionicicum* and *S. propionica* were analyzed in syntrophic co-culture with *M. hungatei* and in tri-culture with additional acetate oxidizing *M. concilii*, respectively. *S. sulfatireducens* was also analyzed in pure culture with sulfate as electron acceptor. Furthermore kinetics of different enriched biogas reactor samples were measured.
Results: For *S. sulfatireducens* the highest K_M and V_{max} values were found in the pure culture with sulfate as electron acceptor (K_M = 15.5 mM and V_{max} = 0.17 mM/h). The addition of an acetate-using methanogen showed no significant difference to the syntrophic co-culture in the V_{max} values (co-culture V_{max} = 0.09 mM/h and tri-culture V_{max} = 0.10 mM/h). However the K_M value of the tri-culture (K_M = 15.3 mM) is comparable with the pure culture while the result for the co-culture (K_M = 11.7 mM) is clearly lower.
Conclusion: These results provide an indication that it is important for model calculations with SPOB to know which kind of methanogenic organisms occur in the microbial community, as this can have an effect on K_M and V_{max} values and the following results.

1. M. Zamanzadeh, W. J. Parker, Y. Verastegui and J. D. Neufeld (2013). Biokinetics and bacterial communities of propionate oxidizing bacteria in phased anaerobic sludge digestion systems. Water research, 47(4), 1558-1569.

SYP12

A review study on microorganisms flora and their population density in feed, intestine and cast of *Eisenia* fetida under different ecological conditions

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Earthworms among different invertebrates intensively disperse diverse microorganism flora. *Eisenia fetida*, one of the most important earthworm species used in decomposition of organic waste materials by symbiont microorganisms. Types of microorganisms and their population density are different in feed, intestine and cast of worm according to different ecological conditions. Sometimes these conditions lead to proliferation or stimulation of growth in microorganisms, affecting the decomposition rate. Hence, about a half a century studies have been carried out with different approaches to investigate diverse microorganisms in the feed to the digestive tract and cast of earthworms. Reports show some differences in the dominant flora regarding to versatilities of applied conditions and techniques. Some show a decline or an increase in the viable microbes due to earthworm activities; others show fluctuations in the microbe density from earthworms' feed to the cast. Moreover, during these activities the metabolic status, viability of microorganisms and nutrient cycles in soil changes. According to these results, bacteria are the main microflora of the worms' intestine and casts. Recently, molecular techniques such as PCR-DGGE and COMPOCHIP (as a complementary method to DGGE) have been employed for investigating the bacterial population densities. By these techniques we can study the abundance of bacterial flora and density in cast (vermicomposts) in different ecological conditions (habitat, temperature, pH, Rh%, feed etc.). The goal of this paper is to study microflora alterations under different ecological conditions in order to apply vermitechnology in a more efficient way.

SYP13

Reduction of root colonization by the endomycotic bacterium *Rhizobium radiobacter* F4 in the gibberellic acid barley mutant

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The Alphaproteobacterium *Rhizobium radiobacter* F4 (*Rr*F4) was originally isolated from the plant growth-promoting fungus *Piriformospora 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*. Significantly, phytohormones such as jasmonates, gibberillins and auxins play a vital role in the tripartite symbiotic association (Schäfer et al. 2009). To assess the impact of gibberellins (GA) on colonization and development of *Rr*F4, the expression of the hormone related genes *ent*-kaurene synthase-1 (*KS1*), *ent*-kaurene synthase like-4 (*KSL4*), and *1-deoxy-D-xylulose-5-phosphate synthase* (*DSX*) was analyzed in barley roots after inoculation with *Rr*F4 using quantitative real-time PCR. We found that all the three genes (*KS1, KSL4* and *DSX*) were up-regulated by *Rr*F4. Consistent with this, proliferation of *Rr*F4 was significantly reduced in barley M640 mutant impaired in GA signaling compared with wild-type barley. Additionally, growth promotion mediated by *Rr*F4 was greatly abolished in the GA signaling M640 mutant. Our results reveal the importance of gibberellin signaling for successful *Rr*F4 development and its biological activity.
Reference:
Schäfer et al. (2009) Manipulation of plant innate immunity and gibberellin as factor of compatibility in the mutualistic association of barley roots with *Piriformospora indica.* Plant Journal 59, 461-474.

SYP14

Phylogenetic analysis of *Hamiltonella* and *Arsenophonus* in wild populations of three whitefly species from South-East Europe

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Introduction: Whiteflies *Bemisia tabaci* (Gennadius), *Trialeurodes vaporariorum* (Westwood) and *Siphoninus phillyreae* (Haliday) are cosmopolitan phloem-feeding pests. *B. tabaci* is a complex of species, some of which are invasive. All whiteflies harbour a primary symbiont *Portiera*, whereas diverse secondary bacterial symbionts (SS) which affect different aspects of insect biology are described in all three whitefly species. SS *Arsenophonus* and *Hamiltonella* are known to be involved in virus transmission and they are the most frequent SS in the whitefly populations from the South-East Europe.
Objectives: In this study we aimed to investigate the evolutionary relationship based on rDNA of the SS *Hamiltonella* and *Arsenophonus* recorded in our study compared with the SS from other worldwide populations.
Materials & Methods: Wild populations of the three whitefly species from South-East Europe were screened for SS infection targeting the 16S rDNA gene for *Hamiltonella* and the 23S rDNA gene for *Arsenophonus*, in order to perform phylogenetic analysis.
Results: 16S rDNA from *Hamiltonella* separated into two groups of identical sequences: one group was composed of sequences belonging to *B. tabaci* (Mediterranean genetic group) and *T. vaporariorum* and the other group was composed of sequences belonging to *S. phillyreae.* Similarly, 23S from *Arsenophonus* separated into two groups: one composed of sequences originating from *T. vaporariorum* and clustering with the corresponding sequence from the GenBank and the other group of sequences originating from *S. phillyreae.* The latter sequences did not cluster with any of the sequences from the GenBank, so they could represent a new and hitherto unknown *Arsenophonus* symbiont.
Conclusion: *B. tabaci* and *T. vaporariorum* share the same (or highly similar) SS *Hamiltonella* and *Arsenophonus*, which suggest horizontal transfer of these SS between these two species, while in *S. phillyreae* these SS are quite distinctive. This study may contribute in investigation of the unknown functions of these SS and clarify the reasons of still not determined presence of the devastating plant viruses in the investigated area.

SYP15

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. Subsequent experiments will focus on more detailed analyses of the expression and regulation of the efflux system and of emrR.
This work was supported by the Deutsche Forschungsgemeinschaft through a Mercator Fellowship.

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SYP16

Does methane induce *Sphagnum*-associated microbial N_2 fixation in peatlands?

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Introduction: Pristine *Sphagnum*-dominated bogs are N-limited and depend on N_2 fixation by diazotrophs. Recently Larmola et al. (2014) hypothesized that methane might induce *Sphagnum*-associated microbial N_2 fixation during early stages of peatland development, thereby stimulating *Sphagnum* growth and increasing its N content. It was found that the *Sphagnum*-associated diazotrophic community consisted up to 30% of methanotrophs (Vile et al. 2014).
Objectives: The objective of this study was to elucidate whether methane actually stimulates N_2 fixation in *Sphagnum* mosses from ombro-, oligo- and mesotrophic field sites and to test whether N_2 fixation is sensitive to oxygen. The second aim was to relate activity measurements to identity of the different diazotrophs including methanotrophs by analysis of total and diazotrophic microbial communities.
Methods: N_2 fixation and CH_4 oxidation activity were studied by incubating mosses with $^{15}N_2$, $^{15}N_2$ + $^{13}CH_4$ or no additions. Furthermore, different oxygen regimes (aerobically, microaerobically (either N_2-He atmosphere or submerged) were applied. DNA and RNA were extracted and analysed for 16S rRNA and nitrogenase genes (nifH) using both qPCR and high-throughput sequencing of amplicons.
Results and Discussion: Tracer studies showed that the diazotrophic communities associated with *Sphagnum* spp. in oligotrophic sites were more active when incubated with both $^{15}N_2$ + $^{13}CH_4$, under microaerobic conditions. Diazotrophic activity in Sphagnum mosses from ombrotrophic and mesotrophic sites was not affected by methane. Furthermore, in mosses from the same site also oxygen did not affect diazotrophic activity inside the *Sphagnum* mosses. Preliminary analysis of both 16S rRNA and nifH genes showed that the mosses harboured a high prokaryotic and diazotrophic diversity. Combined these results imply that in general methane does not induce nitrogen fixation by the *Sphagnum*-associated diazotrophic community, only with specific environmental conditions. Future studies should investigate how much methanotrophic diazotrophs contribute to nitrogen supply of the plant, and which conditions are most favorable to stimulate this contribution.

References: Larmola et al. (2014) *PNAS* 111 (2):734-9
Vile et al. (2014) *Biogeochemistry* 121 (2) 317-328

SYP17

Environment isolates are strongly inhibited by defensive secretion of the flour moth *Ephestia kuehniella*

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Introduction: Insects developed different mechanisms to defend against microbial pathogens. One mode of protection is the release of anti-microbial compounds (AMC) into the environment in order to prevent the growth of potential pathogens before the infection. But the conditioning of the environment comes together with high cost to produce these AMC, therefore the insect needs to find the right balance to defend either against a broad spectrum of microorganisms or to specialise against specific microorganisms in their environment. Not only do insects use their own immune system but also the help of their symbiotic microorganisms to defend against pathogens.
Objectives: The larvae of the flour moth *E. kuehniella* condition their food source with silken webs and faeces. The aim of this study was to find AMC, which condition the environment to prevent growth of pathogenic

microorganisms. Furthermore, we also tested the range of anti-microbial effects and its specificity.
Materials & Methods: Extracts from the larvae of *E. kuehniella* as well as from a mixture of their silk and faeces were taken for the analysis. The analysis of anti-microbial-activity of the extracts was done using zone of inhibition tests and growth assays in liquid medium. The screening was done against either different off-the-shelf test organisms or against environmental bacterial or fungal isolates from the red flour beetle *Tribolium castaneum.*
Results: Extracts from the larvae and from the silk and faeces mixture showed an anti-microbial effect against Gram positive bacteria. Both extracts showed a different anti-microbial pattern with strong inhibition against environmental *Staphylococcus* isolates.
Conclusion: The AMC, which are extracted from the surface of the larvae as well as from the mixture of silk and faeces of *E. kuehniella* show a more specific anti-microbial effect rather than a broad spectrum effect. In conclusion, we assume that *E. kuehniella* has developed a defence mechanism to environmentally present wild type microorganisms, rather than a defence to those which are commercially available. Further, that both extracts consist of a different AMC cocktail but it's still under investigation if its origin is from the insect itself or from their symbionts.

SYP18
Screening for keratin-degrading bacteria in the gut of the common clothes moth *Tineola bisselliella*
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Introduction: Larvae of the common clothes moth, *Tineola bisselliella* are able to digest keratinous structures in form of wool, fur and feathers. Feeding on keratin represents an exceptional form of nutrition, as it is recalcitrant to common digestive enzymes and classified as indigestible. The unusual feeding behavior of the clothes moth larvae suggests an interesting microbial gut community that might be involved in keratin digestion.
Objectives: Culture-dependent screening for keratin-degrading bacteria in the gut of the common clothes moth larvae.
Methods: Larval gut bacteria are cultivated in feather meal media under aerobic and anaerobic conditions. To reflect larval gut conditions cultivation is performed at pH values from 7 - 10. Cultures are separated on feather-meal plates and single bacterial colonies are isolated and identified. To test the keratin-degrading capability, the isolated bacteria are cultivated in nutrient free media supplemented with a keratin azure substrate. Keratinolyis is then indicated by the release of blue azure dye into the medium. Additionally the isolates are cultivated with whole chicken feathers. The capability for keratinolysis is visually investigated on the grade of feather degradation. Both assays are performed in a pH-range from 7 - 10.
Results: Under aerobe cultivation conditions *Bacillus*, *Lysinibacillus* and *Variovorax* spp have already been identified. Several isolates show a partial or whole feather degradation. The keratin azure assay confirms a pH-depending keratinolytic activity of isolated bacteria.
Conclusion: The results suggest that feather-digestion of the clothes moth larvae is mediated by keratin-degrading bacteria and their keratinolytic enzymes. Cultivation of the bacteria and the identification of the responsible enzymes may allow the biotechnological applications in pharmaceutical, animal feeding and bio fuel industries.

SYP19
Diversity and localization of bacterial symbionts from whitefly and parasitoid species collected in Iran
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Introduction: Many whiteflies species such as *Bemisia tabaci*, *Trialeurodes vaporariorum* and *Siphoninus phillyreae* are phloem-feeding insects. Because of this unbalanced diet, those insects harbor the primary symbiont *Portiera*, and a diversity of secondary bacterial symbionts (SS) which impact different aspects of their lifestyle.
Objectives: the aims of this studywere to survey the distribution of whitefly species collected in different provinces in Iran and their infection with SS, and to localize SS inside developmental stages of the whitefly. Whitefly parasitoid populations were also surveyed for infection with SS.
Materials & Methods: Populations of *B. tabaci, T. vaporariorum* and *S. phillyreae,* and the parasitoid species *Encarsia formosa, E. inaron* and *Eretmocerus mundus* were collected in different provinces in Iran, and analysed for genetic identity using Cytochrome Oxidase I sequencing, and for spatial localization inside the whitefly and parasitoid bodies using fluorescence in situ hybridization (FISH).
Results: Only *B. tabaci* B biotype was detected in Iran and exhibited the existence of at least two haplotypes. The three whitefly species were prevalent in all provinces, although *S. phillyreae* was surveyed in some regions. The B biotype was found to be infected with *Hamiltonella* and *Rickettsia, T. vaporariorum* with *Arsenophonus* and *Hamiltonella,* and *S. phillyreae* infection with SS is still ongoing. The majority of the parasitoid species were infected with *Rickettsia* and some with *Wolbachia* and *Cardinium.* Localizations patterns of the different symbionts were similar to previously reported results, however, in some parasitized whitefly samples which showed localization of the whitefly SS inside the developing parasitoid larvae, a leak of *Arsenophonus* outside the bacteriosomes into the hemolymph was observed.
Conclusion: The whitefly species surveyed in this study showed unique and shared patterns of SS infection, suggesting common ancient infections possibly via horizontal transfer. Infection of parasitoids with common bacterial species between whitefly species suggests another mechanism for horizontal transfer. Understanding the mechanisms of SS bacterial distribution and their spread between whitefly species may shed light on their co-evolution and co-adaptation.

SYP20
Microbiome Analysis to Screen for Diversity and Bioactivity in the Sponge *Haliclona* sp.
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Marine sponges (phylum Porifera) act as ecological niches for phylogenetically highly diverse bacteria that fulfill numerous functions for their hosts. Sponges can benefit from, inter alia, bacteria-derived bioactive secondary metabolites to compensate their lack of true physical defense mechanisms. In general, sponges either associate with large and diverse microbial communities (high microbial abundance species, HMA) or affiliate with a less diverse and low microbial load (low microbial abundance species, LMA). We investigated a sponge of the genus *Haliclona* which shows an outstanding defense mechanism which is indicating bioactivity. The abundance and community structure of the sponge associated community was manipulated in a four week stress experiment (dark and light incubations in addition to antibiotics) to test whether bioactive compounds are produced by bacteria. Bioactivity assays were performed in parallel to microbiome studies using denaturing gradient gel electrophoresis and Illumina amplicon sequencing of PCR amplified 16S rRNA gene fragments. Despite major impacts on the host expressed as decreased growth rates and color change, no shifts of bioactivity were detected. The community structure of the microbiome changed; however, typically bioactivity producing bacteria were mostly unaffected. Phylum-level diversity and high relative abundance of indicator species suggest an affiliation to the HMA group, which additionally supports the assumption of bacteria-derived bioactivity. More detailed analyses will follow to identify the responsible bacteria and the compounds they produce.

SYP21
Endophytic bacteria associated to cowpea nodules
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Introduction: Cowpea (*Vigna unguiculata*) tolerates high temperature, drought and low soil fertility and is, hence, one of the most important food legumes in semi-arid regions. Even though under favorable growth conditions high yields have been achieved, at low input managemt practices the productivity of cowpea remains rather low. Inoculation of seeds with plant growth promoting (PGP) bacteria, mainly rhizobia, was proposed as a sustainable alternative to enhance productivity.
Objetives: The aim of this study was to investigate the diversity of bacterial symbionts nodulating cowpea in Brazilians semi-arid soils via high throughput sequencing. Based on these data, new cultivation methods should be developed to allow the isolation of undescribed bacteria.
Material and methods: Nodules, rhizosphere and soil samples were obtained from two soils (Petrolina, PE - argissol and Juazeiro, BA - vertissol) cultivated with the cowpea varieties BRS Acauã (low nodulation) and BRS Pujante (high nodulation). After surface sterilization, DNA was extracted and used for analysis of 16S rRNA genes via T-RFLP and barcode sequencing.
Results: The structure of bacterial communities found in the nodules was determined by the soil type rather than the variety. We detected a high bacterial diversity in the nodules. Most of the detected groups were isolated from cowpea nodules in previous studies. Surprisingly, although *Bradyrhizobium* sequences were highly abundant, OTUs affiliated to *Chryseobacterium* were predominant in all samples.
Conclusions: Our study reveals that the bacterial diversity found in cowpea nodules is much higher previously expected. We speculate that these non-rhizobial endophytic bacteria improve plant fitness by promoting the increasing of infection sites for rhizobial colonization.

SYP22
Electron cryo-tomography discovers a putative archaeal secretion system and its role in host-parasite interaction
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Ignicoccus hospitalis is an unusual hyperthermophilic archaeon that has been isolated from marine hydrothermal vents. The microorganism adheres to surfaces using type IV-like (Iho670) pili, which emanate from a multimegadaton complex. By using electron cryo-tomography (cryoET) we have located this putative archeal secretion system (PASS) at focal contact sites between the inner and outer cell membranes (IM/OCM). Sub-tomogram averaging revealed that the PASS has an unusual cartwheel-like structure with a central channel of 20 nm in diametre. Mass-spectrometry and proteomic profiling is currently beeing employed to dissect the protein composition of PASS.
In its natural environment, *Ignicoccus hospitalis* serves as host to a parasitic archaeon, *Nanoarchaeum equitans*. This parasite lacks genes to regulate essential metabolic pathways and is therefore dependent on importing metabolites, lipids and proteins from *I. hospitalis*. We found that the parasite is equipped with a unique macromolecular transmembrane protein complex, which seems to hook onto Iho670 pili emanating from *I. hospitalis*. By a yet unknown mechanism, *N. equitans* progresses along these pili towards membrane contact sites of its host. At the cell surface, the same complex pierces through the IM and OCM of *I. hospitalis* and thereby establishes a connection between the cytosols of both cells. Confocal fluorescent light microscopy shows that this complex is capable of transporting entire folded proteins. Our observations reveal a novel pathway of intercellular protein transport used by an archaeal parasite to "feed" on its host.

SYP23
Conservation of the type III-secreted effectors in bradyrhizobia isolated from different host plants
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Several studies have demonstrated that the presence of type III secretion systems alters symbiosis between rhizobia and legumes. Secreted effector proteins play a major role in host range determination, and can have positive as well as negative effects on the interaction. The soybean endosymbiont *Bradyrhizobium japonicum* USDA110 was shown to secrete at least 10 proteins in a type III-dependent manner [1].
The legume *Lupinus mariae-josephae* is endemic in Eastern Spain. This plant is of interest especially for its ability to grow on alkaline soil. Several specialized symbionts from the genus *Bradyrhizobium* have been isolated from this legume. These strains were characterized and six rhizobial strains were sequenced. Analysis of the genome sequences revealed the presence of genes encoding putative type III protein secretion systems. *Bradyrhizobium* sp. strain LmjC encodes a type III secretion system in a cluster of 30 genes. In this cluster, a gene coding for the putative effector NopE was identified. This protein shows high similarity to the effector NopE1 of *B. japonicum* USDA110. This effector has been characterized previously [2]. The protein NopE1 of USDA110 contains two metal-ion inducible autocleavage (MIIA) domains. These domains are cleaved in the presence of calcium ions at a conserved cleavage site. In contrast to USDA110 the protein sequence of NopE from LmjC contains only a single MIIA domain. The domain was shown to undergo also autocatalytic cleavage in the presence of several metal ions. A *nopE* deletion mutant of LmjC showed no difference in nodulation with *L. mariae-josephae*. We are further investigating effector proteins of symbiotic bradyrhizobia and their role in symbiotic interaction.

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SYP24
How Streptococcus pneumoniae and Pseudomonas aeruginosa play with hydrogen peroxide when they come together?
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Introduction: Streptococcus pneumonia can cause serious infections. Many other pathogens including Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella penumoniae, Neisseria meningitides, Haemophilus influenzae are frequently detected in pneumococcal infection. It is known that S. pneumonia produces H2O2 to inhibit competing flora1. However, the killing-effect of H2O2 is non-specific. Because S. pneumonia is catalase-negative, it is interesting to know how S. pneumonia reacts to H2O2 if produced at high level under certain conditions? Is there symbiotic effect with other catalase producing pathogens in this regard?
Objectives: To address these questions we studied the growth physiology of S. pneumoniae in an exactly controlled cultivation system in which the dissolved O2 concentration (pO2) was varied. We then examined the potential interaction of S. pneumoniae and P. aeruginosa.
Materials and Methods: Batch cultivations with controlled pH, pO2 and other parameters were performed in a well-equipped parallel bioreactor system. H2O2 production was measured. Celll free supernatant of P. aeruginosa containing catalase was added to S. pneumonia culture to assess potential interactions of these two pathogens.
Results: Microaerobic condition supported the highest cell density of S. pneumoniae compared to anaerobic or aerobic conditions. Under aerobic conditions, cell growth was quickly inhibited by the accumulation of H2O2 which can reach as high as 0.4 mmol/L. The addition of P. aeruginosa cell free supernatant which contains catalyse enhanced the growth of S. pneumoniae significantly under aerobic conditions.
Conclusion: Pure culture of S. pneumoniae can produce too much H2O2 which strongly inhibits its own growth under aerobic conditions. The presence of P. aeruginosa (culture supernatant) can help S. pneumoniae to circumvent this problem. This symbiotic interaction may explain the survival of S. pneumoniae in highly oxygenated environment such as in lung infection.

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SMP01
Metabolic engineering of *Saccharomyces cerevisiae* for cyclic triterpenoid production
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Triterpenoids are terpenoids derived from squalene and consist of six isoprene units (C30). These compounds can be isolated from many different plant sources. They occur in countless variations and can be subclassified into several groups including squalenes, lanostanes, dammaranes, lupanes, oleananes, ursanes, hopanes, cycloartanes, friedelanes, cucurbitacins, and miscellaneous compounds. Many of them or their synthetic derivatives are currently being investigated as medicinal products for various diseases, including cancer. Despite their obvious interest for the industry, their wide applications are often hindered by the presence of these compounds in only minute amounts in natural sources. This poses challenges in a biosustainable production of such compounds since per gram active ingredient produced a high volume of solvent is needed in the purification process.
Within the project TRITERP we establish a biotechnological process for the production of a cyclic triterpenoid, using tailored *Saccharomyces cerevisiae* strains. The plant metabolite has antiretroviral, antimalarial, and anti-inflammatory properties and has potential as an anticancer agent and is of high interest for the pharmaceutical and nutritional industry (Muffler et al. 2011).
The strain re-engineering is accomplished using advanced and modern molecular biology and synthetic biology tools and taking advantage of the genome-scale science, i.e. applying omics technologies for in depth physiological strain characterization and metabolic modeling for data analysis and strain design.
High overproduction of the triterpenoid will reduce downstream processing efforts and will allow the economic and sustainable production of this promising compound.

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SMP02
Construction of plasmid-free *E. coli* cells for the synthesis of human milk oligosaccharides
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Introduction: Beneficial effects of human milk oligosaccharides (HMOs) for infants raised attention for HMOs as potential nutritional additives for infant formula [1]. However, chemical or *in vitro* enzymatic syntheses or purification from human milk are laborious or costly.
Objectives: Our aim was the construction of plasmid-free *Escherichia coli* strains capable of synthesizing HMOs using recombinant glycosyltransferases in combination with augmented supplies of intracellularly generated nucleotide-activated sugars. Strain construction should be followed by HMO production to enable further research on these compounds.
Methods: Strain construction was based on *E. coli* K12 strains and utilized an adapted site-specific λ-red recombineering technique for chromosomal integration of heterologous genes in combination with screening on differential agar plates [2]. Strain evaluation and HMO synthesis was conducted in shake-flask cultivations and fed-batch fermentations. HPLC analysis of intracellular precursor molecule levels allowed their quantification and increase for improvement of productivity.
Results: Plasmid-free *E. coli* strains with up to 6 consecutive integrations in one strain 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 enabled syntheses in fed-batch fermentations without the need for selection markers, resulting in product concentrations, e.g. of 2'-fucosyllactose, of more than 20 g/L [3,4].
Conclusion: Chromosomal integration coupled with screening on differential agar plates is a powerful tool for synthetic microbiology. It allows multiple rapid and site-specific integrations and construction of plasmid-free strains. Utilizing this method, we could demonstrate the combination of specific glycosyltransferases with enhanced intracellular synthesis of nucleotide-activated sugars for the efficient synthesis of HMOs.

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SMP03
Biosensor-based experimental evolution of production strains
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Introduction: Integrated into synthetic regulatory circuits, bacterial transcription factors can be used as biosensors which translate microbial small molecule production into an optical readout (e.g. fluorescence). Recently, we developed a sensor based on the regulator Lrp of *Corynebacterium glutamicum,* which enables the detection of methionine and branched-chain amino acids (1,2). The biosensor was successfully implemented in FACS high-throughput screenings for the isolation of amino acid production strains from mutant libraries (1). However, random mutagenesis typically results in a large number of SNPs in isolated clones, which renders the identification of the effective mutation(s) difficult.
Objectives: Here, we performed a biosensor-based experimental evolution (without chemical mutagenesis) aiming at an improvement of growth and production of the L-valine producer strain *C. glutamicum ΔaceE* (3).
Methods: To this end, Δ*aceE* sensor cells with the highest fluorescence were iteratively isolated and (re-)cultivated using FACS. With this approach, we expected to enrich beneficial and select against detrimental mutations. Isolated strains were analyzed by genome sequencing and further characterized regarding growth and L-valine production by HPLC.
Results: The isolated, evolved *ΔaceE* strains revealed an increased growth rate, a shortened lag-phase and an increased final optical density. The L-valine production of some strains was increased up to 100% compared to the parental strain while the formation of by-products (L-alanine) was reduced. Genome sequencing revealed about 20 different SNPs enriched in the population within six iterative cycles of evolution. Reintroduction of selected mutations into the parental strain revealed a specific mutation which led to a two-fold increased L-valine production of the Δ*aceE* strain.
Conclusion: These results emphasize biosensor-based strain evolution as a straightforward approach to improve growth and productivity of microbial production strains. This novel strategy enlarges the repertoire of engineering techniques and is able to contribute to the establishment of novel cell factories.

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SMP04
Biochemical diversification of insecticidal rhabdopeptides in entomopathogenic bacteria
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Entomopathogenic bacteria of the genera *Xenorhabdus* and *Photorhabdus* are a rich and novel source of natural products with interesting biological activities, such as insecticidal, antifungal, antimalarial or cytotoxic activity. The bacteria live in a mutualistic symbiosis with nematodes of the genera *Steinernema* or *Heterorhabditis*, respectively and together kill the host insects.[1]
Rhabdopeptides are linear nonribosomal peptides, which have been originally identified from *Xenorhabdus nematophila* HGB081.[2] These compounds consist of none-polar amino acids (Val, Leu, Phe) that are often *N*-methylated and carry a phenylethylamine (PEA), a tryptamine (TRA) or an agmatine (AGM) moiety as terminal amine. They show bioactivity against insect cells and *Plasmodium falciparum,* the causative agent of malaria.[2] The rhabdopeptide biosynthetic gene cluster (*rdpABC*) is quite simple and encodes three monomodular nonribosomal peptide synthetase (NRPS) that can be iteratively used to synthesize rhabdopeptides of different lengths. Genomic sequences of additional *Xenorhabdus* and *Photorhabdus* species revealed that similar *rdp* gene clusters are widespread among these bacteria.
In our study, we could clone and heterologously express several of these gene clusters in *Escherichia coli*. Additionally, we also obtained several novel rhabdopeptides via exchange of adenylation (A) domains, coexpression of *rdp* genes from different *Xenorhabdus* strains, and manipulation of the methyltransferases. Thus we were able to mimic the rhabdopeptide diversity that is found in nature and is a result of

flexibilities of individual domains and complete modules being an example for natures' way of creating structural diversity.

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SMP05

Tryptophan *C5*-, *C6*- and *C7*-Prenylating Enzymes revealed a clear Preference for C-6 Alkylation/Benzylation in presence of unnatural DMAPP analogues

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Introduction: Prenyltransferases (PTs) catalyze in nature the attachment of prenyl moieties of prenyl donors, usually dimethylallyl diphosphate (DMAPP) to various aliphatic or aromatic scaffolds (1,2). Previous investigations with tryptophan *C4*- and *C5*-prenyltransferases showed that they also used unnatural methylallyl, 2-pentenyl or benzyl diphosphate as alkyl or benzyl donors (3,4).
Objectives: In this study, the behaviors of two *C5*-prenyltransferases 5-DMATS and 5-$DMATS_{Sc}$, two *C6*-prenyltransferases 6-$DMATS_{Sa}$ and 6-$DMATS_{Sv}$ as well as one L-tyrosine prenyltransferase TyrPT with a tryptophan *C7*-prenyltransferase activity were investigated in the presence of the aforementioned DMAPP analogues.
Methods: In *E.coli* overproduced recombinant proteins were purified and used for enzyme assays. Determination of enzyme activities and product isolation were carried out on HPLC. The structures of the enzyme products were elucidated by NMR and MS analyses.
Results: All of the tested enzymes accepted the DMAPP analogues with different relative activities. The sole *C6*- alkylated/benzylated derivatives were identified as enzyme products of the two 6-DMATS enzymes. 5-DMATS produced *C5*- and *C6*-alkylated/benzylated derivatives and TyrPT and 5-$DMATS_{Sc}$ *C5*-, *C6*- and *C7*-alkylated/benzylated derivatives. However, *C6*-alkylated or benzylated products were found as main products in all of these assays. This demonstrated a clear preference of the five PTs for alkylation/benzylation at C-6 of the indole ring in the presence of unnatural DMAPP derivatives
Conclusion: These results expand significantly the potential usage of tryptophan prenylating enzymes as biocatalysts for Friedel-Crafts alkylation. Further structural analysis of these enzymes in complex with unnatural DMAPP analogues would provide new insights into the substrate binding sites and give a more accurate explanation for this interesting phenomenon.

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SMP06

Selective Oxyfunctionalization of Terpenes using Rieske non-heme Dioxygenases

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The regio- and stereoselective hydroxylation of unactivated C-H bonds is one of the most challenging and highly sought-after reactions in organic chemistry. The selective oxidation of terpenes to their oxygenated derivatives is of particular interest. The resulting terpenoids find broad application in the flavor and fragrance industry and are used as chiral synthons for chemical synthesis in the pharmaceutical industry.
The chemical strategies to oxidize such hydrocarbons are mainly based on heavy metal catalysts, which are not only environmentally toxic, but also lack regio- and stereoselectivity. Furthermore, the formation of undesired byproducts and therefore an increase in downstream costs have to be considered. Enzymatic catalyzed reactions, however, are known for their high selectivity and mild reaction conditions. Especially, the Rieske non-heme dioxygenases (ROs), which are also known as the non-heme analog to the well-known P450-monooxygenases[1], are able to perform mono- as well as dihydroxylations with high regio- and stereoselectivity utilizing an environmentally harmless oxidant such as O_2. These multicomponent systems consist of a reductase and a ferredoxin which are responsible for the electron transport towards the catalytic active oxygenase. The high versatility of the ROs is reflected by the catalysis of over 300 diverse substrates ranging from polyaromatic hydrocarbons to halogenated ethylenes[2]. Consequently, the dioxygenases seem to be suitable enzymes for the efficient production of defined products with high enantiomeric purity.
Here we report the conversion of a wide range of hydrocarbon compounds by different novel and rational engineered dioxygenases which in further steps will be optimized towards these new unnatural substrates.

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SMP07

Development of a citrate biosensor based on the CitAB two-component system

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Introduction: Like most bacteria, the Gram-positive soil bacterium *Corynebacterium glutamicum* employs two-component signal transduction systems (TCS) for sensing and adapting to environmental changes [1]. The CitAB TCS composed of the sensor kinase CitA and the response regulator CitB was previously shown to be required for utilization of citrate as carbon source by activating the expression the citrate transporter genes *citH* and *tctCAB* when citrate is present in the medium [2].
Objectives: Detailed analysis at the single cell level of citrate-dependent activation of the target promoters P_{citH} and P_{tctC} using *eyfp* as reporter gene.
Methods: Construction of plasmid-based transcriptional fusions of P_{citH} and P_{tctC} with *eyfp* and transfer into *C. glutamicum* wild type (Cit^+) and a Cit^- derivative. Analysis of the recombinant strains for citrate-dependent *eyfp* expression by measuring the eYFP fluorescence of entire cultures and of single cells, the latter by flow cytometry. Expression engineering by removing repressor binding sites and altering ribosome binding sites.
Results: The reporter strains showed citrate-dependent eYFP fluorescence, whose strenght could be enhanced by improving the ribosome binding site and by removing the binding site for the global regulator GlxR in the two target promoters. Interestingly, P_{citH} and P_{tctC} differed with respect to their citrate responsiveness. As expected, the kinetics of eYFP fluorescence differed for the Cit^+ and Cit^- strains. Flow cytometry revealed that citrate-induced *eyfp* expression resulted in very uniform populations.
Conclusions: Using *eyfp* as reporter gene, the citrate induction characteristics of the CitAB target promoters P_{citH} and P_{tctC} were determined at the single cell level, the repressing role of global regulator GlxR was shown, and expression strenght was improved by engineering the ribosome binding site.

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SMP08

A cloning-free method for genome editing and enhanced transformation efficiencies boost genome engineering in *Sinorhizobium meliloti* strain Rm1021

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Introduction: Genome analysis of the N_2-fixing plant symbiont *Sinorhizobium meliloti* strain Rm1021 revealed a type-I restriction-modification (R-M) system which is encoded by the *hsdMSR* operon [1]. Whereas HsdMS is necessary for methyltransferase activity, the restriction endonuclease HsdR degrades DNA lacking the appropriate modification [2]. Restrict of the transfer of heterologous DNA by R-M systems hampers genetic engineering. In *S. meliloti* HsdR was shown to reduce the efficiency of conjugation and electroporation drastically [3]. Moreover, common cloning techniques for *S. meliloti* are time-consuming and therefore inappropriate for comprehensive genomic manipulations.
Objectives: It is our aim to establish a sufficient basis for genome engineering in *S. meliloti*. Therefore, we intend to optimize electroporation efficiency in the *S. meliloti* wild type by adjusting methylation of the donor DNA and by employing an *hsdR*-deleted strain. Furthermore, genome engineering in *S. meliloti* requires flexible tools which should be repetitively applicable.
Methods: Electroporation and conjugation of *S. meliloti*; Cre-lox recombination; heterologous gene expression in *E. coli*.

Results: We constructed an *hsdR* deleted strain and determined its transformation efficiency. Furthermore, we achieved at least a 10-fold elevated electroporation efficiency of the *S. meliloti* wild type using plasmid DNA purified from $hsdMS_{S.meliloti}$ expressing *E. coli* cells. To facilitate genome engineering in *S. meliloti*, we established a rapid method for cloning-free genome editing and demonstrated its application by construction of *S. meliloti* knockout mutants and Cre-lox mediated deletions.
Conclusion: Increased transformation efficiency of *S. meliloti* was achieved by use of a strain lacking the *hsdR* gene. Furthermore, DNA which is geared to the needs of the *S. meliloti* R-M system also results in strongly increased electroporation efficiency. Moreover, our cloning-free method facilitates genome editing procedures.

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SMP09

Targeted coexpression of a NRPS gene with different prenyltransferase genes in *Aspergillus nidulans*

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Introduction: Peptides are important secondary metabolites, which can be synthesized by the multifunctional enzymes non-ribosomale peptide synthetases (NRPSs) and further modified by different enzymes, *e.g.* prenyltransferases (PTs). [1-3] The modified derivatives show remarkable diversity in structure and biological activity.
Objectives: To produce prenylated cyclic dipeptides *in vivo*, the cyclic dipeptide-forming NRPS *ftmPS* from *Neosartorya fischeri* was coexpressed with different prenyltransferase genes in *Aspergillus nidulans.*
Methods: The coding sequences of the NRPS and PT genes were cloned into expression vectors with different selection marker, *pyrG* for NRPS or *pyro*A4 for PT, under control of artificial *gpdA* promoter and *trpC* terminator. After protoplast-mediated transformation in *A. nidulans* TN02A7, the potential transformants were selected by complementation of uracil and/or pyridoxine auxotrophy. PCR amplification was used to confirm the ectopic integration into the genome of the transformants. After cultivation, new accumulated products were isolated and identified by HPLC, NMR and MS analyses.
Results: Expression of *ftmPS* alone resulted in the formation of brevianamide F (*cyclo*-l-Trp-l-Pro) with yields of up to 36.9 mg l^{-1}. Introducing the prenyltransferase genes *cdpC2PT*, *cdpC3PT* and *cdpNPT* into a *ftmPS* mutant resulted in *C2*- and/or *C3*-reversely as well as regularly *N1*-prenylated products. The yields of the prenylated products were found to be up to 11.2 mg l^{-1}.
Conclusion: *In vivo* results of this study provide experimental evidence that *ftmPS* from *N. fischeri* catalyzes specifically the formation of brevianamide F and therefore the first pathway-specific reaction step in the biosynthesis of fumitremorgins.[4] Furthermore, this study demonstrated the potential of synthetic microbiology for production of desired compounds by targeted gene expression.

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SMP10

En route to an optimized synthetic promoter environment for *Bacillus subtilis*

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Introduction: The ability of transfering extracytoplasmic function sigma factors (ECFs) and their target promoters between organisms would largely expand our capability to generate orthogonal switches [1]. However, this is challenged by differences in GC content of the coding genes and the poorly understood differences in the architecture of the target promoters [1].
Objectives: It is our goal to develop a *B. subtilis*-optimized synthetic promoter environment (OSPE) to act as a scaffold in which ECF-specific -35 and -10 promoter elements can be embedded. This will allow the implementation of ECF-based orthogonal switches into *B. subtilis*, regardless of their origin.
Methods: We introduced into *B. subtilis* a switch composed of one of three alleles of *Bacillus licheniformis* [2] or *Streptomyces venezuelae* ECF41 proteins along with their target promoters. We investigated the critical promoter size, the feasibility of transfering the characteristic -35 and -10 elements into different promoter environments (*B. licheniformis* P_{ydfG} and/or *B. subtilis* P_{sigW}), the requirement of transfering additional less conserved nucleotides along with the -35 and -10 elements and the tolerance for changes in the spacer length.
Results: We observed that reduction of the UP element and transfer of specific -35 and -10 elements to P_{ydfG} environment had no influence on the promoter activity. However, reduction in promoter activity was observed following removal of the region downstream of the transcriptional start site, transfer of specific -35 and -10 elements to P_{sigW} environment and a reduction in spacer length.
Conclusion: A *B. subtilis* OSPE of significantly reduced size can be based on naturally occurring promoter sequences of other ECFs. Moreover, the characteristic spacer length of the cognate ECF must be preserved.

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SMP11

Adaptation of *Escherichia coli* to L-tryptophan analogs

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Introduction: To expand the repertoire of building blocks for polypeptides and to overcome the limitations of the chemical functions introduced by the 20 canonical amino acids [1], we are using continuous culture techniques to evolve *E. coli* strains able to utilize amino acid analogs.
Objectives: The objective of our work is the generation of descendants of tryptophan-auxotrophic *E. coli* cells that incorporate the non-canonical amino acids 4-aza-L-tryptophan and 4-fluoro-L-tryptophan instead of the canonical amino acid L-tryptophan in their proteome.
Methods: A conditional pulse-feed regime was applied in the Genemat format [2], where alternative pulses of relaxing (i.e., tryptophan-containing) medium and stressing (i.e., analog-containing) medium automatically establish selective conditions which favor variants of *E. coli* that are adapted to usage of the analog.
Results and Conclusion: Genemat cultures fed with media containing the precursors 4-aza-indole and 4-fluoro-indole have been pursued for approximately 11500 and 9000 generations, respectively. Adaptations to these metabolic challenges will be discussed.

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SMP12

Engineering the glycolytic pathway of *Escherichia coli* K12 mutants by gene deletion and introduction of Fructose 6-phosphate Aldolase

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Introduction: Mutations in genes for key enzymes of the Embden-Meyerhof-Parnas route (glycolysis) have a major impact on growth behavior and survival of the cells on distinct C-sources. *Escherichia coli* K12 mutants deficient in phosphofructokinase (PfkA and 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 phosphoglucoseisomerase (PGI) resulted in impaired growth on sugars entering the cell at the level of glucose-6-phosphate [2]. *E. coli* harbors genes for F6P-Aldolase (FSA) [3] which cleaves F6P to dihydroxyacetone and glyceraldehyde 3-Phosphate. This could circumvent growth deficiencies caused by the lack of PfkA and PfkB, but native FSA expression has rarely been described [4].
Objectives: Our aim was to investigate a possible glycolytic shunt using F6P-Aldolases.
Methods: An *E. coli* LJ110 triple mutant (MT2) (lacking PfkA, PfkB and PGI activities) was created by marker-less gene deletion [5]. The strain was characterized and used for analysis of cells overexpressing FSA wildtype gene or its variant A129S.
Results: MT2 showed a complete loss of growth on PTS-substrates as D-mannitol which enters glycolysis at the level of F6P. This defect could

almost be restored by expression of the plasmid FSA variant A129S but not by the WT gene. By long-term cultivation the generation time could be reduced from 5,5 h to 4,5 h. 2D-gel analysis indicated that a strong overexpression of the mutant allele A129S caused 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 of MT2 by FSA A129S expression strongly indicate that the glycolytic shunt was functional. We propose that F6P was accumulated by MT2 when grown on mannitol. Viability was restored however in the A129S strain. Metabolite analyses are underway.

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SMP13
Prenylation of tripeptides by tryptophan prenyltransferases
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Introduction: Naturally occurring prenylated indole alkaloids containing tripeptidyl backbone are found in filamentous fungi. Specific prenyltransferases are responsible for the prenylation of such tripeptides [1].
Objectives: In this study, we investigated the acceptance and conversion of these compounds by prenyltransferases from different gene clusters.
Methods: The overproduced His-tagged prenyltransferases were purified over Ni-NTA affinity chromatography and subsequently applied for incubation with synthesized tripeptides in the presence of dimethylallyl diphosphate. Product formation was monitored by HPLC analysis and structure elucidation of the prenylated products was performed by NMR and MS analyses.
Results: 22 enzyme products were obtained from incubation mixtures of four substrates with four tryptophan prenyltransferases. Different regio- and stereoselective prenylation reactions were observed for the investigated substrates.
Conclusion: Our results showed that tryptophan prenyltransferases can also use tripeptides as prenylation substrates, which was not observed until now. Furthermore, this study expands the potential usage of such enzymes for chemoenzymatic synthesis and synthetic biology.

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SMP14
Systems metabolic engineering of *Escherichia coli* for effective biosynthesis of L-tryptophan
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Introduction: The development of an efficient microbial strain with targeted molecular engineering for the synthesis of L-tryptophan (L-trp) is a challenging task due to the requirement of several precursors and the complex regulations of pathways involved.
Objectives: We attempted to construct a genetically stable and highly productive *Escherichia coli* (*E.coli*) strain for L-trp production by using systems metabolic engineering which should pave the way for synthetic microbiology of L-trp biosynthesis.
Methods: *E. coli* DY330 was used as the starting point for diverse molecular biological work using established methods [1].
Results: Following systematic engineering of the pathways and regulations for L-typ biosynthesis was successfully implemented: (1) blocking the degradation and the intake of L-trp by deleting the gene *tnaA* and by inactivating the trp-specific importers TnaB and Mtr; (2) deregulating the feedback inhibitions of 3-deoxy-D-arabino-heptulosonate-7-phosphate synthase (*aroG*) by L-phenylalanine, D-3-phosphoglycerate dehydrogenase (*serA)* by L-serine and anthranilate synthase (*trpE*) by L-trp; (3) removing the transcriptional attenuation and repression of the *trp*-operon by replacing the native promoter with the *trc* promoter; (4) enhancement of the first enzymatic reaction to catalyze the direct precursors phosphoenolpyruvate and erythrose-4-Phosphate and increasing the availability of the precursor L-serine by using a strong tandem *J23119-PrspL-tac* promoter to control the expression of $aroG^{fbr}$-$serA^{fbr}$. The resulting strain can produce more than 35 g/l of L-trp with a yield of 0.14 (g/g) on glucose in fed-batch fermentation. The production is twice higher than that of the best strains reported and constructed by defined genetic modification.
Conclusion: This is the first rationally engineered L-trp producing strain reported so far the productivity of which is comparable to that of strains developed by time-consuming classic approaches. This rationally engineered *E. coli* strain represents a useful chassis for synthetic microbiology of L-trp synthesis.

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SMP15
Synthetic Secondary Chromosomes in Bacteria to Study Chromosome Maintenance Systems
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Recent advances in DNA synthesis and new DNA assembly techniques now allow construction of whole synthetic chromosomes. Considering a potential design leads almost certainly to the question: Which are the essential parts of a chromosome? Current investigations in this field are mainly focused on the minimal set of genes. However chromosomes are more than arrays of genes. They need so called chromosome maintenance systems to replicate, segregate and organize the entire genetic material. In bacteria several of these systems are known e.g. Nucleoid Occlusion mediated by SlmA or FtsK orienting polar sequences (KOPS). Anyhow there is still a lack of understanding how chromosomes are organized by the maintenance systems.
Here, we present a synthetic biology approach based on our recently established secondary chromosome in *Escherichia coli* to investigate a potential connection between the DNA mismatch repair (MMR) and the segregation protein SeqA. Both systems require hemi-methylated GATCs to maintain the chromosome. However, while a single GATC is sufficient for MMR, SeqA needs at least two GATCs in close proximity. We designed a set of synthetic secondary chromosomes with varied GATC-distribution allowing selective binding of relevant protein subsets to separate their respective functional impact. The building blocks where constructed based on a new software tool that predicts sequences to include chromosome maintenance motifs only at determined positions. The methodology and progress of synthetic secondary chromosome assembly is presented. Upon completion, the chromosomes will be characterized regarding their respective mutation rates in evolution experiments to characterize the impact of SeqA on MMR.
Our long term goal is to provide construction rules for synthetic chromosomes and to use our system to better understand chromosome maintenance of natural chromosomes.

SMP16
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.

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SMP17

L-citrulline production by metabolically engineered *Corynebacterium glutamicum* from glucose and alternative carbon sources

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Introduction: L-citrulline, intermediate of the L-arginine biosynthesis, plays an important role in human health and nutrition. In *Corynebacterium glutamicum* it is a by-product of L-arginine production. *C. glutamicum* is ideally suited for the production of L-glutamate derived amino acids (e.g. L-citrulline) due to its natural ability to secrete L-glutamate. It is well equipped to consume a variety of substrates and its substrate spectrum was enlarged further by strain engineering.
Objectives: Formation of L-citrulline by *C. glutamicum* as sole product has not yet been reported. Here, we present the engineering of *C. glutamicum* for the overproduction of L-citrulline as major product without formation of L-arginine as by-product. In addition, production of L-citrulline from the alternative carbon sources starch, xylose and glucosamine was sought.
Methods: Expression of the L-arginine biosynthesis operon was derepressed by deletion of the arginine repressor gene *argR*. Conversion of L-citrulline towards L-arginine was avoided by deletion of the argininosuccinate synthetase gene *argG*. Moreover, the genes encoding a feedback resistant N-acetyl L-glutamate kinase (NAGK) *argB*^{fbr} and native L-ornithine carbamoylphosphate transferase *argF* were overexpressed. To enable utilization of starch, xylose and glucosamine for L-citrulline production the heterologous genes *amyA*, *xylAB* and *nagB* were expressed ectopically.
Results: L-citrulline production required overexpression of *argB*^{fbr}, which might indicate inhibition of native NAGK by L-citrulline. The final strain accumulated 44.1 ± 0.5 mM L-citrulline from glucose minimal medium with a yield of 0.38 ± 0.01 $g \cdot g^{-1}$ and a volumetric productivity of 0.32 ± 0.01 $g \cdot l^{-1} \cdot h^{-1}$. In addition, production of L-citrulline from the alternative carbon sources starch, xylose and glucosamine could be demonstrated [1].
Conclusion: Engineering of *C. glutamicum* for L-citrulline production required to overcome its conversion to L-arginine as well as feedback-deregulation of NAGK. Strains for production of L-citrulline from a variety of alternative substrates was achieved.

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SMP18

The Right Word in the Right Place – Designing Genes for Optimal Translation by Adapting Codon Usage

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Introduction: Predicting protein expression levels for synthetic genes is a complex task. Adjustment of codon usage is one important aspect. Conventionally, optimization of codon usage by open software was mainly based the replacement of rarely used codons by frequently used codons in highly expressed genes of a given organism. However, this approach is not generally suitable for prediction of the optimal codon usage.
Objectives: In our work we aim at the development of an improved algorithm for prediction of optimal codon substitutions. We predict such substitutions on the basis of translation speed and accuracy.
Methods: In order to find such algorithm we simulate ribosomes that can attach to mRNA, have to wait for matching tRNA and elongation factors, may not overtake one another and can drop off before finishing translation. For each codon the abundance of cognate (exactly fitting according to Watson-Crick binding) and near-cognate (mismatch of one nucleotide) tRNA leads to a codon dependent elongation rate. The rate with which near-cognates are used defines an error rate.
Results: To test our model predictions we have designed synthetic variants of model genes with differently optimized codon usage as predicted by our model and evaluate expression in the background of a *Salmonella enterica* serovar Typhimurium vaccine strain. We have implemented our model within a novel tool which is suitable for codon usage optimization or deoptimization of heterologous genes in E.coli or related bacteria. With this program the user can simply load a gene sequence in a FASTA formatted file and select either translation speed and/or accuracy as optimization criteria. From this input, the program calculates for selected target organisms how much protein is produced per transcript compared to the wild type and the rate at which errors occurs.
Conclusion: With our new model we can fine tune the expression speed and accuracy of arbitrary genes. The method can be easily applied for other organisms, for example the most common expressions systems *E. Coli*, *S. Cerevisiae* and the human HeLa cell line.

SMP19

Establishment of synthetic microcompartments in *Corynebacterium glutamicum*

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Introduction: The integration of synthetic heterologous pathways into chassis organisms is often associated with the intracellular accumulation of the product or the appearance of toxic intermediates. Eukaryotic cells have evolved a wide range of different organelles to encapsulate specific metabolic pathways within the cell to avoid interference with other cytoplasmic processes. Whereas most bacteria lack compartmentalization, some species use protein-coated microcompartments (BMCs) as distinct reaction chambers.
Objectives: The objective of the work is the establishment of synthetic BMCs in *Corynebacterium glutamicum* which allow encapsulation of heterologous pathways within this important industrial platform organism.
Methods: The BMC shell proteins (PduABJKNUT) from *Citrobacter freundii* and eYFP tagged with different BMC targeting peptides were coexpressed in *C. glutamicum* and fluorescence microscopy analyses were performed for the intracellular localization of the fluorescence signal. Heterologously expressed BMCs were purified by sucrose density centrifugation in order to test for the correct assembly of the BMC complexes. The resulting protein fractions were separated via SDS-PAGE and analyzed via MALDI-TOF-MS/MS.
Results: First studies revealed the successful production of *C. freundii* BMC shells in *C. glutamicum*. EYFP tagged with an N-terminal BMC targeting peptide exhibited distinct fluorescent foci in the presence of BMC shell proteins by the use of fluorescence- and super-resolution microscopy. An additional SsrA-degradation peptide at the C-terminus of eYFP confirmed the protection of BMC encapsulated eYFP from cytosolic proteases and resulted in a lower background fluorescence signal due to the degradation of cytosolic eYFP.
Conclusion: The successful heterologous production of BMC shells and the possibility to deliver proteins of interest into the compartment lumen represent first important steps towards the use of BMCs as synthetic nano-bioreactors in *C. glutamicum*.

SMP20

Metabolic engineering of *Corynebacterium glutamicum* for the utilization of L-Rhamnose

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Introduction: *Corynebacterium glutamicum* is a versatile workhorse for the industrial biotechnology. A variety of different carbohydrates like glucose, fructose and sucrose can be used as sole carbon source for the formation of energy and biomass (1). Moreover, *C. glutamicum* was genetically engineered for the utilization of alternative carbon sources that do not have competing applications in the food industry like glycerol, L-arabinose or N-acetyl glucosamine (2-4).
Objectives: The naturally occurring 6-deoxyhexose, L-rhamnose, is a component of the heteropolysaccharide pectine. L-Rhamnose cannot be utilized by *C. glutamicum* as sole carbon source. To enable the consumption of L-rhamnose and to increase the substrate spectrum we genetically engineered *C. glutamicum.*
Methods: We modified genetically *C. glutamicum* by heterologous overexpressing selected genes of the L-rhamnose degradation pathway of *Escherichia coli* (5).

Results: By heterologous overexpression of the *E. coli* L-rhamnose pathway gene products, the L-rhamnose permase (*rhaT*), L-rhamnose mutarotase (*rhaM*), L-rhamnose isomerase (*rhaA*), *L-rhamnulose kinase* (*rhaB*) and L-rhamnulose-1-phosphate aldolase (*rhaD*) *C. glutamicum* can metabolize L-rhamnose as sole carbon source. Furthermore we could show that L-rhamnose can be used for the production of amino acids like L-lysine.
Conclusion: We demonstrated that *C. glutamicum* can be genetically engineered to use L-rhamnose as sole carbon source for the formation of biomass and production of L-lysine. Behind the enzymatic degradation pathway of L-rhamnose we identified that the consumption of L-rhamnose is limited by the L-rhamnose permase RhaT.

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SMP21
Towards synthetic RNA regulators
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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. Therefore, 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 cyanobacteria.

TOP01
Toxin-Antitoxin systems in the human pathogen *Streptococcus pyogenes*
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Introduction: The Gram-positive bacterium *Streptococcus pyogenes*, also known as group A *Streptococcus* (GAS), is an important human pathogen causing a wide spectrum of diseases such as pharyngitis, necrotizing fasciitis, and toxic shock syndrome. In addition to encoding multiple factors for adaptation and pathogenicity, GAS contains several predicted type II toxin-antitoxin (TA) loci. TA systems are widespread in bacterial pathogens and enable bacteria to adapt to rapidly changing environmental conditions and thereby contribute to the pathogenicity of the organisms.
Objectives: To understand the role in physiology and virulence of these putative TAs in *S. pyogenes,* we aim to characterize hypothetical toxin-antitoxins (TAs) and unravel the molecular mechanisms involved in their regulation.
Methods: Operon architecture and expression of the predicted loci was analyzed by RT-PCR, RNA sequencing (RNA-seq), and reporter-fusions. Toxin and antitoxin activity and function was assessed by growth arrest assays, activity assays and microscopy. To validate protein-protein interactions, TAs were purified and detected by Western blotting.
Results: Three out of four predicted TAs cause growth arrest upon overexpression. We show that upon expression, two of the predicted toxins cause a reduction in CFUs but not optical density, which is due to a cell division defect. Expression of the cognate antitoxins was able to relieve the growth arrest. The antitoxin of one of the loci is misannotated, which was demonstrated by creating a series of transcriptional fusions.
Conclusion: Here, we describe functional chromosomal TA loci in *S. pyogenes*. Understanding of TAs roles in *S. pyogenes* and their regulation, will increase our knowledge on pathogenicity, and may reveal key targets for potential novel antibacterial strategies.

TLP01
Occurrence and Localization of Cytochromes *c* in *Ignicoccus hospitalis* and other Archaea
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Introduction: The hyperthermophilic crenarchaeote *Ignicoccus hospitalis* has uncommon cell architecture with an inner membrane encapsidating nucleoid and ribosomes and an outer membrane without a surface layer. Roles and composition of the intermembrane compartment (IMC) are largely unknown. The outer membrane is energized and harbors ATP synthase and the hydrogenase/ sulfur reductase required for energy conservation. Electron transfer pathways are unknown but seem to be mediated by abundantly available cytochromes (cyt) *c*, causing red coloration of *Ignicoccus* cell extracts. Cyt *c* require maturation since their hemes are covalently attached to the protein backbone, a process typically occurring at the positive side of an energized membrane, e.g. in the periplasm of Gram-negative bacteria. Cyt *c* distribution in Archaea and localization of maturation proteins are not known, especially not in double-membrane Archaea like *I. hospitalis*.
Objectives: We will present (1) properties, structure, distribution, and deduced function of cyt *c* from *I. hospitalis* and (2) a survey of the cyt *c* presence in Archaea. Results will be discussed with respect to cell morphology, and places of maturation in single and double-membrane Archaea.
Methods: Multiheme cyt *c* were purified biochemically from *I. hospitalis*. Cyt *c* prediction in Archaea was done with computational tools and structure prediction.
Results: Three distinct multiheme cyt *c*, localized in both membranes were purified chromatographically to apparent homogeneity from *I. hospitalis*[1]. Three other cyt *c* and system I (CCM) maturation proteins were predicted from sequence. In other Archaea, cyt *c* occur in some but not all of the *Desulfurococcales*, *Thermoproteales*, *Archaeoglobi*, *Methanosarcinae* and Halobacteria. Iron-respiring Archaea were rich in cyt *c* genes as well as the uncultured methane-oxidizing ANME-1 and 2 clusters. *Ccm* maturation genes were found in the most of these species.
Conclusions: Results from *I. hospitalis* point to (1) cyt *c* binding in membranes and (2) their maturation in the IMC. Ignicocci are to only double-membrane cyt *c*-bearing Archaea. The distribution of cyt *c* also suggest that anaerobic methane oxidation might couple to iron reduction.

[1]Naß *et al.* (2014) Microbiology 160:1278-89

TLP02
Novel pili-like surface structures of *Halobacterium salinarum* R1 are crucial for surface adhesion
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Introduction: The extremely halophilic archaeon *Halobacterium salinarum* R1 is able to adhere to surfaces and form complex biofilms [1]. Nevertheless nothing is known about the underlying adhesion mechanism. So far the archaellum is the only surface structure described for *Halobacterium* [2].
Objectives: The aim of our study was the identification of the structures mediating adhesion of *Hbt. salinarum* R1 to surfaces.
Methods: Attached cells of *Hbt. salinarum* R1 were investigated by transmission electron microscopy regarding their surface structures. *In silico* analyses were performed to search the *Halobacterium* genome for putative type IV pilus gene loci. Reverse transcription PCR (RT-PCR) analyses were used to characterize transcription of candidate genes. Their transcription in adherent cells was compared to planktonic cells by

quantitative RT-PCR. Gene deletion mutants were generated by a pop-in/pop-out strategy and characterized [3].
Results: Surface attached cells of *Hbt. salinarum* R1 showed surface structures of two different diameters (7.6 nm and 10 nm). The 10 nm filaments constituted the archaella, but the 7.6 nm filaments represented so far undescribed surface structures. A novel gene locus *(pil-1)* putatively encoding *a* type IV pilus biogenesis complex was identified. This locus was cotranscribed and showed a 5-fold induced expression in adherent cells compared to planktonic cells. Deletions of the genes coding for the archaellum assembly/motor ATPase (*flaI*) and additionally the *pil-1* ATPase (*pilB1*) were generated. The *flaI* deletion resulted in non-archaellated cells, which showed a surface adhesion comparable to the wild type but only possessed 7.6 nm surface filaments. The additional deletion of *pilB1* resulted in the lack of surface structures and a 10-fold reduced surface adhesion in comparison to the wild type.
Conclusion: The type IV pilus *pil-1* locus encodes proteins involved in the biogenesis of surface structures that are crucial for surface adhesion of *Hbt. salinarum* R1.

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TLP03
Unraveling the interactions between the inner membrane platform protein PilC, the assembly ATPase PilB and the nucleotide binding protein PilM of the type IV pili assembly system of *Myxococcus xanthus*
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Introduction: Type IV pili (T4P) are surface structures that are important for various processes including twitching motility, biofilm formation, bacteriophage infection, attachment, virulence and natural transformation. They are assembled by a multiprotein complex that spans both the inner and outer membrane [1].
Objectives: The mechanism by which T4P are assembled is not understood. To investigate this mechanism, we studied the inner membrane platform protein PilC, the assembly ATPase PilB and the nucleotide binding protein PilM of the T4P assembly machinery of *Myxococcus xanthus*.
Methods: To test optimal expression of PilC, PilB and PilM the proteins were expressed under various conditions and as fusion-constructs using several fusion proteins [2, 3]. The proteins were purified to homogeneity and their stability and oligomeric state were tested by size exclusion chromatography, blue native PAGE and, for the nucleotide binding proteins, by ATP binding and hydrolysis assays. Moreover, purified PilC was reconstituted into liposomes. The interactions between the proteins were studied by co-purification assays and ATPase assays performed with different combinations of the proteins.
Results: Optimal expression and purification conditions were obtained with PilB fused to the hexameric Hcp1 protein of *P. aeruginosa* [3], PilM fused to the N-terminal 16 amino acids of PilN and with PilC fused to *E. coli* YbeL, which is a short hydrophilic protein previously shown to stabilize membrane proteins [2]. The three proteins were successfully purified and PilC was reconstituted in liposomes. We will report on the interaction between the different purified proteins in co-purification and ATPase assays.
Conclusion: PilB, PilC and PilM of the T4P assembly system of *M. xanthus* were successfully overexpressed and purified. Our results suggest that PilC, PilB, and PilM interact.

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TLP04
The absence of the archaella leads to an increased biofilm formation in *Halobacterium salinarum* strain R1
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Introduction: The attachment to biotic or abiotic surfaces is a key process of Archaea and Bacteria to form biofilms. Surface structures as the archaella and pili were identified to mediate adherence in archaea. For the extremely halophilic archaeon *Halobacterium salinarum* strain DSM3754^T a first hint for the existence of additional surface structures besides the archaella came from electron microscopy analyses of surface attached cells [1]. Recently we identified novel pili-like surface structures with *Hbt. salinarum* strain R1, crucial for surface adhesion [Losensky et al., submitted].
Objectives: The goal of this study was to investigate the influence of the archaella and the novel pili structures on biofilm formation and topology in *Hbt. salinarum* strain R1.
Methods: Deletion mutants in *Hbt. salinarum* strain R1 were constructed by the pop-in/pop-out strategy [2]. Biofilm formation was investigated by a fluorescence based adhesion assay, the biofilm topology by fluorescence- and confocal laser scanning microscopy [1].
Results: Phenotypic characterization of planktonic cells showed that the absence of the archaella or the novel pili structures had no negative effects on cell morphology or growth behaviour. Non-archaellated and non-pilated *Hbt. salinarum* cells showed a 90% reduced surface adhesion over the time course. For wild type and non-archaellated cells significant adhesion signals were obtained after 3 days, a maximum was reached after 10 days of incubation. On the contrary the biofilm topology differed. The wild type cells formed dense primary cell layers on glass surface with embedded microcolonies with 5 to 37 µm in height. The biofilms formed by non-archaellated cells showed an increased amount of dense microcolonies colonies reaching up to 85 µm in height.
Conclusion: Characterizations of the deletion mutants showed that in *Hbt. salinarum* strain R1 pili but not the archaella are important to colonize surfaces. The absence of the archaella leads to an extended biofilm topology.

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TLP05
Phosphorylation of AbfR1 influences biofilm formation and cell motility in *Sulfolobus acidocaldarius*
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Objectives: Uncovering the mechanism of AbfR1 regulating biofilm formation and cell motility
Methods: In frame marker-less deletion; Biofilm culturing and confocal laser scanning microscopy(CLSM)analysis; Swimming motility on semi-solid gelrite plates; Western blotting; RNA isolation and qRT-PCR; Heterologous protein expression and purification; DNA-protein binding assays
Results: Mutation of Y84 and S87 to alanine in AbfR1 led to increased biofilm formation of the *S. acidocaldarius* mutants and decreased expression levels of *flaB* and *flaX* in biofilm associated cells. Moreover, the abfR1^{Y84A} and abfR1$^{Y84A\&S87A}$ mutants exhibited a motility defect that was comparable to that observed in the AbfR1 deletion mutant, at the same time, their FlaB protein levels were also found to be down-regulated. *In vitro* phosphorylation assays showed that AbfR1 was phosphorylated by kinase ArnC, and after phosphorylation, its binding affinity to target genes was notably increased.
Conclusion: These results suggested that the phosphorylation status of AbfR1 plays a vital role in mediating regulation of biofilm formation and cell motility in *S. acidocaldarius*.

TLP06

Structural and enzymatic analysis of TarM from *Staphylococcus aureus* reveals an oligomeric protein specific for the glycosylation of wall teichoic acid

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Introduction: The petidoglycan of the major human pathogen Staphylococcus aureus is functionalized with wall teichoic acids (WTA) serving as virulence factors as well as phage receptor. WTA is further functionalized by sugar moieties. We report the crystal structure of the WTA glycosyltransferase TarM which reveals a unique domain facilitating a trimeric superstructure.
Objectives: We want to understand the biochemical and structural characteristics of WTA glycosylation as well asthe resulting physiological effects in detail.
Methods: In order to study the enzyme's functional properties we solved the crystal structure of TarM and performed structure guided mutagenesis. The constructed mutants were assayed with biochemical methods. Moreover, the catalytic efficiency was determined using a quantitative phage based in-vivo assay.
Results: Our studies revealed crucial amino acids involved in the catalytic mechanism as well as trimerization allowing allow us to propose a catalytic mechanism. Furthermore we demonstrate a putative role for the trimerization domain.
Conclusion: WTA is a crucial determinant for horizontal gene transfer and virulence. The understanding of structural and biochemical properties of WTA glycosylation is crucial for an effective antimicrobial therapy.

TLP07

***Ralstonia eutropha* has three polyphosphate-bound polyphosphate kinases and four soluble polyphosphate dependent kinases**

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Introduction: Polyphosphate (PP) is a linear polymer consisting of ten to several hundreds of phosphate residues and is present in all organisms. PP has more functions than that of a phosphate storage compound [1]. In bacteria, the polymer often forms dense particles, known as volutin or PP granules. *Ralstonia eutropha* H16 is famous for its ability to accumulate large amounts of PHB. However, *R. eutropha* also forms PP granules, though they are much smaller in size than PHB granules. Polyphosphate kinases (PPKs) catalyse the formation of PP from ATP and/or GTP. Two PPKs are annotated in the *R. eutropha* genome. Both possess a PPK1 domain.
Objectives: The locations and physiological roles of PP and of PPKs should be investigated. Furthermore, proteins present on the PP granule surface should be identified.
Methods: PP granules were purified and the bound proteins were identified by proteome analysis. In a separate approach, *in silico* screening of the *R. eutropha* genome sequence for proteins with putative PP-forming activity was conducted. The subcellular localization of identified candidates was determined by fluorescence microscopy after construction of fusions with *eyfp*. Phenotype analysis of deletion mutants was performed.
Results: The *in silico* screening revealed five new putative PPKs all containing a PPK2 domain and differing from the two already annotated PPKs. Four of the eYFP-labelled PPKs were located within the cytoplasm or near one of the cell poles and three co-localised with PP granules. In agreement with this, the same three PPKs were detected by PP granule isolation and proteome analysis of the PP granule fraction. Overexpression or deletion of the three PP granule-binding PPKs resulted in changes of the size and number of the PP granules in the cell.
Conclusions: Apparently, seven PPKs are involved in phosphate homeostasis. At least three of them are responsible for PP granule formation.

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TLP08

Localization of a novel CO_2 fixation pathway in a compartmentalized Prokaryote

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Introduction: In autotrophic eukaryotes, CO_2 is fixed via the Calvin Cycle, a pathway which takes place in the stroma of the chloroplasts; autotrophic prokaryotes, however, have developed five alternative ways of CO_2 fixation which by now were all assumed to be located in the cytoplasm [1]. In the hyperthermophilic Crenarchaeum *Ignicoccus*, CO_2 fixation is proposed to proceed via the unique dicarboxylate/4-hydroxybutyrate cycle and as we could show provided a surprise in its location [2]. *Ignicoccus* cells exhibit an extraordinary ultrastructure. In addition to the cytoplasmic membrane, there is an outer membrane, which encases an intermembrane compartment (IMC). The IMC contains membrane-surrounded vesicular structures and tubes, derived from the cytoplasm. Another curiosity about *Ignicoccus* is the location of its ATP synthase. This enzyme is exclusively found in the outer membrane of *Ignicoccus* cells, thus leading to the assumption that large quantities of ATP are available in the IMC [3].
Objectives: The compartmentalized cell structure of *Ignicoccus* and the unique location of its ATP synthase in the outer membrane raise questions about the subcellular distribution of different steps of the CO_2 fixation pathway. We therefore aim to locate enzymes involved in the CO_2-fixation to track down its route and to get a deeper understanding in the physiology of these highly unusual cells.
Methods: We used different methods of (3D) electron microscopy and immunolabeling in combination with enzymatic assays.
Results: Immunolabeling studies revealed that the Acetyl-CoA synthetase, an enzyme that catalyzes the initial step of the CO_2 fixation, is located in the IMC, tightly associated with the outer membrane. Additionally, we were able to detect PEP carboxylase, malate dehydrogenase, succinate semialdehyde reductase and crotonyl-CoA hydratase/3-hydroxybutyryl-CoA dehydrogenase in the IMC of *Ignicoccus*.
Conclusion: Due to our localization experiments and to the location of the ATP synthase in the outer membrane of *Ignicoccus* we conclude that the whole CO_2 fixation takes place in the IMC of *Ignicoccus*.

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TLP09

A bacterial phospholipid *N*-methyltransferase tubulates model membranes

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Phospholipid *N*-methyltransferase enzymes (Pmts) catalyze the *S*-adenosylmethionine dependent sequential *N*-methylation of the membrane lipid phosphatidylethanolamine (PE) to produce the trimethylated PE-derivative phosphatidylcholine (PC) [1]. In bacterial membranes, PC is a rare component involved in symbiotic and pathogenic microbe-host interactions. *Agrobacterium tumefaciens*, the causative agent of crown gall disease, requires PC for tumor formation on its host plants [2, 3].
We investigated the membrane-binding mechanism of the *A. tumefaciens* PmtA enzyme. Liposome co-sedimentation assays with model membranes containing different lipid compositions demonstrated that anionic lipids enhance membrane-binding affinity of PmtA. We identified two alpha-helical regions in PmtA involved in transient membrane binding via electrostatic and hydrophobic forces. *In vitro* binding assays with liposomes varying in size suggest that PmtA is able to sense membrane curvature. Via transmission electron microscopy we found that liposomes are remodeled by PmtA into thin lipid filaments as evidenced via transmission electron microscopy analyses. This liposome-tubulation activity of PmtA is strictly dependent on the presence of the anionic lipid cardiolipin. We hypothesize that PmtA fulfills a dual function in the cell: de novo PC synthesis and membrane remodeling.

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TLP10
The Influence of Bacterial Cellular Structures on the Immobility and Position of Chemoreceptor Clusters
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Introduction: Chemoreceptor clusters are the largest native protein complexes in the bacterial cell. These clusters are positioned at the poles and laterally along the body of the cell, whereby the polar clusters are more mobile than the lateral clusters despite their larger size. The positioning and immobility of lateral clusters could arise simply from stochastic assembly of the clusters, but also be a consequence of some internal structure inside the cell.
Objectives: Here we investigated the possible involvement of several major structures - nucleoid, cell wall, or membrane domains - in immobilizing clusters at specific positions in *Bacillus subtilis* cells.
Methods: We visualized chemoreceptor clusters using a CheW-YFP fusion. To examine the effect of the nucleoid we blocked nucleoid replication and allowed the cells to grow generating a large anucleoid space inside the cell that contained chemotaxis clusters. To probe the cell wall effect we gently digested the cell wall with lysozyme as well as used antibiotics that interfere with the positioning of the cell wall machinery. To investigate the membrane effect we used membrane active organic solvents and dyes.
Results: We show that the lateral clusters remain fixed even in the absence of the nucleoid. In contrast, gentle digestion of the cell wall with lysozyme lead to a dispersion of lateral clusters. Further experiments suggested that this effect is likely to be mediated by the effects of the cell wall perturbation on the membrane organization. Addition of antibiotics that disrupt the positioning of the cell wall machinery had no effect on the clusters, whereas perturbation of the membrane fluidity disrupted the lateral clusters and reduced the number of clusters overall.
Conclusions: We conclude that the structure of the membrane is a major factor influencing lateral chemoreceptor clusters in the cell, pointing to a higher organization of the bacterial cell that is mediated by the cellular membrane.

TLP11
The Peroxisomal Import Receptor Pex5 in *Ustilago maydis*
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Peroxisomes are ubiquitous organelles that perform important metabolic reactions such as the β-oxidation pathway for degradation of fatty acids. Peroxisomal matrix proteins are translated in the cytoplasm on free ribosomes and are imported in a folded/co-factor bound state or even as oligomers. The majority of peroxisomal proteins is imported via the conserved cytosolic receptor protein Pex5 that recognizes a short C-terminal targeting signal (PTS1) with the consensus sequence S/A/C-R/K/H-L/M/I.
The plant pathogenic fungus *Ustilago maydis* encodes two Pex5 receptors (Pex5a, Pex5b), which show a high extent of sequence similarity. We could show that Pex5a is responsible for the import of proteins with unusual PTS1-motifs, e.g. ANL*, while Pex5b prefers target proteins with canonical PTS1-motifs. Thus, Pex5a and Pex5b appear to import different subsets of peroxisomal matrix proteins. This is corroborated by the fact that deletion of *pex5a* abolished growth on oleic acid but has nearly no effect on pathogenic development. In contrast, Pex5b was found to be important for growth on fatty acids, but also for filament formation and virulence. Interestingly, both Pex5 proteins are differentially expressed during the life cycle of *U. maydis*. This suggests that the peroxisomal proteome and metabolism is modulated during different stages of the life cycle.

TLP12
Dual localization of metabolic enzymes triggered by ribosomal readthrough at a short UGA stop codon context
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In eukaryotic cells single genes often encode distinct proteins that are located in different subcellular locations. This phenomenon is termed dual targeting. We could show that in the basidiomycetous fungus *Ustilago maydis* some glycolytic enzymes are dual localized in the cytoplasm and peroxisomes, e.g. the enzymes glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and phosphoglycerate kinase (PGK). For Pgk1 dual targeting is reached by ribosomal stop codon readthrough during translation creating an elongated protein. This short elongation contains a C-terminal peroxisomal targeting signal (PTS1), which causes import into the peroxisomes.
We established a test system to characterize sequences relating to their effect on stop codon readthrough. This system allows us to identify new candidate proteins for dual targeting via ribosomal readthrough. Furthermore, we could identify a short sequence that induces efficient stop codon readthrough not only in fungi but also in mammals. Genomic data analyses led to the discovery of various protein isoforms with readthrough derived PTS1 motifs. Thus, we speculate that readthrough is a widespread mechanism to create isoforms with different localization or function.

TLP13
Subcellular localization of membrane proteins in *Bacillus subtilis*
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Introduction: Construction and microscopic imaging of protein fusions to fluorescent proteins have improved our understanding of bacterial structure and the function of various biological processes. It has also led us to the realization that despite their small size and absence of compartmentalization, bacterial cells are highly organized. Many proteins show distinctive localization patterns and even dynamic, oriented shifts in localization. In many cases the localization provides important information about protein function.
Objective: To perform a systematic study of the distribution and dynamics of *Bacillus subtilis* membrane proteins.
Methods: We have undertaken a study of the localization of over 200 *B. subtilis* membrane proteins by creating C-terminal fusions of *B. subtilis* proteins with monomeric Venus-YFP and employing different microscopy techniques, e.g. single molecule tracking. Constructs were produced by Gibson assembly [1], which allows for the joining of multiple DNA fragments in a single isothermal reaction. Localization of proteins was determined by TIRF and conventional microscopy. Images were assessed for patterns of fluorescence distribution with the MicrobeTracker software [2].
Results: Proteins were grouped according to their localization pattern. Diffuse, polar, patchy and punctate fluorescent profiles could be observed. Surprisingly, almost 20% of all proteins showing a defined pattern localized to the cell poles, highlighting this subcellular localization as a preferred position for many membrane proteins. The investigation of specific localization patterns of some yet undescribed membrane proteins shed light on their possible subcellular functions, particularly for proteins that are associated with cell poles and/or septa. To gain insight into the dynamic structure of the *B. subtilis* membrane, single molecule tracking was performed and protein diffusion rates were determined for an initial set of proteins. This analysis enabled us to group proteins into different mobility classes and will aid into identifying rules for their temporal localization.
Conclusion: This study is a valuable resource for studying the distribution and dynamics of membrane proteins in *B. subtilis*.

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TLP14
Properties of Small acid-soluble spore proteins of *Clostridium acetobutylicum*
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To escape unfavorable environmental conditions like starvation or stress, *Clostridium acetobutylicum* is capable to initiate the sporulation to release resistant endospores to survive. The small acid-soluble spore proteins (SASPs) play an important role for the resistance and outgrowth of endospores due to their non-specific binding to the spore nucleoid. In the spore core, the DNA is completely saturated by SASPs, leading to its protection against wet and dry heat, desiccation, UV or toxic chemicals [1].
In the genome of *C. acetobutylicum* six SASP candidates SspA, SspB, SspC, SspD, SspH and Ssp-Tlp have been identified. Here we present insights into their regulation and disclose the DNA-Binding capabilities of the respective proteins, which were overexpressed and purified in

Escherichia coli. Using 5' RACE, a sporulation-specific promotor could be identified for every SASP and rho-independent terminator structures could be detected. The expression pattern of the SASPs, investigated by RT (Reverse Transcription)-PCR analysis was restricted to sporulation. For the purified proteins SspA, SspB, SspC and SspD with highest identities to the SASPs of *Bacillus subtilis,* a strong DNA protection could be observed. Only weak DNA binding was restricted to SspH and for Ssp-Tlp no DNA-binding could be detected *in vitro*. Both proteins revealed moderate to weak identities to the highly conserved SASPs. Immunoelectron Microscopy of *C. acetobutylicum* endospores using an Anti-SASP specific antibody confirmed the localization of the SASPs in the core of endospores in close vicinity to the spore nucleoid.

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TLP15

ATPase-independent type-III protein secretion in *Salmonella enterica*

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Question: Type-III protein secretion (T3S) is utilized by bacteria to secrete building blocks of the bacterial flagellum. T3S is also used by gram-negative pathogens to secrete virulence effectors from the cytoplasm into host cells, and the structural subunits that make up the injectisome complex. The flagellar T3S apparatus utilizes both the energy of the proton motive force and ATP hydrolysis to energize substrate unfolding and translocation. The contribution of the T3S-associated ATPase has been unclear.
Methods: We performed a genetic screen and characterized suppressor mutations in a strain deleted for the flagellar T3S-associated ATPase FliHIJ.
Results: We report formation of functional, full-length flagella in the absence of FliHIJ type-III ATPase activity by mutations that increased the proton motive force and flagellar substrate levels. We additionally show that increased proton motive force bypassed the requirement of the *Salmonella* pathogenicity island 1 (Spi1) virulence-associated type-III ATPase for secretion.
Conclusions: Our data support a role for T3S ATPases in enhancing secretion efficiency under limited secretion substrate concentrations and reveal the dispensability of ATPase activity in the type-III protein export process. This finding has important implications for the evolution of the bacterial flagellum and type-III secretion systems, suggesting that a proto ATPase was added to a primordial proton-powered type-III export system with the evolutionary benefit of facilitating the export process.

TLP16

Characterization of the *Ustilago maydis* pathogenicity factor Scp2

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The fungal pathogen *U. maydis* relies on the secretion of effector proteins to establish and maintain a biotrophic interaction with its host plant *Zea mays*. In recent years it has been discovered that proteins lacking a classical signal peptide can also be targeted to the extracellular space by mechanisms that do not depend on the endoplasmic reticulum and the Golgi complex [1]. We want to identify such so called unconventionally secreted proteins in *U. maydis* and investigate their potential function as pathogenicity factors. Our approach is based on affinity purification of tagged candidate proteins, previously detected in the apoplastic fluid (APF) of infected maize leaves [2]. One of the identified candidate proteins is Scp2. Scp2 harbors a peroxisomal targeting signal and shares 36 % amino acid identity with the human sterol carrier protein 2 (SCP2). The *scp2* gene is up-regulated during plant colonization and deletion of *scp2* results in a virulence defect that appears to result from a reduced efficiency of plant penetration. This penetration defect cannot be attributed to a defect in peroxisomal β-oxidation. Based on our finding that peroxisomal targeting of Scp2 is crucial for its virulence-related function we speculate that Scp2 could either function exclusively in peroxisomes or exhibit a dual function in peroxisomes and in the extracellular space.

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TLP17

Intracellular and potential extracellular roles of the *Ustilago maydis* Acyl-CoA-binding protein Acb1

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Conventionally secreted fungal 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 *U. maydis* is the UmAcb1 protein, previously detected in the apoplastic fluid of infected maize. UmAcb1 is an ortholog to the *Dictyostelium discoideum* acyl-CoA-binding protein AcbA, which is unconventionally secreted as full-length protein and extracellularly processed into a small peptide (SDF-2) that triggers terminal spore differentiation upon interaction with a membrane receptor [1].
In our work we attempt to prove the secretion of *U. maydis* Acb1 and its processing into an SDF-2 like peptide, as well as demonstrate a function for the peptide. We could show that *U. maydis* hyphal culture supernatants, as well as apoplastic fluid extracted from *U. maydis* infected plants, trigger spore formation in a *D. discoideum* based bioassay indicating the secretion of an SDF-2-like peptide by *U. maydis*. Deletion of the *acb1* gene in *U. maydis* attenuated growth and led to a mating defect. Both phenotypes could be rescued by suppressor mutations, presumably bypassing the intracellular function. We are now exploiting these suppressors to analyze the putative extracellular function of UmAcb1.
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TLP18

Excretion of cytoplasmic proteins in staphylococci

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Many microorganisms and also eukaryotic cells excrete typical cytoplasmic proteins. As none of the classical secretion systems appears to be involved, this type of secretion has been referred to as "non-classical protein secretion". So far it is neither known by which mechanism nor why cytoplasmic proteins are excreted. Our research is concentrated on why, how, where and when staphylococci excrete cytoplasmic proteins.
In *S. aureus,* approximately 30% of the encoded proteome (totally ± 2,600 proteins) are secreted and among these proteins there is a proportion of typical cytoplasmic proteins, which lack a signal peptide. Our preliminary results have shown that in Staphylococcus aureus more than 20 typical cytoplasmic proteins were excreted. The excretion of cytoplasmic proteins already starts in the early exponential phase and appears to be more pronounced in the clinical isolates than in the non-pathogenic staphylococcal species. We raised antibodies against 4 typical cytoplasmic proteins, to be able to follow the excretion of cytoplasmic proteins in western-blot analyses. In addition we could show that there is no correlation between the quantity of cytoplasmic proteins in the cytoplasm and their release to the extracellular environment. We assume that there exists a selection procedure in the excretion of cytoplasmic proteins. We want to address the question why microorganisms excrete cytoplasmic proteins, which is a loss of resources and energy. Is the excretion of such proteins an accident or is it intentional?

TLP19

Novel insights into the regulation of flagellar type 3 secretion

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Introduction: Most bacteria move by rotating a helical organelle, the flagellum (1). The architecture of the flagellum can be divided into the membrane-embedded basal body and the exterior hook and filament structures. Assembly of the flagellar filament relies on the flagellar type III secretion system (fT3SS) that translocates flagellar building blocks from the cytoplasm to the extracellular assembly point.

Objectives: Besides a cytoplasmic ATPase complex, the fT3SS consists of six integral proteins FliOPQR, FlhA and FlhB. FlhA is the largest fT3SS transmembrane protein and contains a cytosolic domain of 42 kDa (FlhA-C) that serves as a docking platform for the coordinated secretion of late flagellar building blocks (2). However, the way FlhA-C interacts with its clients and enables coordinated export is only poorly understood. We therefore aimed at an in-depth molecular characterization of how FlhA-C recognizes its clients and coordinates fT3S.
Methods: X-ray crystallography, small-angle X-ray scattering (SAXS), hydrogen-deuterium-exchange mass-spectrometry (HDX), biochemical assays, *in vivo* assays
Results: We biochemically reconstituted $FlhA_C$ in complex with its clients for in-depth biochemical and structural characterization. These complexes were analysed by SAXS, HDX and X-ray crystallography. Our analysis reveals similarities but also differences in the molecular mechanisms that allow FlhA to handle its different clients during fT3S.
Conclusion: Our analysis sets the stage for an in-depth molecular understanding of the recognition of clients by the fT3SS.
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LP20
Characterization of the parvulin-type PPIase PsrA2 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*. 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, recruitment of 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 since, many bacterial PPIases have been described in the context of virulence[1]. *C. difficile* has two putative parvulin type PPIases (CD630_15570 and CD630_35000) with predicted extracellular location. Of these, CD630_35000 has the highest sequence homology to the virulence associated PrsA2 of *Listeria monocytogenes*, and was chosen for detailed analysis.
Materials and Methods: CD630_35000 (CdPrsA2) was cloned into the expression vector pSSBM106. Five 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 they were correctly folded as assessed by CD-spectroscopy. 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 a more detailed evaluation of the influence of CdPrsA2 on virulence.

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TLP21
Structure and dynamic 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 A (LipA) revealed that this enzyme requires a cytoplasmic membrane bound steric chaperon called lipase-specific foldase (Lif) to obtains 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 that foldase binds with high affinity (K_d = 5 nM) 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 aim to study the dynamics of these processes *in vitro* by combining biochemical, single molecule fluorescence and computational methods. Six Lif variants, each containing two Cys residues, were constructed and purified. Side chains of Cys residues were labeled with thiol-reactive acceptor and donor fluorescence dyes. This set of variants allows monitoring of relevant movements in the foldase. The analysis of acceptor-donor labeled Lif variants showed that they can promote lipase folding to the level of wild-type Lif. The distances between several Lif domains measured by fluorescence resonance energy transfer (FRET) indicated similar dynamics of foldase in LipA-bound and also LipA-unbound state which supports the hypothesis that the foldase exists in a binding-competent state even in the absence of the lipase. Additionally, we have observed movements in the foldase which indicate the relevance of the C-terminal foldase domain for the release of the lipase. Presently, we further analyse by molecular dynamic simulations the proposed model of lipase activation.

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TLP22
Proteome profiling of *Burkholderia pseudomallei* quorum sensing mutants

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Introduction: *Burkholderia pseudomallei*, the causative agent of melioidosis, is a gram-negative soil bacterium in tropical and subtropical areas. *B. pseudomallei* employs several 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 three orphan *luxR* homologues [3]. The *luxI* homologues encode AHL synthases, which produce specific AHLs binding to the respective transcriptional regulator and thus regulating expression of specific genes involved in virulence like biofilm formation or swarming motility [4].
Objectives: The aim of this study was to dissect the influence of the different QS systems on gene expression by identifying proteins differentially expressed in the wild-type and respective QS mutant strains.
Methods: AHL synthase-deficient mutants were constructed and proteins extracted from different cellular locations of WT and mutant strains. It were subjected to different gel-free and label-free quantitative proteomics approaches. More specifically, the cytosolic proteins were quantified by a DIA approach IMS^E in combination with Hi3 and the extracellular proteins were analyzed by a quantitative GeLC-MS/MS approach. Furthermore, the AHL spectrum, synthesized by the different QS systems was determined by analytical thin layer chromatography employing different AHL-sensor strains.

Results: As expected, *B. pseudomallei* mutants in different synthase-coding genes lost their capability to produce the entire AHL-spectrum of the WT strains. Moreover, the mutation of AHL synthase genes led to differential expression of a significant number of cytosolic as well as extracellular proteins.
Conclusion: QS in *B. pseudomallei* has a strong impact on protein expression by affecting the expression rates of various AHL-dependent genes, some of which also involved in pathogenicity.

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TLP23

The architecture of halobacterial gas vesicles

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Halobacterium salinarum is an archaeal halophilic extremophile that requires very high salt concentrations to survive. Halobacteria produce gas vesicles, air-filled protein pontoons, to provide buoyancy and enable vertical migration to reach favorable growth conditions for photosynthesis. Gas vesicles are predominantly formed by an 8 kDa structural protein, GvpA, which arranges into a tube of 100 - 200 nm in diameter and up to 1.5 µm in length with characteristic conical caps. A minor structural gas vesicle protein, GvpC, is thought to attach to the outside of the rib structures[1] to provide structural stability against pressure variations[2].
Vesicle formation is not well understood. Building blocks of GvpA appear to form regular arrays further arranged into helical ribs with a characteristic pitch of of 4.6 nm. Currently, no crystal structure exists of GvpA, but computational models strongly suggest the protein pairs into stable dimers with hydrophilic α-helices facing outward and β-strands facing inwards to create a hydrophobic surface that excludes diffusion of any polar solvents[3,4,5].
In order to examine the structural details of GvpA, we utilized a 300 keV Titan Krios transmission electron microscope equipped with a post-column energy filter and a sensitive direct electron detector to conduct cryo-electron tomography. Imaging conditions were further improved by a newly developed phase plate that enabled us to work at near ideal focus conditions with significantly increased contrast[6]. We investigated gas vesicles possessing a point mutation resulting in particularly thin tubes[4] that are advantageous for optimizing the resolution of 3D reconstructions.
Through subtomogram averaging we were able to obtain a 3D structure of GvpA from the near-cylindrical parts of gas vesicles and subsequently analyzed the conical tips and structural transition areas.

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TLP24

Three-dimensional arrangements of ribosomes inside fast growing *E. coli* cells

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Introduction:
Translating bacterial ribosomes can form structurally ordered polysomes. The three-dimensional (3D) organization of such polysomes has been described in solution using cryoelectron tomography (CET) and template matching [1]. Ribosomal pairs in a characteristic arrangement has been described in starved *E. coli* cells [2]. However, little is known about how polysomes are organized in their native cellular space when translating cytosolic or membrane proteins during the exponential phase of growth.
Objectives: We propose to examine the 3D arrangement of membrane associated ribosomes *in situ*, for an optimal preservation of polysomes architecture.
Methods or Materials & Methods: To enrich the population of ribosomes specially associated to the inner membrane, we expressed a transmembrane protein fused to a SecM-stalling sequence in slow- and fast-growing *E. coli* cells. The intact cells were rapidly frozen. These vitrified fast-growing cells (>800 nm in diameter) were physically sectioned using focused ion beam (FIB) technology to reduce the sample thickness (<400 nm) [3]. We applied dual-axis tilt CET to the FIB-milled samples. In order to localize 70S ribosomes inside tomograms we used a 3-D pattern recognition algorithm.
Results: Advanced 3D imaging techniques of FIB-milled fast growing *E. coli* cells allowed a novel view of cellular ribosomes. The analysis of cytosolic ribosomes reveals a fraction of particles in similar 3D organization to the previously described for dense polysomes *in vitro* [1]. While *in vitro* studies allowed the visualization of translocating ribosomes bound to isolated membrane vesicles, those vesicles do not resemble the natural curvature of the inner membrane. Here, the *in situ* visualization of translocating ribosome was feasible in slow- and fast-growing cells.
Conclusion: Polysomal organizations, previously described only *in vitro*, are shown here to occur also *in situ*. The described approaches are also adequate to reveal structures of macromolecular complexes inside cells with unprecedented resolutions.

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TLP25

Shuffling genes around in hot environments – structure, function and regulation of the DNA transporter in *Thermus thermophilus*

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Introduction: The DNA translocator of the thermophilic bacterium *Thermus thermophilus* is a leading model system of DNA transporter in thermophilic bacteria. DNA is transported through the outer membrane and the periplasmic space by a unique secretin (PilQ) complex. ComEA transfers the incoming DNA into a DNase resistant state prior to transport through an inner membrane channel comprising of ComEC [1].
Objective: Structure and function of ComEA, ComEC and PilQ and transcriptional regulation of the DNA transporter.
Methods: Adhesion, twitching motility, transformation and piliation analyses unraveled structure/function relationships. Single particle analyses deciphered the structure of PilQ complexes. Electrophoretic mobility shift assays, western blot analyses and qRT-PCR elucidated DNA binding, subcellular localization and regulation of competence proteins.
Results: Recently, we solved the structure of the secretin complex consisting of a "con" and "cup" structure and five staggered rings with a large central channel. Here we report on the identification of ring building motifs and their role in piliation and natural transformation. Type IV pili and DNA translocator genes were found to undergo analogous transcriptional regulation by environmental stress factors [2]. Unexpectedly, ComEC modulates transcriptional regulation of both systems. The membrane anchored ComEA protein was found to bind dsDNA.
Conclusions: The ring structures of the secretin complex play discrete roles in piliation and natural transformation. Transcriptional regulation of both systems in response to environmental signals is mediated by the polytopic inner membrane channel ComEC.

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TAP01

Study of OmpW protein from *Caulobacter crescentus* in *Escherichia Coli*

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Introduction: *C. crescentus*, widely known for its applications as a model organism for studying cell cycle and asymmetric cell division[1], is a Gram-negative organism, mainly found in dilute organic environments such as fresh water and soil.
Background: The genome of *C. crescentus* has been completely sequenced[2], but interestingly, no general diffusion porins of the OmpF/C type or substrate specific porins are coded in the genome[4]. Instead, genes coding for more than 60 TonB dependant outer membrane receptors have been identified[3].

Methods: Against the common belief that no general purpose or substrate specific porin is present in the cell wall of *C. crescentus*, here we report the presence of high channel forming activity in the cell wall extracts of *C. crescentus* in lipid bilayer experiments. The ion permeable channel had a low single channel conductance of about 125pS in 1M KCl. The protein responsible for the channel formation had a molecular mass of 20KDa. Partial sequencing of the protein showed, that it belongs to the well-known OmpW family of outer membrane proteins of Gram-negative bacteria.
Results: Currently, we expressed the gene responsible for the channel protein in *E. coli* with a histidine-tag attached to the N-terminus of the protein. But, unfortunately the protein did not show clear channel forming activity. To obtain good amounts of the cleaved protein was always problematic. So, the protein was expressed in inclusion bodies without any tag and was successfully refolded. Currently, the lipid bilayer experiments are under way. In order to better understand the ion passage and the larger diameter of the channel as compared to OmpW of *E. coli* and OprG of *Pseudomonas aeruginosa*, point mutations will be performed in the gene coding for OmpW of *C. crescentus* (K160W and K161Y).

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TAP02
Role of the Na^+-translocating NADH – quinone oxidoreductase for growth of *Vibrio cholerae*
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Introduction: The Na^+ -translocating NADH:ubiquinone oxidoreductase (Na^+ -NQR) is a membrane bound NADH dehydrogenase found in many pathogens including *Vibrio cholerae*. It is the main entry site for electrons into respiration and couples the free energy of the electron transport to electrogenic pumping of sodium ions across the membrane. This results in the build-up of a sodium motive force required, for example, to drive the flagellar motor [1].
Objectives and methods: To study the role of the Na^+-NQR, a *V. cholerae* deletion strain devoid of the Na^+-NQR was examined with regard to growth in LB with different pH values and varied added NaCl concentrations. In addition, a double mutant lacking the Na^+-NQR and the antiporter NhaA was investigated. Besides, we describe a new method to monitor the level of NQR expression during growth by spectroscopy of cell extracts based on fluorescence of covalently bound FMN of subunits NqrB and NqrC and by *in-gel* fluorescence.
Results: *V. cholerae* lacking the Na^+-NQR did not grow under hypoosmotic conditions at pH 8.2 but resumed growth with the *nqr* genes in trans. When growing at a pH range of 7.7-8.2 with 150 mM NaCl added, the *V. cholerae* mutant lacking both the Na^+-NQR and the Na^+/H^+ antiporter- NhaA showed a strong growth defect when compared to the reference strain or the *nqr* deletion strain. Fluorescence spectroscopic analyzes showed that there was no significant change in the fluorescence intensities in crude extracts obtained from samples during early and late exponential growth of *V. cholerae. In-gel* fluorescence confirmed this result.
Conclusion: The findings suggest that at alkaline pH and limiting Na^+ concentrations, the Na^+-NQR is crucial for the generation of $\Delta\Psi$ (outside positive) to drive the import of H^+ by electrogenic Na^+/H^+ antiporters. *V. cholerae* maintains a constant level of NQR expression during growth with few fluctuations. In alkaline habitats with 150 mM NaCl added, *V. cholerae* depends on Na^+/H^+ antiporters (NhaA, NhaB and NhaD), to keep internal [Na^+] low. The three Na^+/H^+ antiporters encoded by *V. cholerae* transport sodium ions from the cytoplasm to the periplasm in exchange for protons, helping this pathogen to survive in high salinity habitats [2].

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TAP03
Function of GSPII domains and Walker A and Walker B motifs of the AAA -ATPase PilF in natural transformation and pilus biogenesis of *T. thermophilus*
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Introduction: *Thermus thermophilus* exhibits the highest natural transformation frequency known to date and has become a model system to study natural competence in thermophiles. The DNA translocator of *T. termophilus* has a tripartite structure consisting of at least 16 proteins and is functionally linked to type IV pilus biogenesis (1). Translocation of DNA into the periplasm is mediated by a unique secretin complex but also implies pilin-like proteins and the DNA binding protein ComEA. PilF, a unique zinc binding AAA-ATPase, is suggested to power the DNA translocator and type IV pilus assembly (2,3). PilF has an unusual structure with three N-terminal general secretory pathway domains (GSPII), Walker A and Walker B motifs and a tetracysteine motif.
Objective: Functional and structural dissection of the GSPII domains and the Walker A and B motifs of PilF.
Methods: Site directed mutagenesis and generation of GSPII deletions of *pilF* was followed by mutant studies and functional and structural analyses of PilF.
Results: The GSPII domains are essential for generation of intact PilF complexes, natural transformation and type 4 pilus functions such as twitching motility and adhesion. Interestingly, deletion of a single GSPII domain (3rd domain) leads to hypertransformation but reduces piliation. The Walker A and Walker B motif, responsible for ATP binding and hydrolysis, respectively, are essential for DNA uptake and pilus formation. Interestingly, mutation of the Walker A motif leads to decreased heat stability of the PilF complex.
Conclusions: The three GSPII domains are essential for proper assembly and/or folding of the hexameric PilF complex. ATP binding and hydrolysis is required for PilF functionality in DNA translocation and type 4 pilus biogenesis.

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TAP04
Characterization of the nucleoside peptide permease NppABCD from *Pseudomonas aeruginosa* PA14
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Analysis of the genome sequence of *Pseudomonas aeruginosa* PA14 revealed the presence of an operon encoding an ABC-type transporter (NppABCD) showing homology to the Yej transporter of *Escherichia coli*. The Yej transporter is involved in the uptake of peptide-nucleotide antibiotic microcin C, a translation inhibitor that targets the enzyme aspartyl-tRNA synthetase. Furthermore, it was recently shown that a homologous transporter from *P. aeruginosa* PA01 is required for uptake of pacidamycin, an uridyl peptide antibiotic.
We used several approaches to determine the substrate specificity of the NppACDF transporter of *P. aeruginosa* PA14. Growth assays in defined minimal medium containing peptides of various length and amino acid composition as sole nitrogen source, as well as Biolog phenotype microarrays, showed that the NppABCD transporter is not required for the uptake of di-, tri-, and oligopeptides. However, a *nppBCD*-deficient mutant was more resistant to pacidamycin and blasticidin S, a peptidyl nucleoside antibiotic. Furthermore, heterologous expression of the *nppABCD* operon in a *yej*-deficient mutant of *E. coli* resulted in increased susceptibility to albomycin, a naturally occurring sideromycin. Sideromycins are antibiotics covalently linked to iron-chelating siderophores. The antibiotic part of albomycin is a peptidyl nucleoside consisting of a highly modified 4′-thiofuranosyl cytosine moiety.
Our results suggest that the NppABCD transporter of *P. aeruginosa* PA14 is involved in uptake of peptidyl-pyrimidine nucleoside antibiotics.

TAP05
Assembly and Composition of a Type IV Secretion System in *Bacillus subtilis*
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The process of DNA transfer through a direct cell-to-cell contact is known as conjugation and represents the major reason for the dissemination of antibiotic resistance genes among bacteria. The translocation of DNA and/or protein substrates across membranes takes place through macromolecular structures known as type IV secretion systems (T4SSs). Although earlier studies have elucidated the structure and mechanism of T4S in Gram-negative bacteria such as *Escherichia coli* and *Agrobacterium tumefaciens*, less is known about T4SSs of Gram-positive bacteria.
Here, we study the composition and assembly of a T4SS in the Gram-positive bacterium *B. subtilis* by performing a cytological protein-protein interaction screen and a subcellular localization screen with almost all of the proteins encoded by the large conjugation operon of plasmid pLS20. Our protein-protein interaction data reveal that the T4SS mediating the transfer of pLS20 is not a minimized system, because it is composed of several more subunits in addition to the well-conserved VirB-proteins and the coupling protein VirD4. The analysis elucidated many known interactions from Gram-negative T4SSs, but also indicated differences, which will be discussed. In accordance with the previously reported pattern of the conserved Vir-proteins, this previously uncharacterized set of proteins displays a growth phase dependent localization pattern analogously to the transfer kinetics of pLS20. Furthermore, we found that several subunits of the T4SS localized independently of other plasmid encoded factors and that most proteins localized predominantly along the lateral membrane, while VirB4 almost exclusively localized at polar sites of the cell.
The subcellular localization data suggest that the T4SS of pLS20 uses an endogenous molecular scaffold for its spatial assembly and that individual proteins might first subassemble at the lateral membrane before they assemble to fully functional translocation channels at the cell pole. Furthermore, our protein-protein interaction screen allowed depicting a preliminary model for the DNA transfer mediated by the T4SS of pLS20.

TAP06
TatA self-interactions and the importance of the translocon formation for TatA stability
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The Tat pathway translocates folded proteins with specific N-terminal signal peptides across cytoplasmic bacterial membranes. While TatC - in concert with TatB - recognizes the pathway-specific motif in the signal peptide and thus serves as translocon receptor, the TatA component plays a key role in the membrane passage step, in which the globular domain is most likely channeled through TatA assemblies while the signal peptide remains bound to TatBC. Here we describe the effects of inactivating point mutations in TatC on the abundance of all Tat components at wild type expression level. The TatA abundance was strongly affected, which is most likely due to compromised interactions of TatA with TatBC. We also monitor at wild type levels the formation of disulfides in TatA assemblies with engineered cysteine residues that indicate close proximity of the respective positions. Our data suggest that the N-terminal trans-membrane domains of TatA are continuously in close proximity, which agrees with the idea the TatA forms oligomeric complexes by interactions of the trans-membrane-regions. Other positions showed much less interactions, but these interactions may relate to the active translocation process. Together, our data help to further understand the TatA interactions and possible conformational transitions during Tat-dependent translocation.

TAP07
KtrB – a cation channel with low selectivity
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The KtrB subunit of the K^+-translocating system KtrAB from *Vibrio alginolyticus* belongs to the superfamily of K^+ transporters (SKT), which most likely evolved from 2-TM K^+ channels by gene duplication and gene fusion. While the KtrAB complex likely functions as secondary active symporter, its subunit KtrB alone is suggested to have channel-like activities. In this study we focused on the functional characterization of isolated, liposome-reconstituted KtrB. To this aim, we performed electrophysiology measurements based on solid-supported membranes showing that KtrB in fact acts as a channel with low potassium over sodium selectivity.

TAP08
The Role of the T6SS in *Salmonella* Virulence
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Introduction: Secretion is crucial for bacteria to interact with their environment. Especially gram (-) pathogenic bacteria employ secretion to establish an infection. One of these secretion systems ist the recently discovered Type VI Secretion System (T6SS). The T6SS is a important virulence factor, as it translocates effector molecules into eukaryotic cells (e.g. macrophages) (1). However T6SS is also employed by bacteria in competition by delivering bacteriolytic effectors into prey cells (2).
The pathogenic gram (-) *Salmonella enterica* harbors one T6SS. *Salmonella* causes self limiting gastroenteritis (*S.* Typhimurium) but also the life threatening typhoid fever (*S.* Typhi). After ingestion, *Salmonella* reaches the small intestine where the bacteria have to cope with the host microbiota. Following traversal of the epithelium, the cells are taken up by phagocytic cells, where they replicate and disseminate tot he deeper organs like liver and spleen (3).
The T6SS was shown to be involved in *Salmonella* virulence (4). However the regulation, expression and virulence mechanisms are poorly studied.
Objectives: The aim is to elucidate the *in vivo* function of the *Salmonella* T6SS: Regulation and expression as well as its role during infection of macrophages.
Methods: Expression studies: Transcriptomics, qRT PCR, microscopy, *in vivo* imaging. Regulation: Random mutagenesis, gene bank. *In vivo* function: Mice experiments (survival, organ burden) and cell culture (macrophage survival)
Results: Microscopy studies showed, that the T6SS is expressed at later stages during macrophage infection. Deletion of T6SS components and the null mutant resulted in a significant decrease in macrophage survival but showed no phenotype in the used mouse model.
Conclusion: Expression of the T6SS and the phenotype of deletion mutants in macrophage infection underline the importance of the T6SS in virulence. Further analysis and the identification of regulators will lead to a deeper understanding of this fascinating system.

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TAP09
Exploring the mechanism of K(+) translocation by the KtrAB system
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In nature, many intermediates between species exist. At the molecular level, the KtrAB K+ uptake system may represent an intermediate between a channel and a transporter. While the K+-translocating subunit KtrB likely harbors channel-like activity, the complex as a whole may act as a secondary active transporter. The A subunit confers velocity, Na(+) dependency and ion selectivity to the complex. It is possible that, in the course of evolution, a former channel was converted into a transporter by the addition of a non-covalently linked regulatory subunit. Towards a deeper understanding of KtrAB's function, we are performing ITC and EPR measurements, both with the B subunit alone and the KtrAB complex as a whole. Furthermore, we have elucidated the stoichiometry of the KtrAB complex using LILBID-MS. My poster summarizes our current view of the kinetics of the KtrAB system and proposes a model of its function.

Personalia aus der Mikrobiologie 2014

Habilitationen

Susanne Gebhard habilitierte sich am 15. Januar 2014 an der Ludwig-Maximilians-Universität München (Mechanismen der Stressresistenz und deren Regulation in Gram-positiven Bakterien).

Bruno Bühler habilitierte sich am 29. Januar 2014 an der TU Dortmund (Integrated Systems Biotechnology for Efficient Biocatalytic Oxyfunctionalization).

Markus Nett habilitierte sich am 10. Februar 2014 an der Universität Jena (Chemische und biosynthetische Diversität von Naturstoffen aus prädatorischen sowie Pflanzen-assoziierten Mikroorganismen).

Christine Moissl-Eichinger habilitierte sich am 14. Mai 2014 an der Universität Regensburg (Novel insights into the microbiomes of clean rooms, human skin and subsurface biofilms: Archaea everywhere!).

Christiane Baschien habilitierte sich am 17. September 2014 an der Technischen Universität Berlin (Mycodiversität in Fließgewässern).

Tanja Schneider habilitierte sich am 20. Oktober 2014 an der Universität Bonn (Antibiotika – gestern, heute ... und morgen?).

David Schleheck habilitierte sich am 12. November 2014 an der Universität Konstanz (On the Biofilm Life-Cycle and Catabolic Pathways of Bacteria).

Martin Könneke habilitierte sich am 17. Dezember 2014 an der Universität Oldenburg (The discovery of ammonia-oxidizing archaea. (Cultivation and characterization of a novel, globally abundant group of chemolithotrophic archaea).

Prof. Hans-Peter Klenk vom Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH in Braunschweig wurde mit dem Bergey Award 2014 geehrt. Den mit 2.000 US-Dollar dotierten Preis erhielt er in Anerkennung seiner herausragenden Verdienste um die Bakterientaxonomie. Dazu zählen seine Beiträge zur „Sequencing-Orphan-Species Initiative“ (SOS) und zur „Genomischen Enzyklopädie der Bakterien und Archaea“ (GEBA). Die SOS-Initiative erstellte einen „Stammbaum“ aus 16SrRNA-Sequenzen aller bekannten Bakterien und Archaeen. „Vollständige Genomdaten sind noch aussagekräftiger für die Klassifikation von Mikroorganismen als die bisher praktizierte evolutionäre Einordnung über Markergene“, schildert Klenk die weit darüber hinaus gehenden Arbeiten der GEBA. Klenks Team analysiert Gesamtgenom-Sequenzen, um Rückschlüsse auf die Verwandtschaftsverhältnisse und die Evolution der Bakterien mit bislang undenkbarer Präzision zu ziehen. „Dies wird uns erlauben, neue Bakterien, zum Beispiel Krankheitserreger schneller und zuverlässiger zu identifizieren“, so Klenk.

Den Bergey Award verleiht eine Stiftung in Erinnerung an den amerikanischen Arzt und Bakteriologen David Hendricks Bergey (1860–1937), der viele Bakterien identifizierte und das berühmte „Bergey's Manual of Determinative Bacteriology“ verfasste.

(stö)

Ruf angenommen

Richard Splivallo von der Universität Göttingen übernahm am 1. Januar 2014 die Juniorprofessur für Biochemie und Biotechnologie der Pilze an der Universität Frankfurt am Main im Rahmen des LOEWE-Schwerpunkts „Integrative Pilzforschung“.

Alga Zuccaro vom Max-Planck-Institut für terrestrische Mikrobiologie in Marburg übernahm am 1. Februar 2014 die W2-CEPLAS-Professur „Ökologische Genetik der Mikroben“ am Institut für Genetik der Universität Köln.

Martina Schrallhammer von der Technischen Universität Dresden übernahm am 1. März 2014 die Juniorprofessur für den Lehrstuhl Mikrobiologie an der Universität Freiburg.

Dirk Schüler von der Ludwig-Maximilians-Universität München übernahm am 1. April 2014 die W3-Professur für den Lehrstuhl Mikrobiologie an der Universität Bayreuth.

Gunther Döhlemann vom Max-Planck-Institut für terrestrische Mikrobiologie in Marburg übernahm am 15. Mai 2014 die W3-CEPLAS-Professur „terrestrische Mikrobiologie“ am Institut für Genetik, Universität Köln.

Ulrich Nübel vom Robert Koch-Institut übernahm am 1. Juni 2014 die neu eingerichtete DZIF-Professur Mikrobielle Genomforschung am Leibniz-Institut DSMZ.

Julia Bandow von der Universität Bochum übernahm am 12. Juni 2014 die W2-Professur für Angewandte Mikrobiologie an der Universität Bochum.

Charles Franz vom Max-Rubner-Institut Karlsruhe übernahm am 1. Juli 2014 die Institutsleitung am Max-Rubner-Institut in Kiel.

Knut Drescher von der Princeton University begann am 1. Juli 2014 die W2-Max-Planck-Forschungsgruppe „Bacterial Biofilms“ am Max-Planck-Institut für terrestrische Mikrobiologie in Marburg.

Eva Holtgrewe Stukenbrock vom Max-Planck-Institut für terrestrische Mikrobiologie in Marburg übernahm am 1. August 2014 die W3-Professur „Environmental Genomics“ an der Universität Kiel sowie die Leitung einer Max-Planck-Forschungsgruppe am Max-Planck-Institut für Evolutionsbiologie in Plön.

Ilse Jacobsen vom Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie – Hans-Knöll-Institut – übernahm am 20. August 2014 die W3-Professur für Mikrobielle Immunologie an der Universität Jena.

Nicole Frankenberg-Dinkel von der Universität Bochum übernahm am 1. September 2014 die W3-Professur für den Lehrstuhl Mikrobiologie an der TU Kaiserslautern.

Sonja-Verena Albers von der Universität Marburg übernahm am 1. September 2014 die W3-Professur für den Lehrstuhl Mikrobiologie an der Universität Freiburg.

Christine Moissl-Eichinger von der Universität Regensburg übernahm am 1. September 2014 die Professur an der Medizinischen Universität Graz, Österreich.

Nadine Ziemert von der Scripps Institution of Oceanography, UC San Diego in La Jolla/California übernahm am 22. September 2014 die W2-Professur für Applied Natural Products Genom Mining an der Universität Tübingen.

Rainer Meckenstock vom Helmholtz-Zentrum München übernahm am 1. Oktober 2014 die W3-Professur für den Lehrstuhl Aquatische Mikrobiologie an der Universität Duisburg-Essen.

Anett Schallmey von der Universität Amsterdam übernahm am 1. Oktober 2014 die W2-Professur für Biochemie an der Technischen Universität Braunschweig.

Gunhild Monika Layer von der Technischen Universität Braunschweig übernahm am 1. Oktober 2014 die W2-Professur für Stoffwechselbiochemie und Enzymologie an der Universität Leipzig.

Susanne Gebhard von der Ludwig-Maximilians-Universität München übernahm am 1. Oktober 2014 die Lecturer-Stelle am Department of Biology & Biochemistry an der University of Bath, UK.

Mathias Hornef von der Medizinischen Hochschule Hannover, Institut für Medizinische Mikrobiologie und Krankenhaushygiene, übernahm am 1. Oktober 2014 die W3-Professur für den Lehrstuhl Medizinische Mikrobiologie an der Uniklinik RWTH Aachen.

Matthias Brock vom Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie – Hans-Knöll-Institut – nahm am 15. Oktober 2014 den Ruf an die University of Nottingham, UK an.

Hans-Peter Klenk von der Technischen Universität Braunschweig übernahm am 20. Oktober 2014 die Institutsleitung der School of Biology an der Newcastle University, UK.

Susanne Liebner vom Deutschen GeoForschungsZentrum GFZ in Potsdam übernahm am 28. Oktober 2014 die Juniorprofessur für Molekulare Umweltmikrobiologie an der Universität Potsdam als gemeinsame Berufung mit dem GFZ.

Tobias J. Erb von der ETH Zürich übernahm am 1. November 2014 die Leitung einer unabhängigen Max-Planck-Forschergruppe mit dem Thema „Biochemistry and Synthetic Biology of Microbial Metabolism" am Max-Planck-Institut für terrestrische Mikrobiologie, Marburg.

Andreas Schmid von der TU Dortmund übernahm am 1. November 2014 die W3- Professur für Biotechnologie, verbunden mit einer Departmentleitung am UFZ Leipzig.

Christine Skerka vom Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie – Hans-Knöll-Institut – übernahm am 10. Dezember 2014 die apl.-Professur für Immunregulation an der Universität Jena.

Elisabeth Grohmann von der Universität Freiburg übernimmt ab März 2015 die W2-Professur für das Fachgebiet Mikrobiologie im Studiengang Biotechnologie an der Beuth-Hochschule für Technik Berlin.

Pensionierungen

Reinhard Krämer vom Institut für Biochemie an der Universität zu Köln wurde am 1. März 2014 pensioniert.

Ortwin Meyer, Inhaber des Lehrstuhls für Mikrobiologie der Universität Bayreuth wurde am 31. März 2014 pensioniert.

Michael Hecker vom Institut für Mikrobiologie der Universität Greifswald wurde am 1. Oktober 2014 pensioniert.

Frieder Schauer vom Institut für Mikrobiologie der Universität Greifswald wurde am 1. Oktober 2014 pensioniert.

Anton Hartmann von der Abteilung Mikroben-Pflanzen Interaktionen am Helmholtz-Zentrum München ist am 1. November 2014 in den Ruhestand gegangen.

Wolfgang Schilf vom Fachgebiet Mikrobiologie im Studiengang Biotechnologie an der Beuth Hochschule für Technik Berlin wird im März 2015 pensioniert.

Wissenschaftliche Preise

Ulrich Kück, Universität Bochum, Lehrstuhl für Allgemeine und Molekulare Botanik, erhielt am 24. Januar 2014 die Auszeichnung „Fellow of the American Academy of Microbiology".

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 am 25. Februar 2014 den Wissenschaftspreis der Hector-Stiftung für ihre herausragenden Arbeiten zur Erforschung von Tiefseeökosystemen und zugleich ihrem großen Engagement in der akademischen Lehre an einer Exzellenz-Universität.

Sie wurde im Juni 2014 zum Mitglied der EMBO (European Molecular Biology Organization) für Exzellenz in den Lebenswissenschaften gewählt, erhielt am 23. September 2014 die Gustav-Steinmann-Medaille der Geologischen Vereinigung für ihre Verdienste um die marine Geomikrobiologie und am 29. September 2014 die Auszeichnung als David Packard Distinguished Lecturer des Monterey Bay Aquarium Research Institute (MBARI) mit ihrer Vorlesung: The Future of the Arctic Ocean: Scientific and technological challenges in the 4-D exploration of ice-covered deep seas.

Ihr wurde am 05. November 2014 die 12. Exzellenzprofessur der Prof. Dr. Werner-Petersen-Stiftung am GEOMAR Helmholtz-Zentrum für Polar- und Meeresforschung für ihre außergewöhnlichen Leistungen bei der Erforschung von mikrobiellen Lebensräumen am Meeresboden verliehen, und sie erhielt am 17. Dezember 2014 die Auszeichnung als Elected Fellow of the American Geophysical Union AGU für ihre Leistungen auf dem Gebiet der Tiefsee- und Polar-Mikrobiologie und Biogeochemie.

Nicole Dubilier vom Max-Planck-Institut für Marine Mikrobiologie, Bremen erhielt am 14. März 2014 den Gottfried-Wilhelm-Leibniz-Preis der Deutschen Forschungsgemeinschaft DFG für ihre Forschung an Symbiosen zwischen Mikroorganismen und marinen Tieren.

Kathrin Fröhlich von der Universität Würzburg erhielt am 16. März 2014 den Promotionspreis der Unterfränkischen Gedenkjahresstiftung für Wissenschaft für ihre Arbeiten über die Funktionsbestimmung Hfq-abhängiger kleiner RNAs im Modellorganismus *Salmonella Thyphimurium.*

Dr. Kai Papenfort, VAAM-Promotionspreisträger 2011, ist einer der drei Postdoktoranden-Preisträger der Robert-Koch-Stiftung 2014. Er erhielt die mit 5.000 Euro dotierte Auszeichnung für seine Untersuchungen zur Rolle von regulatorischen RNA-Molekülen bei Krankheitserregern. „Er zeigte am Beispiel von *Salmonella typhimurium*, dass sRNAs (small RNAs) ganze Gengruppen steuern", so Laudator Jörg Hacker. Derzeit arbeitet Papenfort im Labor von Bonnie L. Bassler an der Universität in Princeton (USA). *(stö)*

Stephan Binder und **Georg Schaumann** vom Institut für Bio- und Geowissenschaften, Forschungszentrum Jülich, wurden am 10. April 2014 als Preisträger des BMBF-Wettbewerbs „Gründungsinitiative Biotechnologie" gekürt und erhielten am 30. September 2014 den PEP–Award 2014 für ihr Ausgründungsprojekt „SenseUP Biotechnology".

Tom Bretschneider vom Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie – Hans-Knöll-Institut erhielt am 03. April 2014 den Promotionspreis des Beutenberg Campus e.V. für seine Arbeiten über *In vitro*-Charakterisierung nicht kanonischer Ketosynthasen und Imaging-Massenspektrometrie von Naturstoffen.

Swantje Behnken, Keishi Ishida, Florian Kloss und Thorger Lincke vom Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie – Hans-Knöll-Institut erhielten am 28. April 2014 den Preis für den Leibniz-Wirkstoff des Jahres 2014 für ihre Arbeiten über Closthioamid.

Manuel Kleiner vom Max-Planck-Institut für Marine Mikrobiologie, Bremen erhielt am 19. Mai 2014 den Friedrich-Hirzebruch-Preis der Studienstiftung des Deutschen Volkes für seine Dissertation über den Stoffwechsel und die evolutionäre Ökologie der chemosynthetischen Symbionten von marinen Wirbellosen.

Sebastian Suerbaum von der Medizinischen Hochschule Hannover, Institut für Medizinische Mikrobiologie und Krankenhaushygiene, hielt am 22. Mai 2014 die David B. Schauer Lecture über seine Arbeiten zu *Helicobacter pylori* am Massachusetts Institute of Technology, Cambridge, USA; Er wurde zum Fellow der American Academy of Microbiology gewählt.

Qian Chen vom Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie – Hans-Knöll-Institut erhielt am 24. Mai 2014 den Preis der chinesischen Regierung für herausragende Studenten im Ausland (Chinese government award for outstanding self-financed students abroad) für ihre Arbeiten über die Rolle des Komplementsystems bei schwerwiegenden Nierenerkrankungen.

Dominik Esser von der Universität Duisburg-Essen erhielt am 28. Mai 2014 den Scientist Award des Zentrums für Wasser- und Umweltforschung sowie am 25. Juni 2014 den Preis für den besten Promotionsabschluss in der Fakultät Chemie des Jahres 2013.

Gunther Döhlemann vom Max-Planck-Institut für terrestrische Mikrobiologie, Marburg erhielt am 23. September 2014 den Julius-Kühn-Preis für seine Arbeiten über Brandpilze.

Simon Boecker von der Technischen Universität Berlin erhielt am 11. Juni 2014 den Preis des Zukunftsforum Biotechnologie der DECHEMA und am 24. Oktober 2014 den Christian-Wandrey-Preis des Forschungszentrums Jülich für seine Diplomarbeit mit dem Titel „Expression of the Enniatin Synthetase in *Aspergillus niger* and Purification of its Product Enniatin".

Malek Saleh von der Universität Greifswald erhielt am 17. Juni 2014 den „Sanofi Aventis Förderpreis" der Fachgruppe Mikrobielle Pathogenität der DGHM/VAAM für seine in EMBO Molecular Medicine veröffentlichte Arbeit „Molecular architecture of *Streptococcus pneumoniae* surface thioredoxin-fold lipoproteins crucial for extracellular oxidative stress resistance and maintenance of virulence" sowie am 5. Oktober 2014 den Promotionspreis der DGHM für seine Arbeit mit dem Titel „Die Bedeutung der Oberflächen-lokalisierten Thioredoxin-ähnlichen Lipoproteine Etrx1 und Etrx2 für die extrazelluläre oxidative Stressresistenz und Pathogenität von *Streptococcus pneumoniae* ".

Roland Lill von der Universität Marburg und dem Max-Planck-Institut für terrestrische Mikrobiologie, Marburg erhielt am 17. Juli 2014 den „Albrecht-Kossel Prize of the German Chemical Society", GDCh für seine grundlegenden biochemischen Arbeiten zur Biogenese der Eisen-Schwefel-Proteine in Eukaryoten sowie am 8. September 2014 die „Luigi Sacconi Medal of the Italian Chemical Society and Luigi Sacconi Foundation" für seine Arbeiten zur Synthese der Eisen-Schwefel-Cluster in zellulären Systemen.

Nilanjan Pal Chowdhury von der Universität Marburg und dem Max-Planck-Institut für terrestrische Mikrobiologie, Marburg erhielt am 29. Juli 2014 den Vincent Massey Award für seine Arbeiten über "Formation of superoxide and hydrogen peroxide by flavin-based electron bifurcation".

Andreas Küberl vom Institut für Bio- und Geowissenschaften, Forschungszentrum Jülich, erhielt am 10. September 2014 den Josef-Deutscher-Award für „Excellence in Content and Presentation" für seinen Vortrag „Pupylated proteins in *Corynebacterium glutamicum* revealed by MudPIT analysis" auf der „1st International Conference on Post-translational Modifications in Bacteria" in Göttingen.

Hannah Brocke vom Max-Planck-Institut für Marine Mikrobiologie, Bremen erhielt am 21. September 2014 den 1. Preis des Science Slam der Maritimen Woche in Bremen für Wissenschaft kurz und verständlich präsentiert.

Julia Bandow von der Universität Bochum erhielt am 5. Oktober 2014 den VAAM-Forschungspreis.

Axel Brakhage und **Bernhard Hube** vom Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie – Hans-Knöll-Institut erhielten am 5. Oktober 2014 den Hauptpreis der DGHM für ihre Arbeiten über die Infektionsbiologie human-pathogener Pilze.

Yvonne Göpel (Universität Göttingen), **Daniel Pfeiffer** (Universität Stuttgart) und **Volker Winstel** (Universität Tübingen) erhielten am 7. Oktober 2014 den VAAM-Promotionspreis.

Peter F. Zipfel und Christine Skerka vom Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie – Hans-Knöll-Institut erhielten am 16. Oktober 2014 den Galenus-von-Pergamon-Preis für ihre Arbeiten über die Nierenkrankheit MPGN.

Özlem Sarikaya Bayram von der Universität Göttingen erhielt im Oktober 2014 den Elisabeth-Gateff-Preis für ihre Arbeiten über die Neuentdeckung eines zellulären Signalübertragungsweges bei Pilzen.

Volker Schwartze, Ekatarina Shelest, Fabian Horn, Jörg Linde, Vito Valiante und Kerstin Kaerger vom Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie – Hans-Knöll-Institut erhielten am 3. November 2014 den medac-Forschungspreis 2014 für ihre Veröffentlichung Gene expansion shapes genome architecture in the human pathogen *Lichtheimia corymbifera*: An evolutionary genomics analysis in the ancient terrestrial Mucorales (Mucoromycotina).

Sacha Pidot, Keishi Ishida, Michael Cyrulies vom Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie – Hans-Knöll-Institut erhielten am 3. November 2014 den medac-Forschungspreis 2014 für ihre Veröffentlichung „Discovery of clostrubin, an exceptional polyphenolic polyketide antibiotic from a strictly anaerobic bacterium".

Karen Tavares Silva wurde am 20. November 2014 mit dem Preis des Internationalen Clubs der Universität Bayreuth für ihre Arbeiten über mikrobiologische Grundlagen und die Entwicklung einer Methode für die genomweite Transposon-Mutagenese von Magnetbakterien ausgezeichnet.

Tom Bretschneider vom Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie – Hans-Knöll-Institut erhielt am 27. November 2014 den Nachwuchspreis der Leibniz-Gemeinschaft für seine Arbeiten über *In vitro*-Charakterisierung nicht kanonischer Ketosynthasen und Imaging-Massenspektrometrie von Naturstoffen.

Bastian Vögeli vom Max-Planck-Institut für terrestrische Mikrobiologie, Marburg erhielt am 12. Dezember 2014 die Silbermedaille der ETH Zürich für seine Arbeit "Understanding and Engineering Enoyl-CoA Reductases".

Das Bielefelder iGEM-Team mit den Studenten Annika Fust, Birte Hollmann, Boas Pucker, David Wollborn, Janina Tiemann, Julian Droste, Sandra Brosda, Sebastian Blunk, Simon Riedl und Thore Bleckwell hat eine Gold-Medaille im iGEM-Finale in Boston gewonnen.

Promotionen 2014

Universität Bayreuth

Frank Mickoleit: Die Funktion des *coxF*-Genproduktes bei der Reifung des aktiven Zentrums der Kohlenmonoxid-Dehydrogenase in *Oligotropha carboxidovorans*
Betreuer: Ortwin Meyer

Humboldt-Universität Berlin

Steven Wuttge: Untersuchungen zur Regulation der ABC-Transporter für Maltose und Glycerol-3-Phosphat aus *Escherichia coli*
Betreuer: Erwin Schneider

Susann Boerner: Probing reaction conditions and cofactors of conformational prion protein changes underlying the autocatalytic self-propagation of different prion strains
Betreuer: Erwin Schneider

Elena Belogolova: Identification of *Helicobacter pylori* outer membrane protein HopQ as a novel T4SS-associated virulence factor
Betreuer: Thomas F. Meyer

Caspar Schäfer: Proteinbiochemische, spektroskopische und röntgenkristallografische Untersuchung der actinobakteriellen [NiFe]-Hydrogenase aus *Ralstonia eutropha*
Betreuerin: Bärbel Friedrich

Katja Karstens: Untersuchungen zur Funktion sauerstofftoleranter, NAD*-reduzierender Hydrogenasen und ihrer Anwendung in der lichtgetriebenen Wasserstoffproduktion in Cyanobakterien
Betreuerin: Bärbel Friedrich

Universität Bielefeld

Inga Friedenau: ColE1-Plasmidproduktion in *Escherichia coli*: Simulation und Experiment
Betreuer: Karsten Niehaus

Sabrina Grewe: Funktionale Charakterisierung des Lichtsammelkomplexproteins LHCBM9 in stressinduzierten Anpassungsreaktionen von *Chlamydomonas reinhardtii*
Betreuer: Olaf Kruse

Sabine Heider: Characterization and engineering of carotenoid biosynthesis in *Corynebacterium glutamicum* for terpenoid production
Betreuer: Volker F. Wendisch

Stephanie Klatte: Redox self-sufficient amine functionalization of carbonyls and alcohols by *Escherichia coli* whole cell biotransformation
Betreuer: Volker F. Wendisch

Gajendar Komati Reddy: Role of substrate-level phosphorylation in glycolysis of *Corynebacterium glutamicum* for growth and amino acid production
Betreuer: Volker F. Wendisch

Lisa Meier zu Verl: Herstellung und Charakterisierung starklichttoleranter *Chlamydomonas reinhardtii*-Stämme
Betreuer: Olaf Kruse

Almut Mentz: Identifizierung und Funktionsanalysen von kleinen RNAs in *Corynebacterium glutamicum*
Betreuer: Alfred Pühler

Tobias Meiswinkel: Engineering *Corynebacterium glutamicum* for access to alternative carbon sources
Betreuer: Volker F. Wendisch

Rabea Schweiger: Impacts of phytohomone pathway interferences and arbuscular mycorrhizal symbiosis on plant foliar metabolomes
Betreuerin: Caroline Müller

Ahmed Zahoor ul-Hassan: Metabolic engineering of *Corynebacterium glutamicum* for glycolate production
Betreuer: Volker F. Wendisch

Christian Ziert: Metabolic engineering of *Corynebacterium glutamicum* for the production of L-aspartate and its derivatives β-alanine and ectoine
Betreuer: Volker F. Wendisch

Britta Abellan: Genome-wide view and structural close-up on the global transcriptional regulator GlxR of *Corynebacterium glutamicum*
Betreuer: Alfred Pühler

Kyle Lauersen: Heterologous expression of an ice binding protein from the microalgae *Chlamydomonas reinhardtii* with an optimized nuclear gene expression
Betreuer: Olaf Kruse

Marina Vemmer: Encapsulation systems for slow release of CO_2 and antimicrobial plant extracts
Betreuer: Andreas Tauch

Universität Bochum

Roman Moser: Charakterisierung untypischer Phospholipid-Biosynthesewege in Bakterien
Betreuer: Franz Narberhaus

Aaron Overlöper: Charakterisierung kleiner RNAs in *Agrobacterium tumefaciens*
Betreuer: Franz Narberhaus

Philip Möller: RNA-basierte Regulation und RNA-bindende Proteine in *Agrobacterium tumefaciens*
Betreuer: Franz Narberhaus

Kristina Overkamp: Biosynthese offenkettiger Tetrapyrrole und Assemblierung von Phycobiliproteinen in der Cryptophyte *Guillardia theta*
Betreuerin: Nicole Frankenberg-Dinkel

Sabrina Heine: Das Phytochrom-Regulon und eine Phosphdiesterase als Beispiele zur Wahrnehmung von Umweltsignalen in *Pseudomonas aeruginosa*
Betreuerin: Nicole Frankenberg-Dinkel

Julia Böhm: Kreuzungstypgene und Sexualzyklus bei dem Penicillin-Produzenten *Penicillium chrysogenum*
Betreuer: Ulrich Kück

Christina Marx: Ein hochmolekularer RNA-Protein-Komplex ist beteiligt am *trans*-Spleißen chloroplastidären mRNA
Betreuer: Ulrich Kück

Steffen Nordzieke: Molekulargenetische Studien mit PRO45 in *Sordaria macrospora*: Ein Homolog des *sarcolemmal membrane-associated protein* (SLMAP) lokalisiert an der Kernmembran und ist essenziell für die pilzliche Entwicklung
Betreuer: Ulrich Kück

Stefanie Traeger: Funktionelle und vergleichende Genomanalysen zur Untersuchung pilzlicher Entwicklung
Betreuerin: Minou Nowrousian

Universität Bonn

Thomas Weissgerber: Genome, transcriptome, proteome and metabolome based analyses of *Allochromatium vinosum* DSM 180^T
Betreuerin: Christiane Dahl

Yvonne Stockdreher: Analysis of cytoplasmic sulfur trafficking during sulfur globule oxidation in *Allochromatium vinosum*
Betreuerin: Christiane Dahl

Maria Meyer: Produktion und Aufreinigung von Enzymen aus Essigsäurebakterien für biotechnologische Anwendungen
Betreuer: Uwe Deppenmeier

Daniela Münch: The Cell Wall Precursor Lipid II – Structural Variations and Antibiotic Activities
Betreuer: Hans-Georg Sahl

Stefania De Benedetti: Orchestration of the Biosynthesis of Cell Wall Precursors in Chlamydiae
Betreuer: Hans-Georg Sahl

Christian Fritz Otten: Processing of the cell wall precursor lipid II in Chlamydia pneumoniae
Betreuer: Hans-Georg Sahl

Technische Universität Braunschweig

Dagmar Zwerschke: Funktion neuartiger Enzyme der mikrobiellen Häm-Biosynthese
Betreuer: Dieter Jahn, Gunhild Layer

Frederike Lisa Drawert: *Legionella*-Wirtszell-Interaktionen und Analyse neuer Wirkstoffe und Wirkstoffträger
Betreuer: Michael Steinert, Manfred Rohde

Andrea Wesche-Franke: Characterisation of methionine metabolism of *Pseudomonas aeruginosa* under conditions resembling a chronic cystic fibrosis lung infection
Betreuer: Max Schobert, Dieter Jahn

Luisa Roselius: Vergleichende Genomanalyse und mathematische Modellierung von Pathogenitäts- und Regulationsmechanismen bei Bakterien
Betreuer: Dieter Jahn, Dirk Langemann

Ann-Kathrin Meyer: Untersuchung des Einflusses von Tellurit auf die Wirksamkeit von Antibiotika bei uropathogenen *Pseudomonas aeruginosa*-Isolaten
Betreuer: Max Schobert, Dieter Jahn

Ellen Waldmann: Identification and expression analysis of small regulatory RNAs in *Yersinia pseudotuberculosis*
Betreuerin: Petra Dersch

Stephanie Christine Seekircher: Identification of regulatory factors that control the synthesis of the small regulatory RNA CsrC in *Yersinia pseudotuberculosis*
Betreuerin: Petra Dersch

Janina Nikola Georgia Schweer: Molecular function of the cytotoxic necrotizing factor CNF_Y and its impact on the virulence of *Yersinia pseudotuberculosis*
Betreuerin: Petra Dersch

DSMZ/TU Braunschweig

Jan Philipp Meier-Kolthoff: Comparison of nucleotide and protein sequences for genome-based classification and identification
Betreuer: Markus Göker, Hans-Peter Klenk

Katharina Huber: Rolle von Bodenbakterien in den Nährstoffzyklen subtropischer Savannenböden (TFO)
Betreuer: Jörg Overmann

Universität Bremen/MPI für Marine Mikrobiologie Bremen

Wenjin Hao: Bacterial communities associated with Jellyfish
Betreuer: Rudolf Amann

Stefano Romano: Response to phosphate limitation of *Pseudovibrio sp.* FO-BEG1, a versatile bacterium with the potential for a symbiotic lifestyle
Betreuerin: Heide Schultz-Vogt

Christin Bennke: Distribution and function of marine *Bacteroidetes*
Betreuer: Rudolf Amann

Gao Chen: Physiology of the Anaerobic Degradation of Naphthalene by Marine Sulfate-Reducting Bacteria
Betreuer: Friedrich Widdel

Mina Bižić-Ionescu: Polyphasic comparison of limnic and marine particle-associated bacteria
Betreuer: Rudolf Amann

Petra Pjevac: Co-existence and niche differentiation of sulfur oxidizing bacteria in marine environments
Betreuer: Rudolf Amann

Beate Kraft: Competition in nitrate-reducing microbial communities
Betreuer: Marc Strous

Zainab Beiruti: Selective effects of transient oxygen and nitrate exposure on sulfate reducing/fermetative consortia
Betreuer: Marc Strous

Anna Hanke: Continuous Cultivation of Janssand Microbial Communities Response to Varying Oxygen Concentrations and Temperatures
Betreuer: Marc Strous

Stefanie Müller: Experimental evolution of *Paracoccus denitrificans* in anoxic chemostats
Betreuer: Marc Strous

Stefan Häusler: The environment, diversity and activity of microbial communities in submarine freshwater springs in the Dead Sea
Betreuer: Dirk de Beer

Arjun Chennu: Hyperspectral mapping of the microscale dynamics of microphytobenthos
Betreuer: Dirk de Beer

Duygu Sevilgen: Microphytobenthos in cold-water sublitoral systems- their ecological role and response to changing environmental conditions
Betreuer: Dirk de Beer

Hannah Brocke: Little Things Become BIG – Drivers and impacts of benthic cyanobacterial mats on coral reefs
Betreuer: Dirk de Beer

Anna Behrendt: Competition between dissimilatory nitrate reduction to ammonium and denitrification in marine sediments
Betreuer: Dirk de Beer

Mar Fernandez Mendez: Primary productivity in Arctic sea ice and ocean
Betreuerin: Antje Boetius

Marianne Jacob: Influence of Global Change on microbial communities in Arctic sediments
Betreuerin: Antje Boetius

Jessika Füssel: Impacts and importance of ammonia- and nitrite oxidation in the marine nitrogen cycle
Betreuer: Marcel Kuypers

Hannah Marchant: Nitrogen cycling in coastal permeable sediments from eutrophied regions
Betreuer: Marcel Kuypers

Technische Universität Darmstadt

Dennis Petrasch: Untersuchung und biotechnologische Nutzung thermophiler chemolithotropher Mikroorganismen
Betreuer: Arnulf Kletzin

Monique Luckmann: Untersuchungen zum N_2O-Metabolismus des Nitrat ammonifizierenden Bakteriums *Wolinella succinogenes*
Betreuer: Jörg Simon

Technische Universität Dortmund

Kirsten Anna Katrin Köhler: Analysis, design and synthesis of microbial cell factories producing n-butanol
Betreuer: Andreas Schmid

Jianan Fu: Production of Hydroxyproline with recombinant Escherichia coli
Betreuer: Andreas Schmid

Frederik Fritzsch: Envirostat technologies for time resolved single cell analysis in contactless controlled microenvironments
Betreuer: Andreas Schmid

Bartłomiej Tomaszewski: Selective Redox Biocatalysis in Multiphasic Enzyme Reactors
Betreuer: Andreas Schmid

Karsten Lang: Strain and process engineering for the fermentative production of secondary metabolites in *Pseudomonas* sp. Strain VLB120
Betreuer: Andreas Schmid

Nadine Ladkau: Multi-step biocatalysis for the sequential oxy- and aminofunctionalization of renewable DAME – Strain engineering and process design
Betreuer: Andreas Schmid

Technische Universität Dresden

Christian Sattler: Kohlenhydratstoffwechsel in *Methanosarcina*: Genetische und Physiologische Analysen
Betreuer: Michael Rother

Ariane Adam: Reinigung und Charakterisierung der MCAP-3-Halogenase aus *Pseudomonas fluorescens* BL915
Betreuer: Karl-Heinz van Pée

Universität Düsseldorf

Isabel Eichhof: Flavinmononukleotid-basiertes Fluoreszenzprotein als Reporter hypoxischer Genexpression in Pilzen
Betreuer: Joachim Ernst

Quentin Lagadec: Click beetle luciferases as reporters of gene expression in *Candida albicans*
Betreuer: Joachim Ernst

Janpeter Stock: Etablierung eines neuartigen Protein-Expressionssystems in *Ustilago maydis*
Betreuer: Michael Feldbrügge

Parveen Sarkari: Optimizing the expression of antibody formats in protease-deficient *Ustilago maydis* strains
Betreuer: Michael Feldbrügge

Universität Düsseldorf/ Forschungszentrum Jülich

Michael Vogt: Metabolic engineering of *Corynebacterium glutamicum* for production of L-leucine and 2-ketoisocaproate
Betreuer: Michael Bott

Andreas Küberl: Pupylierung in *Corynebacterium glutamicum*
Betreuer: Michael Bott

Andrea Michel: Anaerobes Wachstum von *Corynebacterium glutamicum* durch gemischte Säurefermentation
Betreuer: Michael Bott

Jennifer Pahlke: The 6C RNA of *Corynebacterium glutamicum*
Betreuer: Michael Bott

Sabrina Witthoff: Engineering of *Corynebacterium glutamicum* towards utilization of methanol as carbon and energy source
Betreuer: Michael Bott

Dan Oertel: Tat translocase composition in *Corynebacterium glutamicum* and the effect of TorD coexpression
Betreuer: Roland Freudl

Universität Erlangen-Nürnberg

Sebastian Schmidt: Isolierung und Charakterisierung rtTA2^{s}-M2 regulierender Peptide
Betreuer: Andreas Burkovski

Maria Nikolakakou: Time-resolved quantification of components involved in CcpA-mediated CCR in *Bacillus subtilis*
Betreuer: Andreas Burkovski

Corina Heidrich: Tetrazyklin-vermittelte Regulation der *tetO* Expression durch alternative Faltung der Leader-RNA
Betreuer: Andreas Burkovski

Universität Frankfurt am Main

Feline Benisch: Integration of alternative routes to glycolysis in *Saccharomyces cerevisiae* to optimize product yields
Betreuer: Eckhard Boles

Florian Grundmann: Natural products from entomopathogenic bacteria
Betreuer: Helge B. Bode

Max Kronenwerth: Characterisation, biosynthesis, and synthesis of selected natural products from *Xenorhabdus* and *Photorhabdus*
Betreuer: Helge B. Bode

Wolfram Lorenzen: Identification of biomarkers for the fruiting body formation in *Myxococcus xanthus*
Betreuer: Helge B. Bode

Jessica Schmitt: Translationsregulation des halophilen Archaeon *Haloferax volcanii*
Betreuer: Jörg Soppa

Katrin Gäbel: Molekulare Analyse der Translationsinitiation in halophilen Archaea
Betreuer: Jörg Soppa

Karolin Zerulla: Untersuchung der Polyploidie in Prokaryoten
Betreuer: Jörg Soppa

Verena Hess: Energy conservation acetogenic bacteria: Identification and characterization of key enzymes in Acetobacterium woodii and genome analysis of *Thermoanaerobacter kivui*
Betreuer: Volker Müller

Florian Mayer: Der Nanomotor archaeeller ATP-Synthasen: Na^+-Transport, Ionenspezifität und Membraninsertion
Betreuer: Volker Müller

Carolin Gloger: Heterologe Produktion und Mutationsanalyse der A_1A_O-ATP-Synthase aus *Methanosarcina mazei*
Betreuer: Volker Müller

Universität Freiburg

Rösch, Thomas: Regulation and assembly of the type IV secretion system of plasmid pLS20, and impact of the plasmid on its host, *Bacillus subtilis*
Betreuer: Peter Graumann

Universität Gießen

Linlin Hou: Mechanisms of interaction between archael DnaG and the RNA-processing exosome and role of DnaG in RNA metabolism of *Sulfolobus solfataricus*
Betreuerin: Gabriele Klug

Yannick Hermanns: Die Auswirkung von Umwelteinflüssen auf die mRNA-Stabilitäten in *Rhodobacter sphaeroides*
Betreuerin: Gabriele Klug

Anja Dörrich: Mechanism and function of a non-standard circadian clock system in cyanobacteria
Betreuerin: Annegret Wilde

Universität Göttingen

Clara Hoppenau: Characterization of the pathogenicity relevant genes THI4 and PA14_2 in *Verticillium dahlia*
Betreuer: Gerhard Braus

Özlem Sarikaya Bayram: Role of methyltransferases in fungal development and secondary metabolite production
Betreuer: Gerhard Braus

Hedieh Shahpasandzadeh: Crosstalk between alpha-synuclein posttranslational modification in yeast as model of Parkinson's disease
Betreuer: Gerhard Braus

Chi-Jan Lin: AfuSomA transcriptional network in human pathogen *Aspergillus fumigatus*
Betreuer: Gerhard Braus

Ronny Lehneck: Functional and structural analysis of carbonic anhydrases from the filamentous ascomycete *Sordaria macrospora*
Betreuerin: Stefanie Pöggeler

Felix Mehne: Bildung und Homöostase von c-di-AMP in *Bacillus subtilis*
Betreuer: Jörg Stülke

Christopher Zschiedrich: Nebenwege des zentralen Kohlenstoffmetabolismus von *Bacillus subtilis*: Regulation der Methylglyoxalsynthase und der Zitratsynthase CitA
Betreuer: Jörg Stülke

Universität Greifswald

Malek Saleh: Die Bedeutung der Oberflächen-lokalisierten Thioredoxin-ähnlichen Lipoproteine Etrx1 und Etrx2 für die extrazelluläre oxidative Stressresistenz und Pathogenität von *Streptococcus pneumoniae*
Betreuer: Sven Hammerschmidt

Sylvia Kohler: Unraveling the interaction of *Streptococcus pneumoniae* with human vitronectin
Betreuer: Sven Hammerschmidt

Rasha Aref: Comparative analysis of repressor interaction with pleiotropic corepressors Sin3 and Cyc8 in the yeast *Saccharomyces cerevisiae*
Betreuer: Hans-Joachim Schüller

Felix Kliewe: Interaktion des Repressors Opi1 der Phospholipid-Biosynthese mit Regulatoren des Phosphat-Stoffwechsels und pleiotropen Corepressoren in der Hefe *Saccharomyces cerevisiae*
Betreuer: Hans-Joachim Schüller

Sandra Maaß: Relative und absolute Proteinquantifikation in Bakterien
Betreuer: Michael Hecker, Dörte Becher

Praveen Kumar Sappa: Characterization of the general stress response of *Bacillus subtilis*
Betreuer: Uwe Völker

Kristin Surmann: Charakterisierung von Erreger-Wirt-Interaktionen am Beispiel des Gram-positiven Pathogens *Staphylococcus aureus*
Betreuer: Uwe Völker

Universität Halle-Wittenberg

Johannes Taubert: Untersuchungen zur Interaktion von Tat-Substraten mit den Komponenten des Tat-Proteintransportsystems in *Escherichia coli*
Betreuer: Thomas Brüser

Janine Kirchberg: Die Rolle der Aconitasen als Fe-S-Proteine im Citrat- und Eisenstoffwechsel des phytopathogenen Bakteriums *Xanthomonas camprestris pv. Vesicatoria*
Betreuer: Gary Sawers

Marco Fischer: Die anaerobe Nitratreduktion bei dem obligat aeroben *Streptomyces coelicolor A3(2)*
Betreuer: Gary Sawers

Lydia Beyer: Physiologische Charakterisierung des Formiat-Stoffwechsels in *Escherichia coli*
Betreuer: Gary Sawers

Universität Hamburg

Julia Jürgensen: Identification of an unusual GTase from a non-cultivated microorganism and the construction of an improved *E. coli* strain harboring the *rpoD* gene from *C. cellulolyticum* for metagenome searches
Betreuer: *Wolfgang Streit*

Stefanie Böhnke: A novel function-based screen for detecting RubisCO active clones from metagenomic libraries: elucidating the role of RubisCO associated enzymes
Betreuerin: Mirjam Perner

Boris Nowka: Activity and ecophysiology of nitrite-oxidizing bacteria in natural and engineered habitats
Betreuerin: Eva Spieck

Universität Hannover

Hendrik Osadnik: Funktionelle und strukturelle Charakterisierung des *phage shock protein A* aus *Escherichia coli*
Betreuer: Thomas Brüser

Hananeh Korehi: Microbial diversity in mine taylings and the role of metal sulfide oxidizers in biomining processes
Betreuer: Axel Schippers

Medizinische Hochschule Hannover

Rebecca Geyer: Analysis of the molecular function of invasin-like proteins of *Yersinia pseudotuberculosis* and their role in pathogenesis
Betreuerin: Petra Dersch

Tobias Bönig: Charakterisierung von CagL, dem VirB5-Homolog des *Helicobacter pylori* Cag-Typ-IV-Sekretionssystems
Betreuerin: Christine Josenhans

Pavel Dutow: The role of complement and the receptor for the anaphylatoxin C3a in the defense against Chlamydia AND The effect of chlamydial lung infection on allergic airway disease in mice
Betreuer: Andreas Klos

Eugenia Gripp: *Campylobacter jejuni* genetic and phenotypic diversity and novel host modulatory factors
Betreuerin: Christine Josenhans

Annegret Hampel: Arginine assimilation in *Mycobacterium tuberculosis* and *Mycobacterium smegmatis*
Betreuer: Franz-Christoph Bange

Juliane Krebes: Molecular mechanisms generating genome and methylome variation in *Helicobacter pylori*
Betreuer: Sebastian Suerbaum

Universität Hohenheim

Petra Halang: Stressful environments: Motility and catecholamine response in *Vibrio cholerae*
Betreuerin: Julia Fritz-Steuber

Jennifer Zimmermann: Entwicklung und Validierung schneller und selektiver Verfahren zum Nachweis von *Salmonella enterica*, *Cronobacter* spp. und *Bacillus cereus* in Milcherzeugnissen
Betreuer: Herbert Schmidt

Universität Jena

Francesca Langella: Plant health and growth promoting microorganisms and their effects on metal mobility
Betreuerin: Erika Kothe

Soumya Madhavan: Targeted gene disruption and expression studies for functional analysis of genes in *Schizophyllum commune*
Betreuerin: Erika Kothe

Riya Menezes: Application of Raman spectroscopy and mass spectrometry to study growth and interaction processes of the white-rot fungus *Schizophyllum commune*
Betreuerin: Erika Kothe

Frank Schindler: Vertikale Verlagerungsprozesse innerhalb des Testfelds „Gessenwiese“ im ehemaligen Uranabbaugebiet Ronneburg/Thüringen
Betreuerin: Erika Kothe

Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie – Hans-Knöll-Institut/Universität Jena

Shayista Amin: *In vitro, in vivo* and *in ovo* interactions of human pathogenic fungus *Aspergillus fumigatus*
Betreuer: Hans Peter Saluz

Michael Biermann: Rare Amino Acid Related Physiological Stress Response in Recombinant *Escherichia coli* Fermentations
Betreuer: Uwe Horn

Selina Böcker: Funktionelle Charakterisierung der Interaktion zwischen dem Typ-III-sekretierten Protein IncB aus *Chlamydia psittaci* und dem humanen Protein Snapin
Betreuer: Hans Peter Saluz

Jana Braesel: Sekundärmetabolismus der Basidiomyceten – Molekularbiologische und biochemische Untersuchungen an *Paxillus involutus* und *Suillus grevillei*
Betreuer: Dirk Hoffmeister

Qian Chen: Autoantibodies and a factor H-related hybrid protein deregulate complement in dense deposit disease
Betreuer: Peter Zipfel

Florian Hennicke: Molekularbiologische Untersuchungen zur Stresstoleranz und zur mikrobiellen Kommunikation bei *Candida albicans*
Betreuer: Peter Staib

Christian Hummert: Analyse und Interpretation der Varianz von Genexpressionsdaten
Betreuer: Reinhard Guthke

Sophia Keller: Interaktion der humanpathogenen Mikroorganismen *Aspergillus fumigatus* und *Pseudomonas aeruginosa*
Betreuer: Axel Brakhage

Florian Kloss: Totalsynthese, Struktur-Aktivitäts-Beziehungen und Funktion von Closthioamid, dem ersten Antibiotikum aus einem anaeroben Bakterium
Betreuer: Christian Hertweck

Isabell Kopka: Complement regulation in kidney cells
Betreuer: Peter Zipfel

Martin Kreutzer: Isolation, characterization and structure elucidation of bacterial siderophores
Betreuer: Markus Nett

Kristin Kroll: The hypoxic (low-oxygen) response of the pathogenic mould *Aspergillus fumigatus* and its relevance to pathogenicity
Betreuer: Axel Brakhage

Peter Kupfer: Dynamic modeling of stimulated cells involved in cartilage and bone degenerative diseases
Betreuer: Reinhard Guthke

Katrin Lapp: Die Rolle von reaktiven Stickstoffspezies in der Interaktion von *Aspergillus fumigatus* mit humanen neutrophilen Granulozyten
Betreuer: Axel Brakhage

Anne-Catrin Letzel: Genome Mining von anaeroben Bakterien zur Entdeckung von Wirkstoffen
Betreuer: Christian Hertweck

Claudia Lüdecke-Beyer: Investigation of Bacterial Adhesion Mechanisms on Nanorough Biomaterials Surfaces Using a Novel In Vitro Testing Device
Betreuer: Uwe Horn

Juliane Macheleidt: Proteinkinase A-regulierte Biosynthese eines neuen, immunsupprimierenden Naturstoffs durch den humanpathogenen Pilz *Aspergillus fumigatus*
Betreuer: Axel Brakhage

Sven Micklisch: Funktionelle Charakterisierung von Age-Related Maculopathy Susceptibility 2 – ARMS2
Betreuerin: Christine Skerka

Nadine Möbius: Molekulare Untersuchungen zu Symbiosefaktoren und Toxin-Biosynthese in *Burkholderia-Rhizopus*-Interaktionen
Betreuer: Christian Hertweck

Sarbani Mohan: Complement Escape Mechanisms of *Streptococcus pneumoniae*
Betreuer: Peter Zipfel

Christian Otzen: Untersuchungen zum Fettsäure- und Propionatmetabolismus in *Candida albicans*
Betreuer: Matthias Brock

Julia Pauly: Molekularbiologische und biochemische Untersuchungen zum Sekundärstoffwechsel des Bakteriums *Ralstonia solanacearum GMI1000*
Betreuer: Dirk Hoffmeister

Katja Tuppatsch: Assoziierte Proteine und regulatorische Funktionen des CAAT Bindekomplexes in *Aspergillus nidulans*
Betreuer: Axel Brakhage

Nico Ueberschaar: Mutasynthese und Charakterisierung von antitumoralen Wirkstoffen aus Streptomyceten
Betreuer: Christian Hertweck

Sebastian Vlaic: Integrated knowledge-assisted genome-wide transcriptome data analysis of the non-alcoholic fatty liver disease
Betreuer: Reinhard Guthke

Anja Wartenberg: *In-vitro*- und *In-vivo*-Mikroevolutionsstudien zum Anpassungsvermögen von *Candida albicans* an wirtsspezifische Umgebungen
Betreuer: Bernhard Hube

Emerson Zang: Droplet-based microfluidic screening for novel antibiotics from *Actinobacteria*
Betreuer: Uwe Horn

Universität Karlsruhe, KIT

Ines Schulze: Microbial Lipid Production with Oleagineous Yeasts
Betreuerin: Anke Neumann

Martin Pöhnlein: Investigation on the enzymatic synthesis of novel glycolipids
Betreuer: Christoph Syldatk

Marius Henkel: Layered Modeling and Simulation of Complex Biotechnological Processes
Betreuer: Christoph Syldatk und Rudolf Hausmann

Kerstin Dolch: Stabilität und Leistungsfähigkeit von gezielt gezüchteten Biofilmen auf Anoden
Betreuer: Johannes Gescher

Gunnar Sturm: Periplasmatische Elektronentransfer-Reaktionen in *Shewanella oneidensis* MR-1
Betreuer: Johannes Gescher

Katrin Sturm-Richter: Etablierung und Intensivierung von Mikroben-Anoden-Interaktionen in synthetischen und natürlichen exoelektrogenen Stämmen
***Betreuer:** Johannes Gescher*

Nikola Strempel: Response of *Pseudomonas aeruginosa* to components of the innate immune system
Betreuer: Jörg Overhage

Beatrix Tettmann: Genregulation und Biofilmbildung in *Pseudomonas* – Untersuchungen zu Sigma-Faktoren und Quorum Quenching
Betreuer: Jörg Overhage

Helmholtz-Zentrum für Ozeanforschung – GEOMAR/Universität Kiel

Johanna Silber: Characterization of new natural products from fungi of the German Wadden Sea
Betreuer: Johannes F. Imhoff

Mien Thi Pham: Community of soft coral *Alcyonium digitatum* associated bacteria and their antimicrobial activities
Betreuer: Johannes F. Imhoff

Universität Kiel

Daniela Langfeldt: The importance of bacterial virulence factors in natural biofilms at different stages of maturation for the immune response of gingival epithelial cells
Betreuerin: Ruth Schmitz-Streit

Jan-Moritz Sutter: Pentosestoffwechsel in halophilen Archaea. Abbauwege, Enzyme und transkriptionelle Regulation
Betreuer: Peter Schönheit

Universität Köln

Benjamin Roennecke: Die Glukosylglycerolphosphatsynthase aus dem Cyanobakterium *Synechocystis* sp. PCC 6803: Mechanismus der Aktivitätsregulation
Betreuer: Reinhard Krämer

Andreas Uhde: Kontrolle des Aminozuckerstoffwechsels in *Corynebacterium glutamicum*
Betreuer: Reinhard Krämer

Katja Kirsch: The impact of CO2 on inorganic carbon supply and pH homeostasis in *Corynebacterium glutamicum*
Betreuer: Reinhard Krämer

Alexander Eck: Trehalose Transport in *Corynebacterium glutamicum* and its Significance for Cell Envelope Synthesis
Betreuer: Reinhard Krämer

Anna Bartsch: Identification and functional characterization of cation/proton antiport systems in *Corynebacterium glutamicum*
Betreuer: Reinhard Krämer

Universität Konstanz

Dirk Oehler: Energy conservation in syntrophic acetate oxidation
Betreuer: Bernhard Schink

Michael Weiß: Bacterial degradation pathways for xenobiotic and natural organosulfonates
Betreuer: David Schleheck

Alexander Schmidt: Syntrophic oxidation of butyrate and ethanol
Betreuer: Bernhard Schink

Universität Leipzig/ Helmholtz Zentrum für Umweltforschung-UFZ

Judith Schuster: Analyse funktioneller Gene des Abbaues tertiärer Etherstrukturen in dem Bakterienstamm *Aquincola tertiaricarbonis* L108 anhand von *Knockout*-Mutanten
***Betreuer:** Hauke Harms*

Helmholtz Zentrum für Umweltforschung-UFZ/ Uppsala University

Inga Richert: Environmental filtering of bacteria in low productivity habitats
Betreuer: Annelie Wendeberg/Stefan Bertilsson

Helmholtz Zentrum für Umweltforschung-UFZ/ Utrecht University

Mehdi Gharasoo: Reactive transport simulations of microbial activity and biogeochemical transformations in porous environments: development and application of a pore-network model
Betreuer: Martin Thullner/Philippe van Cappellen

Universität Potsdam/ Deutsches GeoForschungs Zentrum GFZ

Paloma Serrano: Methanogens from Siberian permafrost as models for life on Mars: Response to simulated martian conditions and biosignature characterization
Betreuer: Dirk Wagner

Universität Mainz

Esther Gasser: Mikrobielle Synthese von Biopolymeren aus der nachwachsenden Rohstoffquelle Weizenstroh
Betreuer: Helmut König

Christian Monzel: Signaltransduktion in der membranständigen Sensor-Histidinkinase DcuS von *Escherichia coli*
Betreuer: Gottfried Unden

Anna Petri: Entwicklung und Evaluierung von Methoden zur zeitnahen Identifizierung weinrelevanter Mikroorganismen
Betreuer: Helmut König

Philipp Steinmetz: Signalerkennung und Signaltransduktion im DctA/DcuS-Sensor-Regulatorkomplex von *Escherichia coli*
Betreuer: Gottfried Unden

Universität Marburg

Lisa Lena Carlotta Debnar-Daumler: Aldehyd-oxidierende Enzyme im anaeroben Phenylalanin-Stoffwechsel von *Aromatoleum aromaticum*
Betreuer: Johann Heider

Huan Li: (*R*)-Indolelactyl-CoA dehydratase, the key enzyme of tryptophan reduction to indolepropionate in *Clostridium sporogenes*
Betreuer: Wolfgang Buckel

Nilanjan Pal Chowdhury: On the mechanism of electron bifurcation by electron transferring flavoprotein and butyryl-CoA dehydrogenase
Betreuer: Wolfgang Buckel

Binbin He: Study of a sociable molecule: Mapping the binding interfaces of the cell division regulator MipZ in *Caulobacter crescentus*
Betreuer: Martin Thanbichler

Max-Planck-Institut für terrestrische Mikrobiologie, Marburg

Yi Ding: Host-related nitrogen availability affects the lifestyle of *Piriformospora indica*
Betreuer: Alga Zuccaro

Carina Holkenbrink: The analysis of programmed cell death and sporulation in *Myxococcus xanthus* developmental program
Betreuerin: Penelope Higgs

Edina Hot: The small G-protein MglA connects the motility machinery to the bacterial actin cytoskeleton
Betreuerin: Lotte Søgaard-Andersen

Beata Jakobczak: Identification and characterization of a novel cell-envelope subcomplex crucial for A-motility in *M. Xanthus*
Betreuerin: Lotte Søgaard-Andersen

Urs Lahrmann: Genomics and Transcriptomics of the sebacinoid fungi *Piriformospora indica & Sebacina vermifera*
Betreuer: Alga Zuccaro

Kristina Lang: Diversity, ultrastructure, and comparative genomics of *Methanoplasmatales*, the seventh order of methanogens
Betreuer: Andreas Brune

André Nicolai Müller: Funktionelle Charakterisierung des *Ustilago maydis* Effektorproteins Pit2
Betreuer: Gunther Doehlemann

Victoria Desiree Paul: Identifizierung und Charakterisierung neuer Faktoren der zytosolischen Fe-S Proteinbiogenese
Betreuer: Roland Lill

Nicole Rietzschel: Rolle des mitochondrialen Carriers Rim2 und des Transkriptionsfaktors Yap5 im Eisenmetabolismus von *Saccharomyces cerevisiae*
Betreuer: Roland Lill

Katja Siewering: One ring to rule them all: Identification and characterization of the type IV pili secretin associated protein TsaP and analysis of the type IV secretion system of *Neisseria gonorrhoeae*
Betreuer: Chris van der Does

Carl-Eric Wegner: Metatranscriptomic analyses of methanogenic plant polymer breakdown in paddy soil
Betreuer: Werner Liesack

Tomasz Neiner: Deciphering the subunit interactions in the crenarchaeal archaellum
Betreuerin: Sonja-Verena Albers

Technische Universität München

Richard Landstorfer: Comparative transciptomics and translatomics to identify novel overlapping genes, active hypothetical genes, and ncRNAs in *Escherichia coli* 0157:H7 EDL933
Betreuer: Siegfried Scherer

Daniela Kaspar: Molekulare Effekte von Nitrit auf *Listeria monocytogenes* und die temperaturabhängige Regulation des Stickstoffmetabolismus in *Listeria monocytogenes*
Betreuer: Siegfried Scherer

Jan Kabisch: Mikrobiologische Sicherheit von Rohwurstprodukten, Wirkung von Natriumnitrit auf Lebensmittelinfektionserreger
Betreuer: Siegfried Scherer

Gernot Rieser: Biodiversität von Mikroorganismen im Produktionsumfeld pharmazeutischer Betriebe
Betreuer: Siegfried Scherer

Jie Luo: Detection, identification, and quantification of aflatoxin producing fungi in food raw materials using loop-mediated isothermal amplification (LAMP) assays
Betreuer: Rudi F. Vogel

Frank Jakob: Novel fructans from acetic acid bacteria
Betreuer: Rudi F. Vogel

Ekaterina Minenko: Einfluss des FgHyd5 Proteins auf Morphologie, Wachstum und Virulenz von *Fusarium graminearum* Schwabe
Betreuer: Rudi F. Vogel

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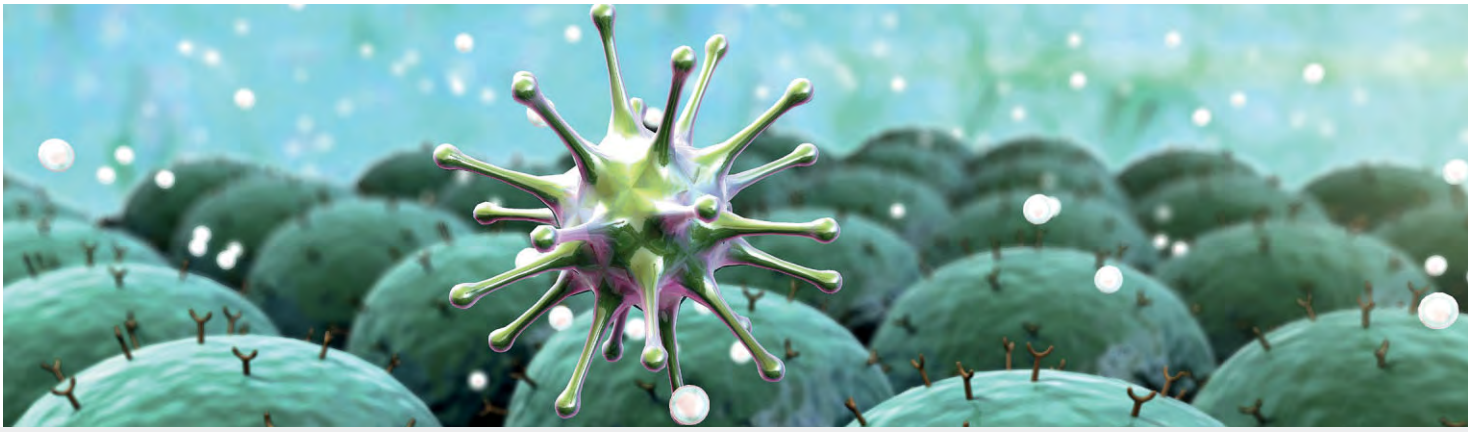

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