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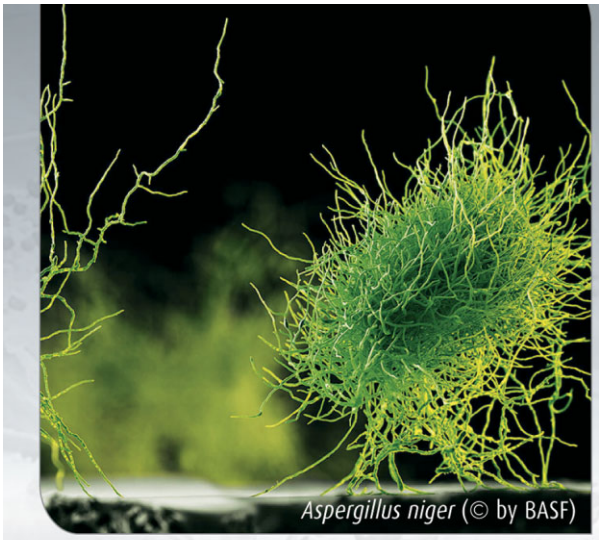
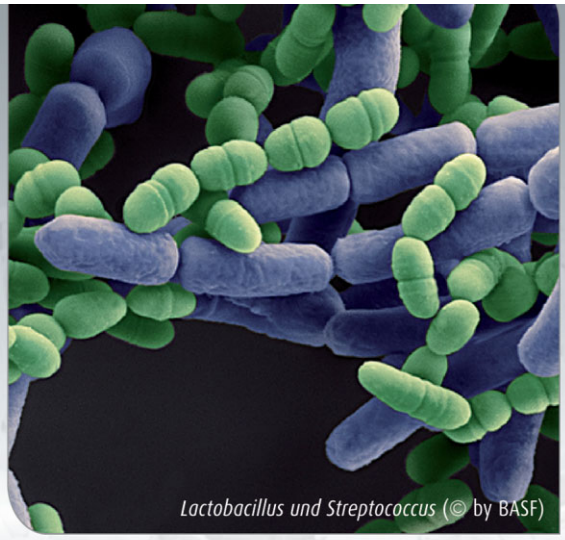

Aspergillus niger (© by BASF)


Lactobacillus und Streptococcus (© by BASF)

2011
Sonderausgabe

Tagungsband zur

VAAM-Jahrestagung 2011

Karlsruhe, 3.–6. April 2011

Spektrum Akademischer Verlag

VAAM

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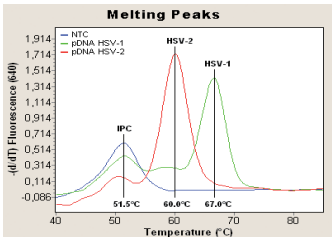
40-0282-16	Roseolovirus	HHV-6A/B	HHV-6
40-0198-16	Kaposi's sarcoma	HHV-8	HHV-8



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Vereinigung für Allgemeine und Angewandte Mikrobiologie (VAAM)

Tagungsband zur VAAM-Jahrestagung 2011

3. bis 6. April in Karlsruhe

Conference President: Reinhard Fischer

Scientific Committee: *Karlsruhe Institute of Technology (Campus South):* Reinhard Fischer, Natalia Requena, Jörg Kämper, Christoph Syldatk, Tilman Lamparter, Josef Winter, Clemens Posten; *Karlsruhe Institute of Technology (Campus North):* Ursula Obst; *Max Rubner Institute:* Rolf Geisen; *BASF SE:* Claus Bollschweiler, Marvin Karos, Oskar Zelder; *nadicom GmbH*: Bernhard Nüßlein

Zum Titelbild:

Der filamentöse Pilz *Aspergillus niger* wird zur Phytaseproduktion eingesetzt. Das linke Bild zeigt eine Myzelflocke. Das rechte Bild zeigt *Lactobacillus paracasei* und *Streptococcus mutans*. Das probiotische Bakterium soll zum Kariesschutz eingesetzt werden (pro-t-action™). *L. paracasei* verklumpt mit *S. mutans* so dass die Bakterien leicht aus dem Mund gespült werden können.

Das Karlsruher Schloss (unten) ist das Zentrum des Karlsruher Fächers. Karlsruhe wurde am 17. Juni 1715 von Markgraf Karl Wilhelm von Baden-Durlach an der Stelle gegründet, an der er der Sage nach bei der Jagd geschlafen und von seiner neuen Stadt geträumt hatte, daher der Name - Karlsruhe. Das Zentrum bildet das klassizistische Residenzschloss, zu dem strahlenförmig 32 Straßen führen.

Welcome address of the President of the Karlsruhe Institute of Technology (KIT)

On behalf of the Karlsruhe Institute of Technology I am delighted to welcome you to the Annual Conference of the Association for General and Applied Microbiology – the VAAM 2011.

In the last five years the appearance of Karlsruhe in the international educational landscape has changed dramatically. After three years of preparation, on October 1st 2009, the state Technical University Karlsruhe, Fridericiana, and the federal Research Center Karlsruhe, became one legal unity. This newly created institution is a complete novelty in the German science system where state and federation have very different sovereign rights. Two strong and equal partners have merged by creating an organization which has the potential to influence and change the research community profoundly. With about 8.700 administrative employees and about 384 Professors KIT is now one of the largest research and education centers in Europe and it is well equipped to tackle all kinds of scientific problems.

KIT research is primarily based on the capacities and knowledge of the scientists. In 157 institutes – thereof 27 Helmholtz-institutes – our scientists work in an excellent and worldwide unique scientific infrastructure. This gives us the opportunity to cooperate on completely new levels with other research centers, universities and industry partners. At KIT research, teaching and innovation are linked closely. We see our strength in the connection between people, infrastructure and know-how.

The work of every single scientist at KIT contributes to the KIT idea – to create new possibilities for research; to cooperate on new levels with scientists all over the world; to bring the cream of the crop together for outstanding research goals. Therefore we are honored to welcome every single participant of the VAAM 2011 at Karlsruhe. This annual conference provides insights into the latest developments of General and Applied Microbiology and gives the chance for the international research community to amplify their knowledge. In this spirit we wish you interesting and fruitful discussions. Enjoy your stay!

Prof. Dr. Horst Hippler
President of the Karlsruhe Institute of Technology

Greeting of the President of the VAAM to the Annual Meeting 2011

Dear Microbiologists,

the VAAM is committed to promote both fundamental and applied microbiological research and, as a result, has established considerable contacts with companies. This connection is mirrored by the annual meeting, this year to be held in Karlsruhe and organised by scientists of both the Karlsruher Institute of Technology and the BASF SE. This inter-disciplinary environment is also emphasised by the research areas covered this year. These topics include cell biology, environmental microbiology, food microbiology, microbial interactions, stress response and white biotechnology. This very interesting program reflects our success in attracting both national and international scientists. Because the VAAM now has more than 3300 members, we expect more than 1000 participants to attend the annual meeting in Karlsruhe. World-wide leading scientists have been recruited to present their recent data in plenary talks. These plenary sessions will also include overviews of the current state of the art in various aspects of microbiology. As president of the VAAM, I am particularly glad that the meeting attracts so many junior members, who will be offered the possibility to present their work.

In addition, the meeting will host the traditional career symposia, to highlight career paths and encourage professional development.

I would also like to invite you to the general assembly of VAAM members, where we will elect a new executive board and new honorary members will be proposed and elected. Here, the presentation of awards and PhD lectures will take place.

On behalf of the VAAM, I would like to thank the president of the meeting, Reinhard Fischer, all the members of the organizing committee of the Karlsruher Institute of Technology, the Max Rubner Institute, the nadicom GmbH and the BASF SE, as well as the many assistants for their great effort in preparing this interesting meeting. I am really pleased about the offer of child care during the program, that will allow parents with young children to attend the VAAM meeting. I would also like to express my gratitude to all participants, whose scientific contributions are essential for the success of such a meeting. I am sure that the meeting in Karlsruhe will be of the highest scientific quality and will also give the opportunity for enjoyable scientific exchange and interactions.

Prof. Dr. Axel Brakhage
President of the VAAM

Die VAAM dankt den Sponsoren der Promotionspreise

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Welcome address of the organizing committee

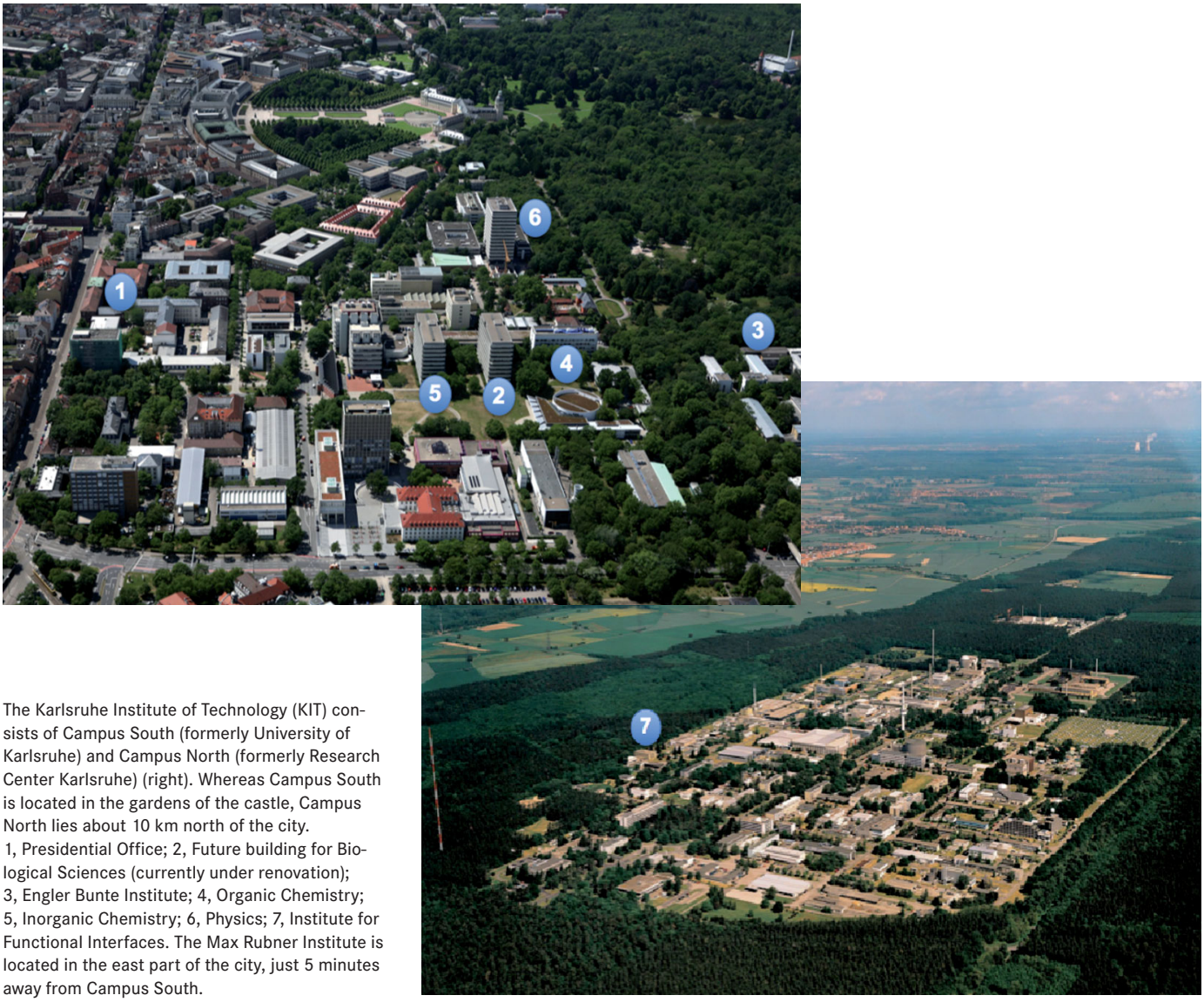
It is a great pleasure for us to welcome you to the annual meeting of the VAAM at the Karlsruhe Institute of Technology (KIT). More than 900 abstracts from all fields of microbiology have been submitted and promise a very interesting and fruitful meeting. The topics of the main sessions reflect the broad microbiological research interests in Germany and are represented by leading national and international scientists of the field of microbiology. In addition to the plenary sessions, a large number of concurrent sessions have been set up, where primarily young scientists will be encouraged to highlight their results. As a scientific society, we continuously rely on excellent students who are fascinated by the microbial world, and we do hope that this meeting in Karlsruhe will help to stimulate their enthusiasm.

The entire meeting will be held without distraction in the same building, at the Stadthalle, which will allow direct interaction with all participants during the breaks between sessions. Posters will be located in the break area, and thus will attract the most attention of the participants.

The location of the Stadthalle, in the center of Karlsruhe, is within walking distance of the castle, KIT's South Campus, the city's pedestrian area and the large and modern shopping center Ettlinger Tor, which offers the opportunity to experience the city beyond science. If you have one or two extra days of time, you can travel around Karlsruhe: within a few minutes you can cross the Rhine and be in France; or Strasbourg and the beautiful wine villages in Alsace or Pfalz are only one hour away.

We will do our best to organize a most interesting and enjoyable meeting in Karlsruhe!

Reinhard Fischer, Jörg Kämper, Tilman Lamparter, Clemens Posten, Natalia Requena, Christoph Syldatk, Josef Winter, Ursula Obst, Rolf Geisen, Claus Bollschweiler, Marvin Karos, Oskar Zelder, Bernhard Nüßlein



The Karlsruhe Institute of Technology (KIT) consists of Campus South (formerly University of Karlsruhe) and Campus North (formerly Research Center Karlsruhe) (right). Whereas Campus South is located in the gardens of the castle, Campus North lies about 10 km north of the city.
1, Presidential Office; 2, Future building for Biological Sciences (currently under renovation); 3, Engler Bunte Institute; 4, Organic Chemistry; 5, Inorganic Chemistry; 6, Physics; 7, Institute for Functional Interfaces. The Max Rubner Institute is located in the east part of the city, just 5 minutes away from Campus South.

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General Information
Annual Conference 2011 of the VAAM

Venue

Stadthalle Karlsruhe
Festplatz 9
76137 Karlsruhe
Germany

Address for correspondence

Conventus Congressmanagement & Marketing GmbH
Isabelle Lärz/Martin Singer
Carl-Pulfrich-Straße 1
07745 Jena, Germany
Phone +49 (0)3641 311 63 -20/-10
Fax +49 (0)3641 311 62 41
www.vaam2011.de
vaam2011@conventus.de

Opening hours

Sunday	03.04.2011	14:30 – 19:30
Monday	04.04.2011	07:30 – 19:00
Tuesday	05.04.2011	07:30 – 19:00
Wednesday	06.04.2011	08:00 – 12:00

Travelling to Karlsruhe

By train

Karlsruhe is a hub for ICE, InterCity, EuroCity and InterRegio connections and is located directly on the ICE route from Hamburg to Basel through Frankfurt. The west-east route, from Karlsruhe to Munich through Stuttgart, begins here. It takes about 3 hours with a TGV train from Paris to arrive in Karlsruhe.

The congress centre is in walking distance (10 min) from the main station.

By public transport in Karlsruhe

You can take tram S1, S4, S11, and 2 from the main station to the tram stop "Kongresszentrum." From the city centre, take any of the same trams to the tram stop "Kongresszentrum" or tram 5 to the tram stop "Konzerthaus".

From motorway A5/A8

From the A8 from Stuttgart continue onto the A5 toward Frankfurt. Take exit 45 "Karlsruhe-Mitte" and continue on the B10. Follow the sign-posting to Karlsruhe. From the B10, take exit 2 towards the city centre and follow the signs to the congress centre (Kongresszentrum).

Address for navigation systems: Kongresszentrum, Festplatz 9, Karlsruhe.

From motorway A65

The A65 turns into the B10. Continue on the B10. Take exit 2 and continue towards the congress centre (Kongresszentrum).

Address for navigation systems: Kongresszentrum, Festplatz 9, Karlsruhe.

Car park

There is space for approximately 1,000 vehicles in the underground parking at the congress centre.

Address for navigation systems: Park House 1 – Hermann-Billing-Straße 1, Karlsruhe; Park House 2 – Beiertheimer Allee 9, Karlsruhe.

Hotel reservation

We have reserved a contingent of rooms at special rates in Karlsruhe. Please find the reservation fax on our conference homepage www.vaam2011.de.
Hotel rooms may also be looked through: www.karlsruhe.de

Registration and conference fees

Online registration is possible till 28 March 2011 through the conference homepage www.vaam2011.de. Registrations after this date are possible only on-site. Beside cash payments we also accept credit cards at the conference reception desk (Master/ Euro, VISA, American Express and JBC) as well as EC-Cards.

Mixer

The Mixer will take place on Tuesday, 05 April 2011 at 19:30 at the "Weinbrenner-Saal" at the Main Floor in the Stadthalle Karlsruhe. Accompanying persons may purchase a ticket for the mixer at the conference reception desk.

Posters

Posters are to be presented in English and in the format DIN A0 (84.1 cm × 118.9 cm, unlaminated). Authors are asked to attach to the posters the time when they will be available for discussion. The posters will have to be fixed by pins. Materials will be provided.

The posters may be hung from 14:00 on Sunday, 03.04.2011 and should be removed before 12:00 on Wednesday, 06.04.2011.

The poster sessions will be held on:

- Monday, 04.04.2011, 15:15 – 17:30
- Tuesday, 05.04.2011, 15:30 – 17:30

Registration fees (all days)

VAAM-Members	
Regular	170 €
Student*	85 €
Non-members	
Regular	240 €
Student*	110 €

Fee for day tickets (Monday, Tuesday, Wednesday)

Member	90 €
Non-member	115 €
Student member	40 €
Student non-member	70 €

* Please provide confirmation and quote VAAM 2011 as the reference.

Social programme

Welcome reception** (03 April 2011)	included
Mixer** (05 April)	included

** Registration required.

Presentation of the Honory Award, PhD Awards, and Poster Prizes

The presentation of the Honory Award will take place on 04.04.2011 at 11:00.

The presentation of the PhD Thesis prizes will take place on 05.04.2011 at 18:30.

The presentation of the Poster Prizes will take place on 06.04.2011 at 11:30.

All awards will be presented in Brahms-Saal, Stadthalle.

Short lectures

The length of short lectures has been fixed at 10 minutes plus 5 minutes for discussion. Due to the fact that there will be up to 7–8 parallel sessions please adhere to the total time of 15 minutes per presentation.

Short lectures are to be held in English. Data projectors are available in each of the lecture halls. In each lecture hall there will be an assistant for technical support. We ask all lectures to make use of the computer facilities located at our **media check-in** to check their presentations in advance.

Please submit your presentation in at least 120 minutes before your lecture will start. You are asked to clearly label your CD/memory stick and the file with your short lecture code number and the name of the person giving the talk. All presentations will be loaded onto our computers and will be deleted after the talks.

General Tips for Authors and Presenters

Presentation Submission

Follow the signs or ask at the check-in desk how to find the media check-in.

Time Allotment

To ensure smooth running of the entire programme, all speakers are encouraged to adhere to their allocated speaking time. The chair persons of the sessions are urged to cancel discussions in delay. Contact your chair person before your session begins and advise of any changes or special wishes.

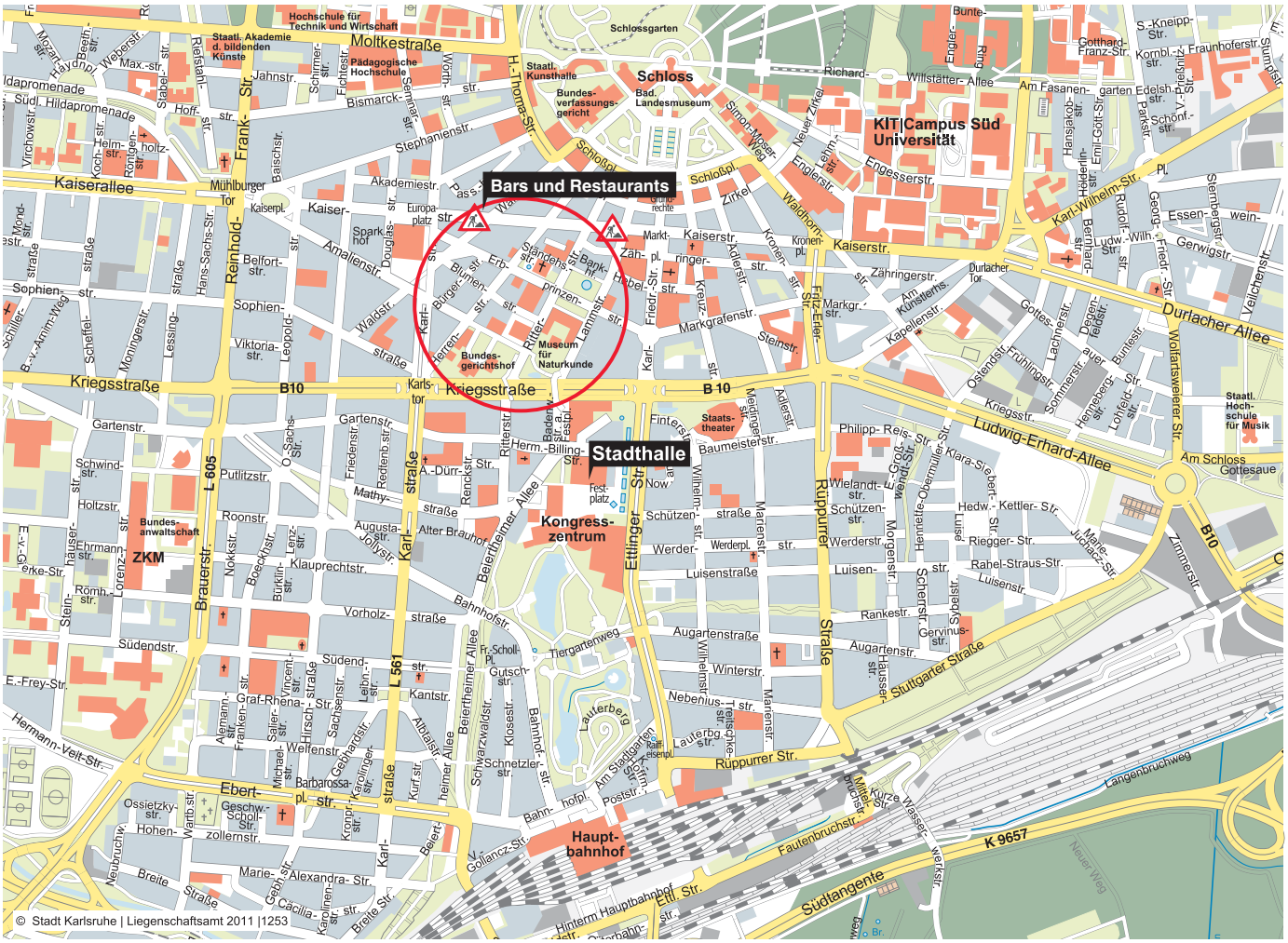
Presentation Form and Submission of Presentation

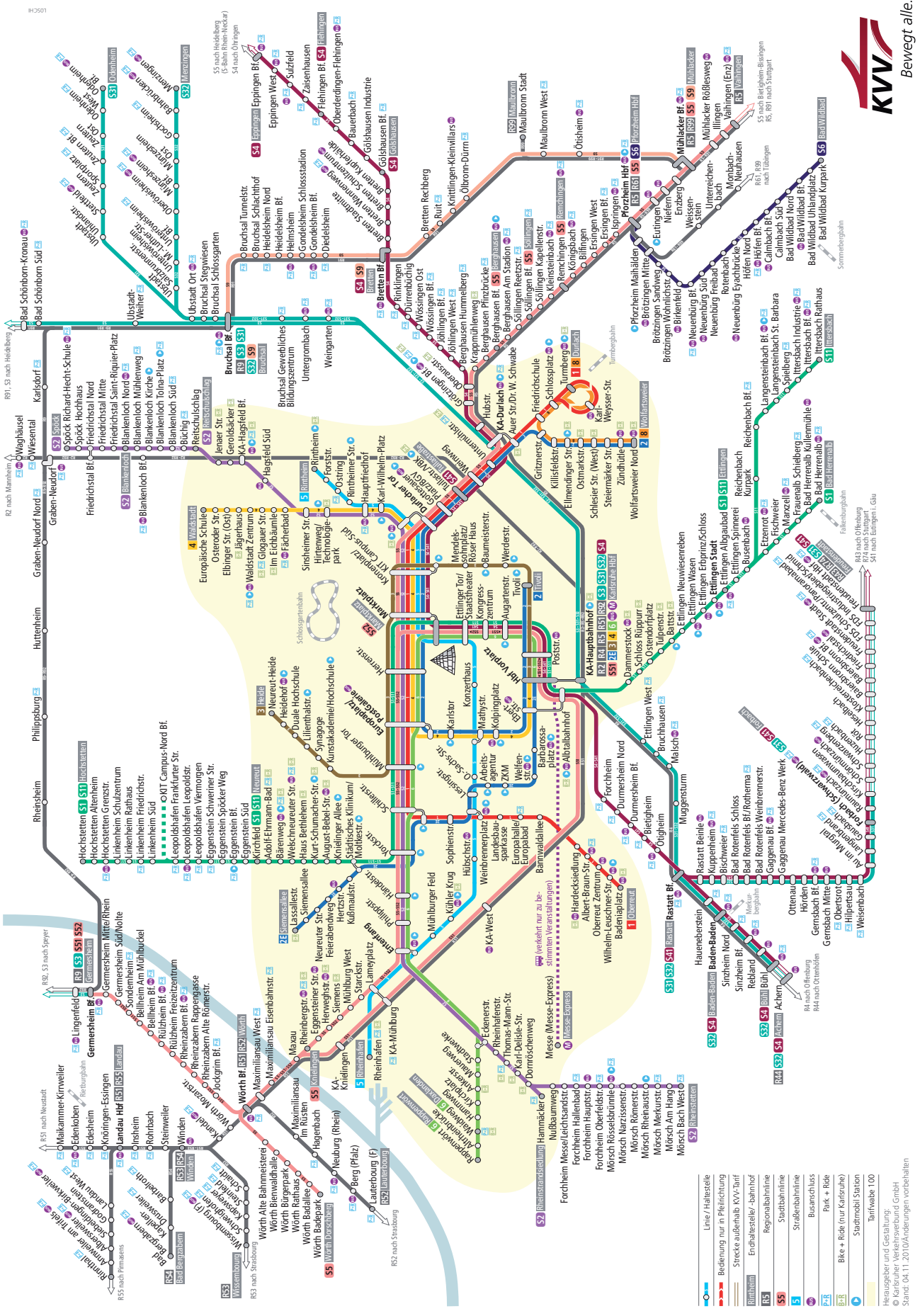
PDF and PowerPoint presentations are permitted. Open Office formats may also be used. Required technical equipment will be available at the congress. Please make sure that any required CODEC files for any videos are also submitted.

The use of Macintosh or Open Office formats as well as the use of a personal laptop for a presentation is not planned, but possible. If necessary, please contact us by 23 March 2011 at vaam2011@conventus.de.

For video and audio files please submit AVI, WMV and MPG files only as a separate file.

Please note: If you use a USB stick to save your files, do not protect it with software.

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Evonik Degussa GmbH (Hanau/Wolfgang)
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Federation of European Microbiological Societies (FEMS)
www.fems-microbiology.org

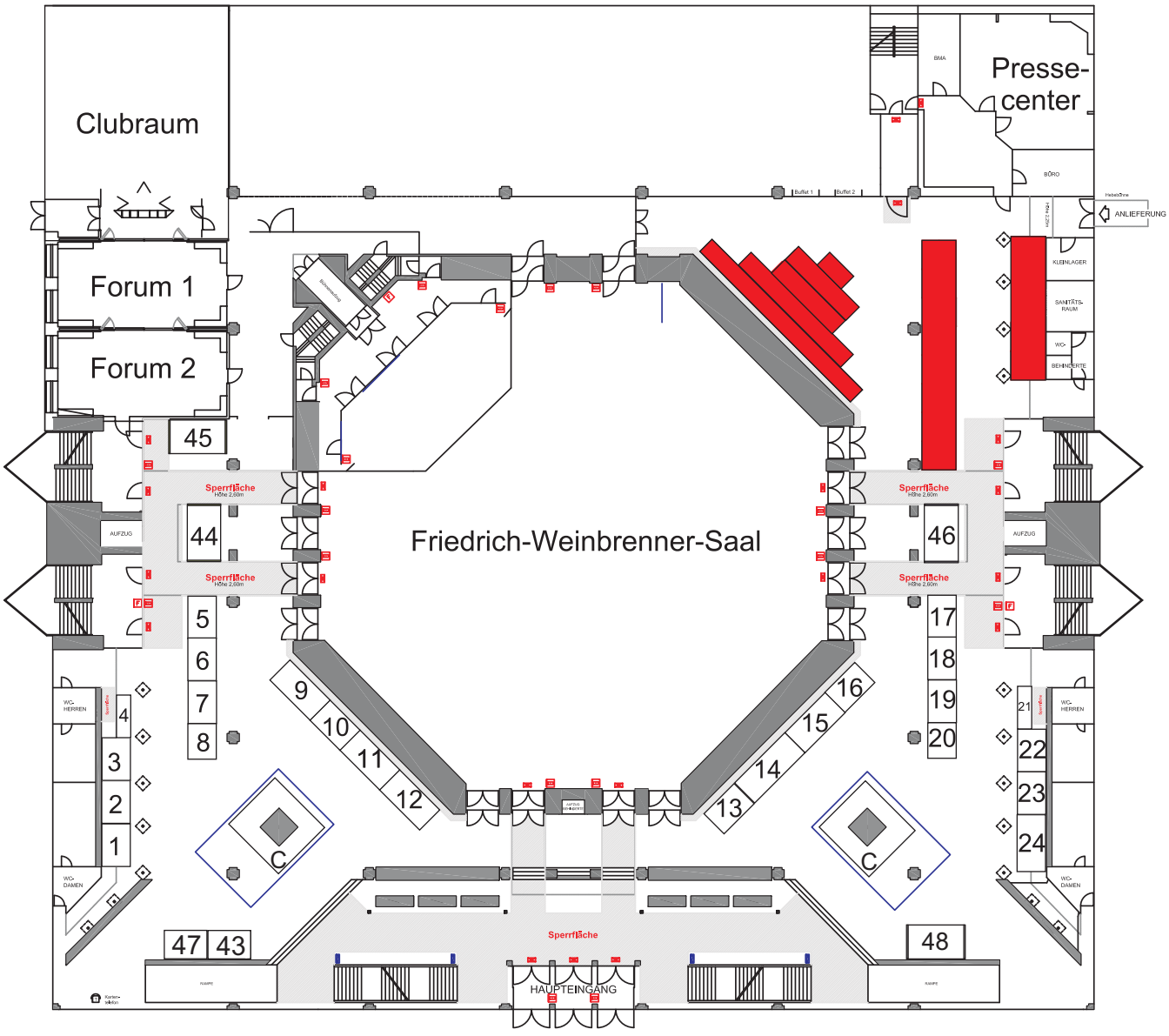
Exhibitors

alphabetically

by booth number

Ausstellungsplan
Ebene 0/Erdgeschoss

Jahrestagung der Vereinigung für Allgemeine und Angewandte Mikrobiologie
03.–06. April 2011

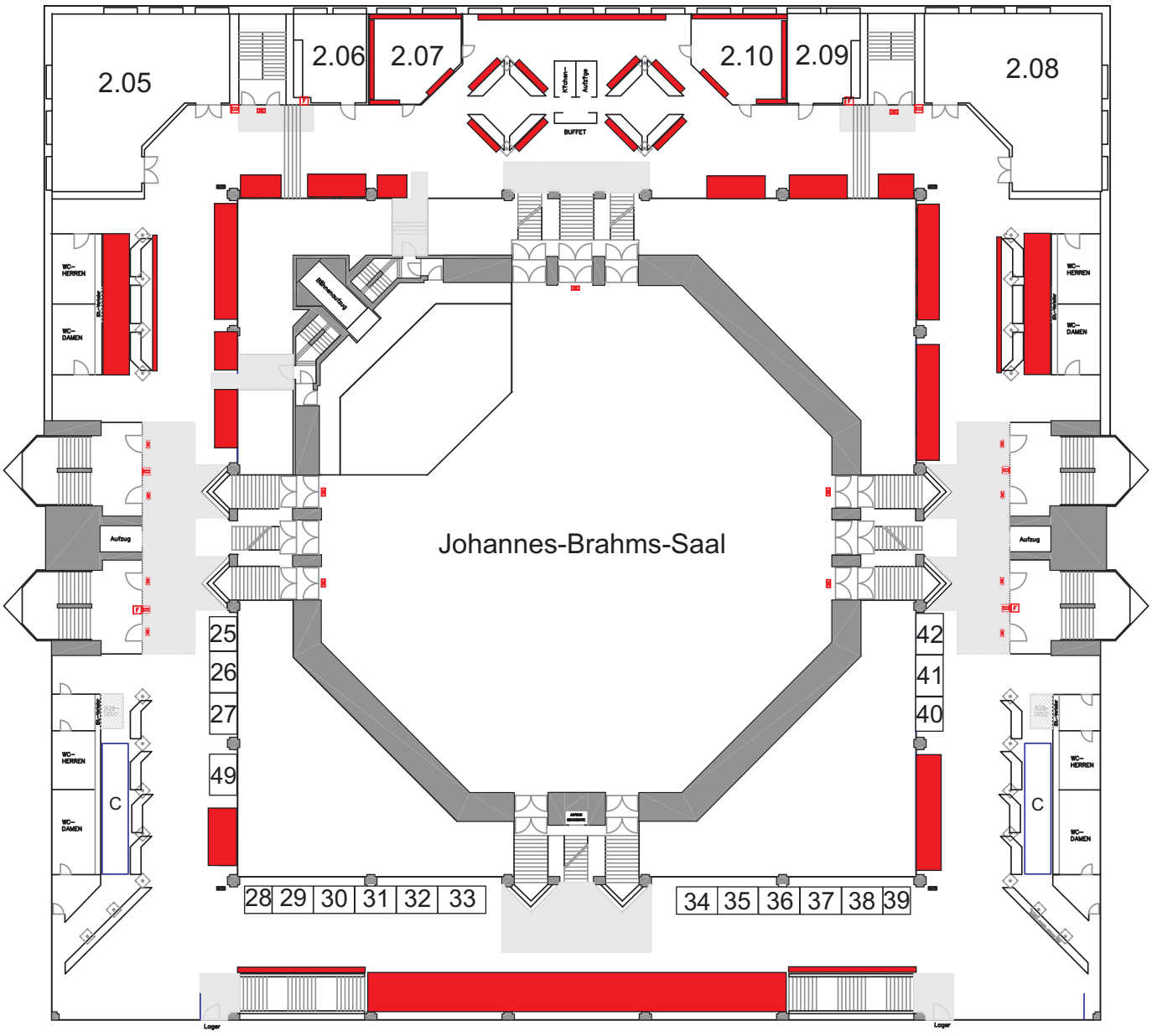


C = Catering

= Poster

Ausstellungsplan
Ebene 2/Obergeschoss

Jahrestagung der Vereinigung für Allgemeine und Angewandte Mikrobiologie
03.–06. April 2011



C = Catering

= Poster

Einladung zur Mitgliederversammlung der VAAM

■ Hiermit lade ich alle Mitglieder der VAAM zur Mitgliederversammlung ein. Sie wird am **Dienstag, den 5. April 2011, um 17.30 Uhr** in der Stadthalle in Karlsruhe stattfinden.

Vorläufige Tagesordnung:

1. Festlegung der Tagesordnung und Genehmigung der Niederschrift der Mitgliederversammlung vom 30. März 2010 in Hannover (siehe BIOspektrum 4/10, Seiten 460 und 461)
2. Bericht aus dem Vorstand, u.a. Haushalt 2010 und Haushaltsplan 2011, Ort und Zeit der nächsten Jahrestagung, Aktivitäten der Fachgruppen, VBIO
3. Neustrukturierung der Mitgliedsbeiträge
4. Bericht der Kassenprüfer
5. Entlastung des Vorstandes
6. Wahl des Präsidiums (Präsident, 1. Vizepräsident, Schatzmeister, Schriftführer) und des Beirats (geheime Wahl während der Mitgliederversammlung)
7. Ehrenmitgliederwahl
8. Verschiedenes

Im Anschluss findet die **Verleihung der VAAM-Promotionspreise 2011** statt.

Hiermit bitte ich alle Mitglieder, Vorschläge zur Wahl des Präsidiums und des Beirats 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 am **Dienstag, den 5. April 2011, von 15.00 – 17.00 Uhr** sowie am **Mittwoch, den 6. April 2011, von 10.00 – 12.00 Uhr** im Tagungsbüro abgeholt werden. ■

Hubert Bahl
Schriftführer

Symposium by Eurofins MWG Operon (Ebersberg)

Tuesday, 5 April 2011, 16:00–17:30, Clubroom

Latest developments in NGS Sequencing

■ Eurofins MWG Operon, a global genomics service provider with the longest experience for Roche/454 sequencing technology in the market has updated his sequencing machine park and is offering now sequencing with Roche GS Junior and enhanced TAT service.

Built around meanwhile 3 Roche sequencing machines Eurofins MWG Operon offers a broad portfolio for de novo sequencing of viral, bacterial and fungal genomes, as well as bioinformatics analysis services like SNP identification, strain comparison and annotation of genomes.

Sequencing experience with bar-coded samples allows multiplexing of different samples like BAC clones, phages or small genomes, but also multiplexing of different types of libraries in one lane, like shotgun and long paired-end libraries.

As transcriptome analysis and expression profiling by next generation sequencing becomes more and more important, the construction and sequencing of cDNA libraries for the identification of full length mRNAs and expression profiling is an important part of the portfolio. Meanwhile the protocols for normalised cDNA and small RNA libraries, originally developed for GS FLX sequencing, are now also available for Illumina HiSeq 2000 technology.

Illunima HiSeq 2000 services have become part of our broad product portfolio. Qualitative and quantitative expression profiling is offered with specially designed 3'-fragment cDNA libraries, but also with standard mRNA sequencing protocols. Non-coding cDNA libraries with longer than the standard 29 bp allow the analysis of non coding RNAs that are not detectable with alternative sequencing technologies.

As a certified NimbleGen service provider for targeted enrichment and sequencing, the sequencing of any genomic region of choice, as well as the proprietary complete exome sequencing is offered. In a exclusive worldwide strategic partnership with Genomatix Software GmbH high quality data mapping and data mining of target enriched sample sequencing and data analysis is available.

A selected number of examples will be presented during the seminar. ■

Karrieresymposium

Montag, 4. 4. 2011
15.45 – 17.15 Uhr
Clubraum

Ulrike Gerischer, Göttingen
Wissenschaftskoordinatorin am MPI für biophysikalische Chemie
"Akademische Laufbahn: Plan B"

Anja Störiko, Hofheim
Wissenschaftsjournalistin
Formulieren statt Forschen
"Von den Biowissenschaften in den Journalismus"

Martin Langer, Ute Dechert, Zwingenberg
B.R.A.I.N
"Wachstum mit Weißer Biotechnologie:
Karrieren und Chancen bei BRAIN"

EINLADUNG/INVITATION

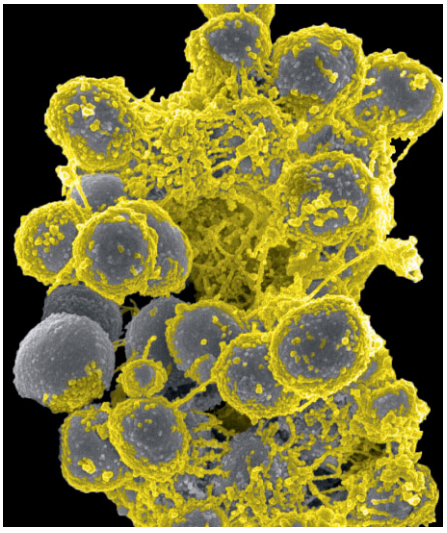
Jahrestagung der Vereinigung für Allgemeine und Angewandte Mikrobiologie

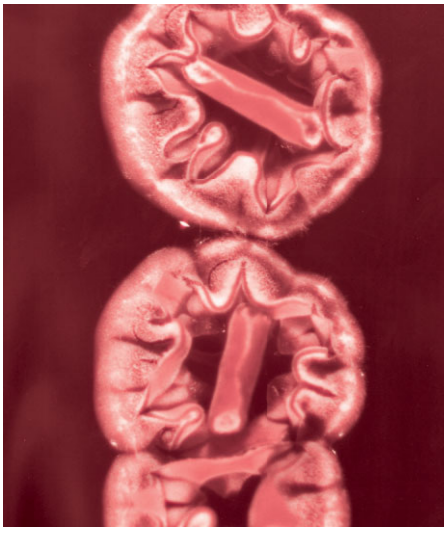
Annual Conference of the Association for General and Applied Microbiology (VAAM)

18–21 MARCH 2012 • TÜBINGEN

Topics

- Bacterial Differentiation
- Cell Envelope
- Human Microbiota
- Metabolic Regulation and Signalling
- Microbial Pathogenicity
- Microbial Survival Strategies
- Secondary Metabolites
- Soil Microbiology





www.vaam2012.de

EBERHARD KARLS
UNIVERSITÄT
TÜBINGEN

Fachgruppe: Fungal Biology and Biotechnology/ Experimentelle Mykologie

Es ist das Ziel unserer Fachgruppe, jungen Nachwuchswissenschaftlern, d.h. Doktoranden, Post-Docs und Habilitanden, zweimal im Jahr ein Forum zur Diskussion eigener Ergebnisse zu bieten.

Im Jahr 2010 wurde dieses Ziel mit dem Herbst-Symposium "Biotransformation by Fungal Cells or Fungal Enzymes" in Senftenberg und mit dem Minisymposium "Fungi in the Environment" im Rahmen der Frühjahrstagung in Hannover erreicht.

Zur Frühjahrstagung in Karlsruhe findet das Fachgruppensymposium "Fungal Development and Pathogenicity Mechanisms" statt. Der frisch habilitierte Nachwuchswissenschaftler Matthias Brock (Jena) wird es zusammen mit Stefanie Pöggeler (Göttingen) am Montag, den 4. April 2011, leiten. Als Gastsprecher wurde Dr. Pieter van West (Aberdeen) gewonnen, der einen Vortrag über Oomyceten und ihre Pathogenität halten wird. Anschließend werden Kurzvorträge von Doktoranden stattfinden, die anhand der eingereichten Abstracts ausgewählt wurden.

Im Anschluss an das Minisymposium wird die Fachgruppensitzung stattfinden. Auf der Tagesordnung steht die Wahl der Fachgruppensprecher. Zudem sollen Ideen für künftige Veranstaltungen diskutiert werden, insbesondere Themenvorschläge für ein Mini-Symposium zur VAAM-Frühjahrstagung 2012 in Tübingen.

Für den Herbst 2011 ist bereits die Traditionstagung "Molecular Biology of Fungi" in Planung. Vom 11.–14. September lädt dazu Michael Bölker nach Marburg ein. Informationen unter: birgit.niedziella@staff.uni-marburg.de

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Fachgruppe: Funktionelle Genomanalyse

Die Fachgruppe ‚Funktionelle Genomanalyse' wird bei der Jahrestagung in Karlsruhe ihr zwölftes Minisymposium abhalten, mit dem Thema "Standards for Large Scale (Meta-) Genomics and Metadata". Die Veranstaltung wird einen Einblick in die Arbeit des "Genomic Standards Consortium" bieten (FO Glöckner, MPI Bremen), sowie neue Wege der Publikation und Verbreitung standardisierter Genominformation aufzeigen (G. Garrity, Univ. Michigan, USA). Beispiele angewandter Metagenomik zur Charakterisierung von Candidatus-Stämmen aus Metagenomsequenzen (F. Meyer, Argonne National. Laboratory, USA) und kombinierter Genom-Proteom-Analyse (L. Wöhlbrand, ICBM, Oldenburg) runden das Programm ab.

Die Mitglieder der Fachgruppe sowie Interessenten an der künftigen Gestaltung der Fachgruppenveranstaltungen werden im Anschluss an das Symposium zur Mitgliederversammlung der Fachgruppe eingeladen, bei der die Wahl der Fachgruppenvertreter für die nächsten Jahre stattfinden wird.

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Fachgruppe: Hefen

Hefen haben eine große Bedeutung in der Biotechnologie, und sie sind als Eukaryoten wichtige Modellorganismen in der Zellbiologie. Darüber hinaus nimmt die Bedeutung einiger Hefen als human- und pflanzenpathogene Infektionskeime stetig zu. Die Fachgruppe Hefe fasst die Mitglieder der VAAM zusammen, die mit Hefen als Mikroorganismus an diesen Fragestellungen arbeiten und umfasst zurzeit 69 Mitglieder. Bei der letzten VAAM-Tagung in Hannover wurde gemeinsam mit Fachgruppe Eukaryotische Krankheitserreger der DGHM ein Symposium mit dem Thema "Infectious agents and model organisms in medical research" durchgeführt. Des Weiteren haben Mitglieder der Fachgruppe Hefen das Jubiläumssymposium zum 10-jährigen Bestehen des französischen Hefekonsortiums Genolevure (10.11.2011) mit organisiert.

In 2011 soll im Herbst eine gesonderte Fachgruppentagung stattfinden. Für 2013 wird unter Mitwirkung der Fachgruppe Hefe erstmalig seit 1976 die internationale Hefekonferenz wieder in Deutschland (Frankfurt) stattfinden.

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Fachgruppe: Mikrobielle Pathogenität (gemeinsam mit der DGHM – Deutschen Gesellschaft für Hygiene und Mikrobiologie)

■ Die Fachgruppe "Mikrobielle Pathogenität" zählt zu den ältesten Fachgruppen der VAAM und ist ebenfalls die älteste und mit 350 Mitgliedern auch die größte Fachgruppe der DGHM. Molekulare Mechanismen der Erregervirulenz stellen den wissenschaftlichen Schwerpunkt dar. Neben klassischen Themen der mikrobiellen Pathogenität (Toxinfunktionen, Adhäsion, Regulation von Virulenzgenen) ist die zelluläre Mikrobiologie eine bedeutende Forschungsrichtung; Fragen zur Wechselwirkung zwischen mikrobiellen Erregern und eukaryotischen Wirtszellen stehen dabei im Mittelpunkt.

Wie auch in den Jahren zuvor hat die Fachgruppe aktiv zentrale Veranstaltungen zu Fragen mikrobieller Pathogenität mitgestaltet. So war die Fachgruppe im März des Jahres 2010 wesentlich an der erfolgreichen Organisation und Durchführung der gemeinsamen Jahrestagung von DGHM und VAAM beteiligt. Gemeinsam wurden drei Sessions zu wichtigen aktuellen Themen wie "Pathogen-induced host cell signalling", "The cell envelope in bacterial infections" und "Bacterial metabolism and infection" ausgerichtet. Daneben fanden gemeinsame Sessions mit der Fachgruppe "Gastrointestinale Infektionen" der DGHM ("Microbial pathogenesis and gastrointestinal infections") und der Fachgruppe "Regulation und Signaltransduktion in Prokaryoten" der VAAM ("Cell-cell communication") statt. Zudem wurde aus der Fachgruppe heraus eine Vielzahl qualitativ hochwertiger Poster präsentiert. Zukunftsweisend war die gemeinsame Fachgruppensitzung der zuvor nur lose interagierenden Fachgruppen "Mikrobielle Pathogenität" der DGHM und VAAM. Hier wurden die großen Gemeinsamkeiten dieser beiden Fachgruppen herausgearbeitet und der Wille bekräftigt, sich zukünftig als gemeinsame Fachgruppe zu verstehen und für ein engeres Zusammengehen der beiden Fachgesellschaften einzutreten.

Ein wichtiges sichtbares Zeichen für diese enge Zusammenarbeit war die Ausrichtung der alle zwei Jahre stattfindenden Fachgruppentagung in Bad Urach vom 21.6. bis 23.6.2010. Die Tagung bot bereits zum fünften Mal gerade jungen Nachwuchswissenschaftlern aus Medizin und Naturwissenschaften die Möglichkeit, ihre Ergebnisse in entspannter und diskursiver Atmosphäre zu präsentieren und zu diskutieren. Als Zeichen der besonderen Bedeutung der Förderung junger Wissenschaftler wurde im Rahmen der Tagung zum zweiten Mal der "Sanofi-Aventis Förderpreis" verliehen. Preisträger sind Dipl. Biol. Inga Jensch, Greifswald und Dipl. Biol. Marc Burian, Tübingen. Die Fortsetzung der Veranstaltungsreihe 2012 ist in Planung.

2011 wird sich die Fachgruppe schwerpunktmäßig mit der Organisation der DGHM-Jahrestagung in Essen und der VAAM-Jahrestagung in Karlsruhe beschäftigen. Im Rahmen dieser Veranstaltungen sollen auch weitere Möglichkeiten gemeinsamer Aktivitäten und Initiativen diskutiert werden. ■

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Sprecher DGHM:
Volker Kempf, Universität Frankfurt;
Sven Hammerschmidt, Universität Greifswald;

Fachgruppe: Qualitätssicherung und Diagnostik

■ Die Fachgruppe beschäftigt sich mit aktuellen Fragen und Entwicklungen aus dem Themenbereich Qualitätssicherung und Diagnostik. Zur mikrobiologischen Qualitätssicherung gehören beispielsweise Prüfungen von Rohstoffen und Medien (z. B. Wasser) für die Herstellung von pharmazeutischen Wirkstoffen oder die Überwachung von Reinräumen. Wenn es hier zu Grenzwert-Überschreitungen kommt, müssen aufgrund der mikrobiologischen Analyseergebnisse Lösungen für korrigierende und vorbeugende Maßnahmen gefunden werden. Erfahrungen dazu werden innerhalb der Fachgruppe diskutiert, und anhand derer können Lösungen erarbeitet werden.

Die Identifizierung (un)bekannter Kolonien auf einer Agarplatte in möglichst kurzer Zeit ist eine ständige Herausforderung nicht nur in der Industrie und daher ein Schwerpunkt innerhalb der Fachgruppe. Die Erfahrungen mit sich ständig weiterentwickelnden diagnostischen Systemen tauschen wir ebenfalls aus. Hier ergeben sich auch immer wieder Kontakte zur Forschung und Entwicklung sowie zu Unternehmen aus dieser Branche.

Mikrobiologen und andere Fachkollegen, die sich für derartige Fragestellungen interessieren, sind als neue Mitglieder in der Fachgruppe herzlich willkommen. Auch VAAM-Mitglieder, die sich für eine entsprechende Position in der Industrie interessieren oder eine solche gerade angetreten haben, sind eingeladen, an diesem Erfahrungsaustausch teilzuhaben.

Zur Jahrestagung der VAAM 2010 in Hannover fand eine Fachgruppensitzung gemeinsam mit der entsprechenden Fachgruppe der DGHM statt. Sechs Vorträge aus dem Themenbereich molekularer Diagnostik (PCR) und Fragen der Qualitätssicherung stießen auf ein breites Interesse. Insgesamt nahmen etwa 30 Teilnehmer an dieser Veranstaltung teil. Durch das gemeinsame und offene Diskutieren der Themen verlief dieses Treffen sehr erfolgreich, da sich zudem die Mitglieder der beiden Fachgruppen einander besser kennenlernen konnten.

Im November fand das jährliche und gut besuchte Treffen der VAAM-FG in Berlin statt. Hier diskutierten 25 Teilnehmer anhand von acht Vorträgen "*Probleme und Erfahrungen mit diagnostischen Systemen*", und die Teilnehmer knüpften untereinander neue Kontakte.

Im Rahmen dieses Treffens wurden auch Fachgruppensprecher und -stellvertreter neu gewählt. So wurden der langjährige Fachgruppenleiter Dr. Gerhart Heinz und sein Stellvertreter Dr. Michael Rieth verabschiedet und mit Prof. Dr. Steffen Prowe (Leitung) und Dr. Andreas Seiffert-Störiko (Vertreter) ein neues Sprecherteam gewählt.

Zukünftig will die Fachgruppe weiterhin mindestens einmal jährlich zusammenkommen. Hierzu sollen möglichst auch Wissenschaftler und Kollegen aus der Industrie angesprochen werden, die in thematisch ähnlich ausgerichteten anderen Fachgruppen aktiv sind. Dabei wird an eine Vertiefung der Kontakte zur DGHM und dem Curriculum der pharmazeutischen Mikrobiologen (CPM) als auch Kooperationen mit weiteren Fachverbänden gedacht. Zudem soll hierdurch u. a. die regelmäßige Kommunikation zwischen den Mitgliedern aktiviert werden. Das nächste Treffen findet im September 2011 in Frankfurt in den Räumen der Provadis-Hochschule statt. ■

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Fachgruppe: Regulation und Signaltransduktion in Prokaryoten

■ Im Rahmen der Fachgruppe fand im Oktober des vergangenen Jahres das 28. Symposium "Mechanisms of Gene Regulation" statt, das dankenswerter Weise Gottfried Unden, Universität Mainz, organisierte. Auch dieses Mal hatte dieses Symposium einen äußerst großen Zuspruch, was sich in fünf Plenarsprecher/innen und mehr als 100 teilnehmenden Doktoranden/innen und Postdoktoranden/innen widerspiegelte. Das nächste 29. Symposium wird 2012 in München unter der Leitung von Thorsten Mascher, München, organisiert werden.

Während der VAAM-Tagung 2011 in Karlsruhe findet ein interessantes Symposium zum Thema "Second Messengers" statt, eine Gemeinschaftsveranstaltung unserer Fachgruppe und der Fachgruppe "Mikrobielle Pathogenität". Second Messenger sind niedermolekulare Verbindungen, die der Signalübertragung innerhalb von Zellen dienen. Dabei beeinflussen Second Messengers eine Vielzahl von intrazellulären Signaltransduktionsprozessen, was gleichzeitig der Signalverstärkung und –integration dient. Zur Thematik der Second Messenger ist es uns gelungen, zwei in diesem Gebiet international führende Wissenschaftler zu Übersichtsvorträgen einzuladen: Urs Jenal, Biozentrum Basel, Schweiz, wird über c-di-AMP im Zusammenhang mit Beweglichkeit, Zellzyklus und Differenzierung sprechen. Andrew Camilli, Tufts University School of Medicine, Boston, USA, wird über die Bedeutung von Second Messengern in *Vibrio cholerae* berichten. Das Programm des Symposiums wird mit einem Kurzvortrag von Sven DeCausmacker, Universität Gießen, zum Thema c-di-GMP in der Phototaxis von *Synechocystis* komplettiert.

Im Anschluss an dieses Fachgruppensymposium findet die diesjährige Mitgliederversammlung unserer Fachgruppe statt, zu der ich Sie herzlich einlade. ■

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Fachgruppe: Struktur und Mikroskopie

■ Die Fachgruppe widmet sich Themen der mikrobiellen Strukturforschung und Methoden der Mikroskopie und bietet mit ihren Minisymposien Einblicke in aktuelle Entwicklungen. In diesem Jahr behandelt das Fachgruppen-Symposium *"Die Struktur des Cytoplasmas"*. Das mikrobielle Cytoplasma steht schon lange im Verdacht, geordneter zu sein, als es sich uns vermeintlich darbietet. Die Schwierigkeit besteht darin, die Anordnung und Interaktion der Makromoleküle sichtbar zu machen und ihre dynamische Entwicklung zu verfolgen, sodass auch weiträumige Strukturen und Muster im Kontext der Fülle von Proteinkomplexen in intakten Zellen erkennbar werden. Fluoreszenzmikroskopie und ihre hochauflösenden Varianten (STED, PALM, STORM) können Informationen über die Lokalisation markierter Proteine in Mikroorganismen liefern, die Methode der Kryo-Elektronentomographie die dreidimensionale Verteilung und Struktur größerer Makromolekülkomplexe. Inzwischen eröffnet sich auch die Möglichkeit, die strukturellen Daten in Simulationen (von Teilen) des Cytoplasmas zu nutzen und seine Dynamik auf molekularem Niveau zu untersuchen. Drei Übersichtsvorträge befassen sich mit der gegenwärtigen Forschung zu diesen Aspekten:

Julio Ortiz (MPI für Biochemie, Martinsried) wird über die Bildung von Ribosomen-Clustern in stoffwechselaktiven und hungernden Bakterien berichten, die mit Kryo-Elektronentomographie nachgewiesen und in ihrer 3D-Struktur untersucht wurden. Johan Elf (Universität von Uppsala, Schweden) verfolgt mit Einzelmolekül-Nachweisen im Fluoreszenzmikroskop die Diffusion und Reaktionskinetik markierter Proteine in Zellen und simuliert ihr Verhalten. Zan Luthey-Schulten (University of Illinois, Urbana, USA) gibt einen Einblick in die jüngsten Ergebnisse ihrer Simulationen der Dynamik des mikrobiellen Cytoplasmas, die nur mit fortschrittlicher Rechnerarchitektur zu bewältigen sind und einen faszinierenden Ausblick auf zukünftige Entwicklungen bieten. Die Vortragenden sind führende Experten in ihren Forschungsgebieten, die nicht unbedingt im Zentrum der Mikrobiologie angesiedelt sind, uns aber Zugang zur inneren Struktur und Organisation mikrobieller Zellen verschaffen.

Im Anschluss an das Symposium veranstalten wir ab 19.30 Uhr im gleichen Raum ein Mitgliedertreffen der Fachgruppe. ■

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Fachgruppe: Symbiotische Interaktionen

■ Die derzeit jüngste VAAM-Fachgruppe (gegr. Dezember 2009) umfasst aktuell ca. 60 Mitglieder aus Universitäten, Forschungseinrichtungen und der Industrie. Primäres Ziel der Fachgruppe ist es, einen regelmäßigen Kontakt und Austausch zwischen deutschen und internationalen Arbeitsgruppen zu fördern, die Arbeiten der Fachgruppe international sichtbar zu machen und gemeinsame Fortbildungsveranstaltungen für den wissenschaftlichen Nachwuchs durchzuführen. Die Forschungsaktivitäten sind, ebenso wie die Fachgruppe selbst, an der sich neben Mikrobiolog/inn/en auch Mediziner/innen und Tiermediziner/innen aktiv beteiligen, stark interdisziplinär ausgerichtet. Im Vordergrund stehen die vielfältigen Interaktionen von Mikroorganismen mit tierischen oder pflanzlichen Wirten, die pathogen, mutualistisch oder kommensal ausgeprägt sein können. Die Themenfelder der Fachgruppe umfassen beispielsweise die Stoffwechselinteraktionen zwischen mikrobiellen Symbionten und Wirt sowie der Mikroorganismen untereinander, die Bedeutung des Quorum Sensings für die Bakterien-Wirt-Interaktion und die Aufklärung der Interaktionen im bakteriellen Biofilm des gesunden Wirtes. Dabei stehen die Vorstellungen der jeweiligen Modellsysteme, der Abgleich der Symbiose-Systeme untereinander und die angewendeten Methoden im Zentrum der Aktivitäten. Nach dem ersten VAAM-Fachgruppen-Treffen im November 2009 in München fand das zweite Meeting im September 2010 in Würzburg statt. An diesem Treffen nahmen über 90 Teilnehmer teil und präsentierten ihre Arbeiten in 25 Vorträgen und 22 Postern. Die Fachgruppe präsentiert sich im Jahr 2011 auf der VAAM-Jahrestagung in Karlsruhe. Die Mitglieder der Fachgruppe sowie Interessenten an der künftigen Gestaltung der Fachgruppe sind dazu herzlich eingeladen.

Weitere Informationen unter: http://www.helmholtz-muenchen.de/en/symbiotic-interactions/home-aims/index.html ■

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Fachgruppe: Umweltmikrobiologie

■ Die Fachgruppe Umweltmikrobiologie bietet ein breites Forum, das von der Mikrobiellen Ökologie bis zu umweltrelevanten Stoffwechselwegen oder praktischen Anwendungen reicht. Ein Ziel der Fachgruppe ist, den intensiven Austausch zwischen diesen Fachbereichen zu fördern und mit Veranstaltungen zu neuen Themen zu unterstützen.

Umweltmikrobiologie ist bereits bei den Plenarvorträgen ein Schwerpunkt dieser VAAM Tagung. Zusätzlich findet am Montag, 04. April, von 17:30 bis 19:30 ein Fachgruppensymposium zum Thema "Relevance of ecological principles in environmental microbiology" statt. Mit Christoffer van der Gast, UK, und Alban Ramette, MPI Bremen als eingeladene Redner sowie weiteren Beiträgen verspricht dieses sehr interessant zu werden.

Im September 2011 findet das International Symposium on Subsurface Microbiology (ISSM) in Garmisch-Partenkirchen statt, zu dem alle Mitglieder herzlich eingeladen sind (www.issm2011.com). Hier wird mikrobielle Ökologie von marinen Sedimenten über tiefe terrestrische Habitate bis zu flachen Grundwasserleitern diskutiert. Für 2012 ist es geplant, zusätzlich zu einem Fachgruppensymposium auf der VAAM-Jahrestagung eine spezielle Fachgruppentagung zu organisieren. Interessierte können sich gerne mit Themenvorschlägen an den Sprecher wenden.

Bisher wurde die Fachgruppe von Prof. Engesser geleitet, der mit der VAAM-Tagung 2011 auf eigenen Wunsch ausscheiden möchte. Deshalb wird im Anschluss an die Fachgruppenveranstaltung ab 19.30 Uhr die Wahl des neuen Sprechers stattfinden, zu der wir herzlich einladen. ■

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Der VAAM-DECHEMA-Gemeinschaftsausschuss: Biotransformationen

■ Im November 2008 haben die seit 1996 bestehende VAAM-Fachgruppe "Biotransformationen" mit zuletzt 130 gemeldeten Mitgliedern und der DECHEMA-Arbeitsausschuss "Grundlagen der biotechnologischen Stoffproduktion" in Frankfurt am Main gemeinsam den neuen Gemeinschaftsausschuss "Biotransformationen" gegründet. Gewählte Sprecher sind von DECHEMA-Seite zurzeit Prof. Dr. Andreas Liese (TU Hamburg-Harburg) und von VAAM-Seite Prof. Dr. Christoph Syldatk (Karlsruher Institut für Technologie KIT). Im gemeinsamen Ausschuss sind neben Mitgliedern aus der Industrie maßgeblich auch Vertreter der Gesellschaft Deutscher Chemiker (GDCh) und der Gesellschaft für Fettwissenschaften (DGF) engagiert. Dieses Zusammengehen verschiedener Fachdisziplinen lag nahe: Die wachsende Nachfrage nach ökonomischen, ökoeffizienten und ressourcenschonenden Prozessen in der Chemie-, Pharma-, Energie- und Lebensmittelindustrie erfordert verstärkte Anstrengungen, Forschungsergebnisse in Produkte umzusetzen. An biokatalytischen Verfahren führt dabei kein Weg vorbei, sie bilden die Grundlage der modernen Industriellen Biotechnologie. Das sehr interdisziplinäre Forschungsgebiet der Biotransformationen erlebt zurzeit ein rasantes Wachstum, dem auch moderne Methoden wie Metagenomanalyse und Protein-Engineering große Impulse verleihen. Durch den Zusammenschluss der beiden Gremien der großen Fachgesellschaften kann dieses Arbeitsgebiet nun wesentlich besser und effektiver vertreten werden und es können Forscher aus Universitäten, Forschungseinrichtungen und der chemisch-pharmazeutischen Industrie nun noch enger zusammengeführt werden, was bereits in einer Reihe von Veranstaltungen gemeinsam mit anderen Ausschüssen der DECHEMA und Fachgruppen der VAAM geschehen ist, über die im Biospektrum berichtet wurde. Ziel dabei ist vor allem, durch spezielle Symposien für Doktoranden, Habilitanden und Juniorprofessoren junge Forscher zu fördern.

Für 2011 sind die Beteiligung an einer DECHEMA-Vortrags- und Diskussionstagung zum Thema "Bioverfahrenstechnik an Grenzflächen" vom 30.05. bis 01.06.2011 in Potsdam geplant, sowie die Durchführung einer internationalen interdisziplinären Sommerschule für Promovierende und junge Wissenschaftler aus der Industrie zum Thema "Biokatalyse" vom 22. bis 25.08.2011 in Bad Herrenalb. ■

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SYSTEMATIC AND APPLIED MICROBIOLOGY

A Journal of Microbial Diversity

www.elsevier.com/locate/syapm
ISSN 0723-2020

From the Contents Volume 33, Issue 5

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Aims & Scope

Scope of the Journal: Systematic and Applied Microbiology deals with various aspects of microbial diversity and systematics of prokaryotes. It focuses on Bacteria and Archaea; eukaryotic microorganisms will only be considered in rare cases. The journal perceives a broad understanding of microbial diversity and encourages the submission of manuscripts from the following branches of microbiology:

Systematics: Theoretical and practical issues dealing with classification and taxonomy, i.e. (i) new descriptions or revisions of prokaryotic taxa, including descriptions of not-yet cultured taxa in the category Candidatus (ii) innovative methods for the determination of taxonomical and genealogical relationships, (iii) evaluation of intra-taxon diversity through multidisciplinary approaches, (iv) identification methods.

Applied Microbiology: all aspects of agricultural, industrial, and food microbiology are welcome, including water and wastewater treatment.

Comparative biochemistry and genomics: studies concerning biochemical/metabolic and genomic diversity of cultured as well as yet-uncultured Bacteria and Archaea.

Ecology: descriptions of the microbial diversity in natural and man-made ecosystem; studies quantifying the size, dynamics, and function of microbial populations; innovative research on the interaction of micro-organisms with each other and their biotic and abiotic environment.

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Microbiology in Karlsruhe's technical environment

■ The University of Karlsruhe, presently known as the Karlsruhe Institute of Technology (KIT), was founded in 1825 as a polytechnical school. In 1832, the first biological discipline, the school of Forest Science, was incorporated into the Polytechnical School. The chair of Botany, held by Alexander Braun (1833 – 1846), lectured topics in botany and zoology. In 1872, Pharmacy was added, and before the end of the 19th century, the Botany and Zoology departments were split into two teaching units. In 1890, Walter Migula, a pioneer in bacterial systematics, habilitated in botany and served as professor until 1904; he described the important bacterial genus *Pseudomonas* in 1895. Forestry was later moved to Freiburg and Pharmacy to Heidelberg. After World War II, teaching started again, and in 1956 H. Kühlwein was appointed as the Chair of Botany. Although a professor of botany, Kühlwein became internationally recognized for his work on Myxobacteria. One of his Ph.D. students, H. Reichenbach, continued research with these gliding bacteria at the GBF in Braunschweig. Kühlwein also studied the physiology of some wood-degrading fungi.

In 1967, the university was restructured and renamed *University of Karlsruhe*, and several new Chairs were appointed, among them a new Chair of Microbiology, W. Zumft. One of the charms of KIT is that within Campus South (the former University of Karlsruhe) microbiologists are not only members of the Faculty of Chemistry and Biosciences, but also of the Faculties of Chemical Engineering (Institute of Life Sciences Engineering), Civil Engineering, and Geo- and Environmental Sciences (Institute of Biology for Engineers and Biotechnology of Wastewater Treatment). They are also situated at Campus North (formerly Forschungszentrum Karlsruhe), at the Institute for Functional Interfaces, and close to Campus South, at the Max-Rubner Institute (formerly Bundesanstalt für Ernährung und Lebensmittel). Other microbiology research groups are located in the Water Technology Center (TZW) Karlsruhe or at Geilweiler Hof. Since various groups employ microorganisms in a range of technical processes, there are many established collaborations with industry. This is also reflected in the fact that researchers from the BASF SE company are integrated into the teaching program of the University and are members of this organizing committee. These wide microbiological interests, ranging from basic research with bacteria and fungi, pathogenic and symbiotic interactions, to applied aspects and bioengineering, offer a broad education for the students and the possibility to transform ideas into products at KIT. We are very happy to host the annual VAAM meeting this year in Karlsruhe!

Prof. Dr. Reinhard Fischer,
Cell biology of filamentous fungi
Institute for Applied Biosciences, Dept. of Microbiology, Faculty of Chemistry and Biosciences; www.iab.kit.edu/microbio/
Filamentous fungi are extremely polarized eukaryotic cells that continuously elongate their hyphal tips. The *Aspergillus* research group studies the cell biology underlying polarized growth, which is driven by the concerted action of microtubules, actin and the corresponding motor proteins that deliver enzymes for cell wall biosynthesis to the cortex. Recently, it was discovered that at least two different populations of microtubules exist in the hyphae of *Aspergillus nidulans*.

A. nidulans is able to initiate different morphogenetic programs and develop either asexual or sexual spores. One environmental trigger for these processes is light. The group recently identified phytochrome as one of the important photoreceptors in this fungus, which shows that phytochrome also functions outside the plant kingdom.

Prof. Dr. Jörg Kämper,
Molecular Phytopathology
Institute for Applied Biosciences, Dept. of Genetics, Faculty of Chemistry and Biosciences; http://genetics.iab.kit.edu/
The basidiomycete *Ustilago maydis* is a ubiquitous pathogen of maize and a well-established model organism for the study of plant-microbe interactions. This fungus belongs to the group of biotrophic parasites that depend on living tissue for proliferation and development. Pathogenic development in *U. maydis* is linked to a dimorphic switch from budding to filamentous growth. The main interest of the Molecular Phytopathology group is to understand the regulatory networks that link pathogenic development and the morphological changes of the fungal cell. Expression profiling, in combination with reverse genetic approaches, has led to the identification of various novel pathogenicity factors. Another focus of the group is the metabolic reprogramming of the maize plant by *U. maydis*, and the utilization of carbon sources by the fungus during pathogenic development.

Prof. Dr. Natalia Requena.
Molecular biology of plant-fungal interactions
Botanical Institute, Dept. of Plant-Microbe Interactions, Faculty of Chemistry and Biosciences; http://www.iab.kit.edu/heisenberg/
Microorganisms often live in association with plants either in mutualistic symbioses or as parasites. The focus of the plant-fungal interactions group is the arbuscular mycorrhizal symbiosis that involves the fungi of the Glomeromycota phylum and most plant roots. The colonization of a root by arbuscular mycorrhizal fungi involves a deep reorganization of the plant cell to accommodate the symbiont and to provide the fungus with photoassimilates. The group of Natalia Requena is interested in unraveling the recognition mechanisms and involved molecules that characterize this symbiosis. How have plants learned to distinguish between pathogenic and mutualistic fungi? How have some pathogens learned to escape the defense response of the plant? The group uses as model organisms the arbuscular mycorrhizal fungus *Glomus intraradices* and the hemibiotrophic pathogenic fungus *Magnaporthe oryzae*.

Prof. Dr. Tilman Lamparter,
Photobiology of plants and bacteria
Botanical Institute, Faculty of Chemistry and Biosciences; http://www.rz.uni-karlsruhe.de/~db127/
Agrobacterium tumefaciens is a soil bacterium and a plant pathogen that transfers genes into plants. The photobiology research group is interested in the photobiology of *A. tumefaciens* and of related nitrogen-fixing Rhizobia. The two phytochromes of *A. tumefaciens* serve as model proteins for biochemical studies on chromophore protein interaction, photoconversion, modulation of histidine-kinase activity and tertiary structure. Initial studies have led to the discovery of the chromophore binding site of bacterial phytochromes, which differs from that of plant phytochromes. *Agrobacterium tumefaciens* contains also two photolyases, flavoproteins that repair UV-damaged DNA; these photolyases are also analyzed as recombinant proteins. One of the photolyases is closely related to plant cryp-

tochromes, while the other probably provides an evolutionary link between photolyases and other DNA repair enzymes.

Prof. Dr. Christoph Syldatk,
Institute of Engineering in Life Sciences (IBLT), Dept. of Technical Biology (Section II); http://tebi.blt.kit.edu/index.php
The Section II – Technical Biology research group is part of the Faculty of Chemical and Process Engineering, and was founded in 2003 as part of the new Institute of Engineering in Life Sciences. It is responsible for providing the fundamentals biology to the "Engineering in Life Sciences" (BIW) students, and trains students of "Applied Biology" in enzyme technology and biochemical engineering. The primary research topics for around 20 researchers in this group are Microbial Biotechnology and Industrial Biocatalysis. Here, microorganisms can be cultivated under S-1- and S-2-conditions, up to 30-liters per day. These facilities include the possibility of downstream processing of microbial cultures by centrifugation, filtration, cell disruption and immobilisation of microorganisms and enzymes, chromatographic purification of proteins, and the basic equipment for molecular biological and genetic engineering experiments, such as standard HPLC-, TLC- and GC-analytical equipment, as well as a special laboratory dedicated to studies on the cultivation of marine sponges and associated microorganisms.

Researchers working in the field of Microbial Biotechnology are currently developing a process to produce microbial bio-surfactants. They are also investigating aspects of the "*in-vitro*"-cultivation of marine sponges and the role of associated microorganisms, as well as the production of special yeast and fungal metabolites. In the field of Industrial Biocatalysis, the focus is on production of unnatural α- and β-amino acids, the enzymatic hydrolysis of linear and cyclic amides, as well as enzymatic modification and synthesis of surface- and interfacial-active compounds, including aspects of enzyme immobilisation, such as to magnetic carriers, and process development.

Prof. Dr. Josef Winter,
PD Dr. Claudia Gallert,
Institute of Biology for Engineers and Biotechnology of Wastewater Treatment (IBA); http://www.iba.kit.edu
The research of IBA, Faculty of Civil Engineering, Geo- and Environmental Sciences of KIT, is directed towards practically and technically relevant aerobic and anaerobic processes for water, wastewater and bio-waste treatment, microbial product recovery and soil sanitation procedures. In this context, the following topics are currently under investigation: bioremediation of soil that was contaminated with anti-knocking agents from leaded fuel production, biogas and bio-hydrogen production from energy crops, bio- and market waste fractions, degradation of aromatic/phenolic compounds in industrial wastewater, microbial chitin deproteination and decalcification of shrimp shells of *C. crangon* (North Sea) or *P. monodon* (from Indonesia), and nitrification of saline, ammonia-loaded wastewater. In addition, the fate of arsenic and selenium in the groundwater of the Bengal delta and in northern India, and the fate and the effect of antibiotics in domestic wastewater are investigated. Our research is funded by DFG, BMBF, BMZ, AiF and industry.

Prof. Dr.-Ing. Clemens Posten,
Photo- and Particle Biotechnology
Institute of Engineering in Life Sciences (IBLT), Dept. of Bioprocess Engineering, Faculty of Chemical Process Engineering; http://bvt.blt.kit.edu/
Microalgae show enormous potential for the production of high value products in the pharmaceutical and cosmetic industries, but are increasingly discussed with regards to their production of middle and low cost products, such as fine chemicals, food or animal feed. Closed photo-bioreactors are employed to produce light and CO_2 in large scale production.

In the research group of Clemens Posten, growth and product formation kinetics are studied to understand the specific behavior of microalgae, such as *Porphyridium* or *Chlamydomonas*, under production conditions. Reactor design is optimized based on intra- and extracellular measurements; thus, the reactors are developed "around the cells".

Prof. Dr. Ursula Obst,
Interface Microbiology
Institute for Functional Interfaces, Campus North, Faculty for Chemical and Process Engineering; http://www.ifg.kit.edu/58_188.php
Biofilms are assemblies of adhesive microorganisms on surfaces, and are ubiquitous in the environment. As biological filters, they are part of natural and technical large-scale processing systems; however, under certain circumstances biofilms also represent germ reservoirs that cause contamination as hygienically relevant bacteria. In medicine, bacterial biofilms are the causative agents of implant infections and chronic wounds that are difficult to treat. As an extremely flexible life form, resistant to a variety of external stressors, natural biofilms are characterized by our research group by focusing on their stress and survival strategies, with application fields ranging from environment to biotechnology and medicine.

Prof. Dr. Rolf Geisen,
Molecular Food Mycology
PD Dr. Charles Franz,
Plant Food Fermentation
Max Rubner Institut; http://www.mri.bund.de/
The Max Rubner Institut is a governmental research institute whose task is the analysis and assessment of health-promoting constituents of foods, as well as food quality and safety assurance. Within the Department of Safety and Quality of Fruit and Vegetables, which focuses on metabolomics of nutritionally important plant secondary metabolites, as well as microbiological safety of plant-derived products, the group of Prof. Dr. Rolf Geisen is working on the regulation of fungal mycotoxin production in foods. The role of food-relevant environmental conditions and transmittance via signal transduction pathways to regulate transcription of mycotoxin biosynthetic genes are being analyzed. The research group of PD Dr. Charles Franz focuses on plant food fermentation and the development of starter cultures for malolactic fermentation of wine, to improve the quality of wines. Other research is centred on the effect of secondary plant compounds on human gut microflora biodiversity, as well as the metabolism of these compounds by gut bacteria.

Dr. Andreas Tiehm,
Environmental Biotechnology
Dr. Beate Hambsch,
Drinking Water Microbiology
DVGW-Technologiezentrum Wasser (TZW); www.tzw.de
The TZW is part of the Institute for Applied Water Research of the DVGW, the German Gas- and Waterworks Association. The TZW provides a link between the fundamental research undertaken at universities and water treatment companies. It is contact point for authorities, ministries and associa-

Max Rubner Institute

tions with all questions concerning water quality, resource protection and water technology.

Current research projects focus on intrinsic and stimulated bioremediation of chlorinated and tar oil pollutants, biodegradation of emerging pollutants, such as pharmaceutical residues, reuse of waste water and aquifer recharge, detection/identification of pathogens and indicator organisms, and development and application of bio tests and molecular biological identification methods.

Dr. Bernhard Nüßlein
nadicom GmbH; http://www.nadi-com.com
nadicom is a leading international GMP-certified biotechnology company in the field of genetic identification of bacteria and fungi. nadicom specialises in molecular-biological identification of pure bacterial and fungal cultures for the pharmaceutical, food, and cosmetic industries, as well as the research sector. We also prepare DNA fingerprints from pure cultures, environmental samples and complex mixed cultures to screen for genetic identity. We have special expertise in contracted research in environmental and agricultural microbiology, directed at solving client-defined problems.

DFG Research Unit 1341,
http://www.for1334.kit.edu/
This research unit was founded in 2010 by Meritxell Riquelme (Ensenada, Mexico) and Reinhard Fischer (KIT) and is a collaborative action among five groups from Mexico and nine groups from Germany. The topic of this new alliance is molecular analysis of the polarized growth of different filamentous fungi. Filamentous growth is adapted to different growth and developmental conditions, and is highly modulated by internal and external signals. These signaling processes are part of our research. Funding for the German groups is provided by the DFG and by CONACYT for the Mexican groups.

DFG Research Unit 831 "Dynamic capillary fringes"
This interdisciplinary research unit was formed in 2007 to investigate hydrogeological, hydraulic, soil physical, hydro chemical and microbiological interactions in the capillary fringe (CF). It is a cooperation program of KIT, the universities of Tübingen and Heidelberg, and Helmholtz Center of Environmental Research, Halle. One of the main goals is to elucidate the influence of biofilm formation or bio surfactant excretion by microorganisms on hydraulic, soil water- and geochemical parameters in the CF, and to develop a mathematic model that describes these interactions under changing conditions.

Priority research group of the Baden Württemberg Stiftung;
http://www.iab.kit.edu/
A grant from the Baden Württemberg Stiftung was used to create a priority program on secondary metabolites at the Karlsruhe Institute of Technology, South Campus. Groups from the Faculty of Chemistry and Biosciences, the Faculty for Bioengineering and the MRI collaborate to understand the genetics, production and toxicology of alternariol, a mycotoxin produced by *Alternaria alternata*.

Monday, 04 April 2011

	Brahms Hall	Mombert Hall	Clubroom	Room 2.05	Room 2.08	Hebel Hall	Forum 1	Forum 2
08:30–10:30	Environmental Microbiology I	Pathogen Metabolism & Physiology	Cellular Systems in Biotechnology	Plant-Microbe Interactions	Standards for Large Scale (Meta-)Genomics and Metadata	Fungal Biology and Development	Oxidative Stress Responses	Special Session Science and Infrastructure
	p. 35	p. 35	p. 35	p. 36	p. 31	p. 36	p. 36	p. 37
	Coffee break/Industrial exhibition							
11:00–12:45	Award Session/ Plenary Session Cell Biology							
	p. 28							
	Lunch break/Industrial exhibition							
14:15–15:15	Plenary Session Stress Response							
	p. 29							
15:15 –17:30	Poster Session I/Coffee break/ Industrial exhibition		15:45–17:15 Karriere-Symposium	Poster Session I/Coffee break/ Industrial exhibition				
17:30–19:30	Fungal Development and Pathogenicity Mechanisms	Enzymatic Systems in Biotechnology	Relevance of Ecological Principles in Environmental Microbiology	Second Messengers in Bacteria	Anaerobic Metabolism	Structure of the Microbial Cytoplasm	Liquids as Part of Cellular Processes (Attention: Session takes place in Forum 1 & 2)	
	p. 31	p. 37	p. 32	p. 33	p. 38	p. 33	p. 34	

■ Special Group Mini-Symposia □ Short Lecture

Tuesday, 05 April 2011

	Brahms Hall	Mombert Hall	Clubroom	Room 2.05	Room 2.08	Hebel Hall	Forum 1 & 2
08:30–10:30	Cell Biology I	Virulence Factors	Food Microbiology	Fungal Biotechnology	Environmental Microbiology – ISME	Experimental Progress and Molecular Tools	Archaea
	p. 38	p. 39	p. 39	p. 39	p. 40	p. 40	p. 40
	Coffee break/Industrial exhibition						
11:00–12:30	Plenary Session White Biotechnology						
	p. 29						
	Lunch break/Industrial exhibition						
14:00–15:30	Plenary Session Microbial Ecology						
	p. 30						
15:30 – 17:30	Poster Session II/Coffee break/ Industrial exhibition		Industrial Symposium Eurofins MWG Operon	Poster Session II/Coffee break/ Industrial exhibition			
17:30–19:30	VAAM Annual General Meeting/ PhD Awards						
19:30	Friedrich-Weinbrenner-Hall						
	Mixer						

■ Special Group Mini-Symposia □ Short Lecture

Wednesday, 06 April 2011

	Brahms Hall	Mombert Hall	Clubroom	Room 2.05	Room 2.08	Hebel Hall	Forum 1 & 2
09:00–11:00	Environmental Microbiology II	Microbial Diversity	Hydroxylation, Oxygenation and Oxidation Reactions	Cell Biology II	Other Stress Responses	Symbiotic Interactions	Physiology/Regulation
	p. 41	p. 41	p. 34	p. 42	p. 42	p. 42	p. 43
	Coffee break/Industrial exhibition						
11:30–11:45	Poster Awards						
11:45–13:15	Plenary Session Microbial Interactions						
	p. 30						
13:15–13:30	Closing Remarks						

Special Group Mini-Symposia Short Lecture

CONFERENCE PROGRAMME

VAAM 2011 Jahrestagung Karlsruhe (03.04.–06.04.2011)

Sunday, 03.04.2011

14:00–18:30 Registration and mounting of the posters — **Foyer Stadthalle Karlsruhe**

PUBLIC LECTURE — **Brahms-Saal**

15:30–16:00 **Welcome Addresses**
V. Saile
Karlsruhe Institute of Technology (KIT), Institute of Microstructure Technology, Karlsruhe, Germany

R. Fischer
Karlsruhe Institute of Technology (KIT), Institute of Applied Biosciences – Department of Microbiology, Karlsruhe, Germany

16:00–17:00 ISV01: **H.C. Flemming**
University Duisburg-Essen, Faculty of Chemistry – Biofilm Centre, Duisburg, Germany
"Die letzten Meter bis zum Wasserhahn: Mikrobiologie in der Trinkwasserleitung"

17:00–17:30 Coffee break

PLENARY SESSION: ENVIRONMENTAL MICROBIOLOGY — **Brahms-Saal**
Chair: Josef Winter

17:30–18:00 ISV02: **A. Boetius**
Max Planck Institute for Marine Microbiology, Bremen, Germany
"Microbial consumption of hydrocarbons in the deep sea: From methane seeps to oil spills"

18:00–18:30 ISV03: **D. Cowan**
University of the Western Cape, Department of Biotechnology, Cape Town, South Africa
"Metagenomics and Gene Discovery"

19:30 Welcome Reception — **Rathaus Stadt Karlsruhe**
Welcome Address
K. Stapf, City Mayor Karlsruhe

Monday, 04.04.2011

08:00–19:30 Industrial exhibition — **Ground floor/ 2nd floor**

08:30–10:30 **Special Groups Mini Symposia** (see page 31) — **various**
(followed by General Meeting of the Special Group Functional Genomics)

08:30–10:30 **Short lectures** (see page 35) — **various**

10:30–11:00 Coffee break/Industrial exhibition — **Ground floor/ 2nd floor**

PLENARY SESSION: CELL BIOLOGY
Chair: Jörg Kämper

11:00–11:45 **VAAM Honary Award Session**
ISV04: **M. Thanbichler** — **Brahms-Saal**
Max Planck Institute for terrestrial Microbiology, Marburg, Germany
"Spatial regulation in *Caulobacter crescentus*"

11:45–12:15 ISV05: **P. Graumann** — **Brahms-Saal**
Albert-Ludwigs Universität Freiburg, Faculty of Biology, Freiburg, Germany
"Intrinsic properties guide the function of bacterial cytoskeletal elements"

VAAM 2011 Jahrestagung Karlsruhe (03.04.–06.04.2011)

12:15–12:45	ISV06: **S. Osmani** *Ohio State University, Department of Molecular Genetics, Columbus, USA* "Mitotic restructuring of the nucleus in the filamentous fungus *Aspergillus nidulans*"	**Brahms-Saal**
12:45–14:15	Lunch break/Industrial exhibition	**Ground floor/ 2nd floor**

PLENARY SESSION: STRESS RESPONSE
Chair: Tilman Lamparter

14:15–14:45	ISV07: **R. Hengge** *Freie Universität Berlin, Faculty of Biology, Berlin, Germany* "The general stress response, biofilm formation and c-di-GMP signalling in *Escherichia coli*"	**Brahms-Saal**
14:45–15:15	ISV08: **E. Bremer** *Philipps-Universität Marburg, Faculty of Biology, Germany* "Driving up the pressure: genetic and cellular responses of *Bacillus subtilis* to osmotic stress"	**Brahms-Saal**
15:15–17:30	Coffee break/Industrial exhibition	**Ground floor/ 2nd floor**
15:45–17:15	**Karrieresymposium** (see page 14) Vielfältige Berufsbilder in den Biowissenschaften – Anregungen und Tipps	**Clubraum**
15:15–17:30	Poster Session I	**Ground floor/ 2nd floor**
17:30–19:30	**Special Groups Mini Symposia** (see page 31) (followed by the General Meetings of the Special Groups: Fungal Biology, Environmental Microbiology, Structure and Microscopy, Regulation and Signal transduction in Prokaryotes)	**various**
17:30–19:30	**Short lectures** (see page 37)	**various**

Tuesday, 05.04.2011

08:00–19:00	Industrial exhibition	**Ground floor/ 2nd floor**
08:30–10:30	**Short lectures** (see page 38)	**various**
10:30–11:00	Coffee break/Industrial exhibition	**Ground floor/ 2nd floor**

PLENARY SESSION: WHITE BIOTECHNOLOGY
Chair: Clemens Posten

11:00–11:30	ISV09: **C. Wittmann** *Technische Universität Braunschweig, Institute of Biochemical Engineering, Braunschweig, Germany* "Tailor-made cell factories for a sustainable bio-economy"	**Brahms-Saal**
11:30–12:00	ISV10: **C. Kubicek** *Vienna University of Technology, Institute of Chemical Engineering, Vienna, Austria* "Genome-wide aspects of cellulase regulation in *Trichoderma reesei*"	**Brahms-Saal**
12:00–12:30	ISV11: **C. Wilhelm** *Universität Leipzig, Institute for Biology I, Leipzig, Germany* "Energy balances from photon to biomass: lesson for biofuel production"	**Brahms-Saal**
12:30–14:00	Lunch break/Industrial exhibition	**Ground floor/ 2nd floor**

CONFERENCE PROGRAMME

VAAM 2011 Jahrestagung Karlsruhe (03.04.–06.04.2011)

PLENARY SESSION: MICROBIAL ECOLOGY
Chair: Ursula Obst

14:00–14:30	ISV12: **S. Wuertz** *University of California, Department of Civil & Environmental Engineering, Davis, USA* "Monitoring of human pathogens and source identifiers in discharges across the United States: QMRA from source to bathing site"	**Brahms-Saal**
14:30–15:00	ISV13: **M. Wagner** *University of Vienna, Department of Microbial Ecology, Vienna, Austria* "New nitrifiers: Surprising diversity and unexpected physiological properties"	**Brahms-Saal**
15:00–15:30	ISV14: **A. Ulrich** *Karlsruhe Institute of Technology (KIT), Institute of Organic Chemistry – Department of Biochemistry, Karlsruhe, Germany* "Biomembrane barriers and their role in survival: case studies on molecular transport and lethal damage"	**Brahms-Saal**
15:30–17:30	Coffee break/Industrial exhibition	**Ground floor/ 2nd floor**
15:30–17:30	Poster Session II	
16:00–17:30	**Industrial symposium Eurofins MWG Operon (Ebersberg)*** (see page 14)	**Clubraum**
17:30	**VAAM Annual General Meeting** (see page 14)	**Brahms-Saal**
ca. 18:30	**PhD Awards** Sponsored by BASF SE, Sanofi Aventis Deutschland GmbH, Bayer Schering Pharma, New England Biolabs GmbH, Evonik Degussa GmbH	**Brahms-Saal**
20:00	Mixer	**Friedrich-Weinbrenner-Saal**

* Snacks and beverages provided

Wednesday, 06.04.2011

08:30–12:00	Industrial exhibition	**Ground floor/ 2nd floor**
09:00–11:00	**Special Groups Mini Symposia & Short Lectures** (see page 34; 41)	**various**
11:00–11:30	Coffee break	
11:30–11:45	**Poster Awards** Sponsored by nadicom GmbH	**Brahms-Saal**

PLENARY SESSION: MICROBIAL INTERACTIONS
Chair: Natalia Requena

11:45–12:15	ISV15: **J. Boch** *Martin-Luther-University Halle-Wittenberg, Institute for Genetics, Halle, Germany* Xanthomonas TALEs – from plant pathogen weapon to biotech hype	**Brahms-Saal**
12:15–12:45	ISV16: **C. Hertweck** *Hans-Knöll Institute Jena, Jena, Germany* "Toxin producing endofungal bacteria"	**Brahms-Saal**
12:45–13:15	ISV17: **P. Bonfante** *University of Torino, Plant Biology Department, Turin, Italy* "Plants and arbuscular mycorrhizal fungi – Born to be friends"	**Brahms-Saal**
13:15–13:30	**Closing remarks**	**Brahms-Saal**

ACTIVITIES OF THE SPECIAL GROUPS

Mini-Symposia of the Special Groups: Monday, April 4, 08:30–10:30

Special Group Funktionelle Genomanalyse
Topic: "Standards for Large Scale (Meta-) Genomics and Metadata"

Organisation: H.P. Klenk, German Collection of Microorganisms and Cell Cultures (DSMZ), Braunschweig, Germany
Room 2.08

ISV18 **08:30** **F.O. Glöckner**
School of Engineering & Science (SES), Jacobs University, Bremen, Germany
"The genomic standards consortium: Bringing standards to life"

ISV19 **09:00** **G. Garrity[1]*, N. Kyrpides[2], D. Field[3], P. Sterk[3,4], H.-P. Klenk[5]**
[1] Microbiology & Molecular Genetics, Michigan State University, East Lansing, USA
[2] DOE Joint Genome Institute, Walnut Creek, USA
[3] Centre for Ecology & Hydrology, Molecular Evolution and Bioinformatics Group, Oxfordshire, UK
[4] The Sanger Institute, Welcome Trust Genome Campus, Hinkston Down, UK
[5] German Collection of Microorganisms and Cell Cultures (DSMZ), Braunschweig, Germany
"Standards in Genomic Sciences: A standards compliant open-access journal for the 'omics community"

ISV20 **09:30** **F. Meyer**
Institute for Genomics and System Biology National Laboratory Mathematics and Computer Sciences Division, Argonne, USA
"Reversing the paradigm – The genome sequence of *Candidatus Sulfuricurvum* sp. derived from a complex short-read metagenome with more than 300 OTUs enables detailed studies of the novel epsilon-proteobacterium"

FGV001 **10:00** **L. Wöhlbrand[1]*, J. Jacob[2], M. Kube[3], A. Beck[3], R. Reinhardt[4], R. Rabus[1,2]**
[1] Institute for Chemistry and Biology of the Marine Environment (ICBM), General and Mocelular Microbiology, Oldenbrug, Germany
[2] Max Planck Institute for Marine Microbiology, Bremen, Germany
[3] Max Planck Institute for Molecular Genetics, Berlin, Germany
[4] Max Planck Institute for Plant Breeding Research, Cologne, Germany
"Genome and proteome of *Desulfobacula toluolica* Tol2, a sulfate-reducing aromatic compound degrader"

Mini-Symposia of the Special Groups: Monday, April 4, 17:30–19:30

Special Group Experimentelle Mykologie/Fungal Biology and Biotechnology
Topic: "Fungal Development and Pathogenicity Mechanisms"

Organisation: S. Pöggeler, Institute for Microbiology and Genetics, University of Göttingen, Göttingen, Germany; M. Brock, Leibniz Institute for Natural Product Research and Infection Biology, Hans-Knöll-Institute, Jena, Germany
Brahms Hall

ISV21 **17:30** **P. van West*, S. Wawra, S. Grouffaud, C. R. Bruce, N. R. Horner, J. Bain, A. Matena, C. MM Gachon, I. de Bruijn, K. L. Minor, J. A. Boddey, S. C. Whisson, P. Bayer, P. R.J. Birch, A. J. Porter, C. J. Secombes**
Aberdeen Oomycete Laboratory, University of Aberdeen, Aberdeen, Scotland
"Translocation of *Oomycete* effectors into host cells"

FBV014 **18:00** **R. Kumar*, S. Sathya, B.P. Venkatesh**
Microbial Biotechnology, Bharathiar University, Coimbatore, India
"Antifungal and antibacterial activity of marine actinomycetes strains isolated from east and west coastal regions of India"

ACTIVITIES OF THE SPECIAL GROUPS

Mini-Symposia of the Special Groups: Monday, April 4, 17:30–19:30

MPV018 18:15 **K. Lapp[1,2]*, T. Heinekamp[1,2], I. Jacobsen[2,3], H.-M. Dahse[2,4], A.A. Brakhage[1,2]**
[1] Leibniz-Institute for Natural Product Research and Infection Biology, Hans-Knöll-Institute, Molecular and Applied Microbiology, Jena, Germany
[2] Friedrich-Schiller-University, Jena, Germany
[3] Leibniz-Institute for Natural Product Research and Infection Biology, Hans-Knöll-Institute, Microbial Pathgenicity Mechanisms, Jena, Germany
[4] Leibniz-Institute for Natural Product Research and Infection Biology, Hans-Knöll-Institute, Infection Biology, Jena, Germany
"Functional characterisation of hemolysins of *Aspergillus fumigatus*"

MPV019 18:30 **M. Vranes[1]*, T. Langner[1], M. Scherer[2]**
[1] Institute for Applied Biosciences (IAB), Genetics, Karlsruhe Institute of Technology, Karlsruhe, Germany
[2] Qiagen, Hilden, Germany
"Regulating early infection and *in planta* development of *Ustilago maydis*"

SIV002 18:45 **S. Kloppholz*, H. Kuhn, N. Requena**
Botanical Institute, Plant-Microbial Interactions, Karlsruhe Institute of Technology (KIT), Karlsruhe, Germany
"An effector protein from the symbiotic fungus *G. intraradices* suppresses plant early defense responses"

FBV015 19:00 **M. Boenisch*, W. Schäfer**
Biocenter Klein Flottbek, Molecular Phytopathology and Genetics, University of Hamburg, Hamburg, Germany
"Infection structures and mycotoxin induction of *Fusarium graminearum* on wheat florets"

FBV016 19:15 **O. Voigt*, S. Pöggeler**
Institute for Microbiology and Genetics, Genetics of eukaryotic Microorganisms, Göttingen, Germany
"The Role of the autophagy related genes *Smatg4* and *Smatg8* in the sexual development of *Sordaria macrospora*"

Special Group Umweltmikrobiologie
Topic: Relevance of Ecological Principles in Environmental Microbiology

Organisation: T. Lueders and R. Meckenstock, German Research Center for Environmental Health, Helmholz-Centre Munich, Munich, Germany
Clubroom

ISV22 17:30 **C. van der Gast**
Natural Environment Research Council Centre for Ecology & Hydrology Population and Community Ecology Section, Oxford, UK
"Applying ecological principles to microbial systems: Partitioning core and satellite taxa from within bacterial communities"

ISV23 18:00 **A. Ramette**
Max Plank Institute for Marine Microbiology, Bremen, Germany
"Effects of space and ecosystem type on the structuring of marine microbial communities at the global scale"

EMV009 18:30 **M. Schloter**
Helmholtz-Centre Munich, Terrestrial Ecogenetics, Oberschleissheim, Germany
"Dynamics and drivers of ammonia oxidizing microbes in soil"

EMV010 18:45 **A. Chatzinotas*, R. Schäwe, M. Saleem, I. Fetzer, H. Harms**
Environmental Microbiology, Helmholtz-Centre for Environmental Research – UFZ, Leipzig, Germany
"Microbial model systems and ecological theory: How does increasing environmental stress affect microbial interactions and ecosystem services?"

EMV011 19:00 **G. Pilloni[1]*, A. Bayer[1], B. Anneser[1], M. Engel[2], C. Griebler[1], T. Lueders[1]**
[1] Helmholtz-Centre Munich, Groundwater Ecology, Neuherberg, Germany
[2] Helmholtz-Centre Munich, Terrestrial Ecogenetics, Institute of Soil Ecology, Neuherberg, Germany
"Disturbance ecology controls natural attenuation in contaminated aquifers"

Mini-Symposia of the Special Groups: Monday, April 4, 17:30–19:30

EMV012 **19:15** **D.P.R. Herlemann[1]*, M. Labrenz[1], K. Jürgens[1], S. Bertilsson[2], J.J. Waniek[1], A.F. Andersson[3]**
[1] Leibniz Institute for Baltic Sea Research, Warnemünde, Biological Oceanography, Rostock, Germany
[2] Department of Ecology & Genetics, Limnology, Uppsala University, Uppsala, Sweden
[3] KTH Royal Institute of Technology, Science for Life Laboratory, Stockholm, Sweden
"The Baltic Sea microbiome: bacterial transitions along a 2000 km salinity gradient"

Special Group Regulation und Signaltransduktion in Prokaryoten/Mikrobielle Pathogenität
Topic: Second Messengers in Bacteria

Organisation: P. Dersch, Department for Molecular Infection Biology, Helmholtz Centre for Infection Research, Braunschweig, Germany; K. Jung, Biozentrum Department Biologie I, Bereich Mikrobiologie, Ludwig-Maximilians-Universität, Martinsried, Germany
Room 2.05

ISV24 **17:30** **U. Jenal**
Biozentrum, University of Basel, Basel, Switzerland
"Mechanisms of c-di-GMP mediated cell cycle control in *Caulobacter crescentus*"

ISV25 **18:00** **A. Camilli**
Department of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, USA
"Dynamic cyclic di-GMP signaling in *Vibrio cholerae* during infection"

RGV001 **18:30** **S. de Causmaecker*, A. Wilde**
Institute of Micro- and Molecular Biology, Justus-Liebig-Universtiy, Gießen, Germany
"The role of the c-di-GMP in phototactic motility of Synechocystis sp. PCC 6803 cells"

Special Group Struktur und Mikroskopie
Topic: Structure of the Microbial Cytoplasm

Organisation: H. Engelhardt, Max-Planck-Institut für Biochemie, Martinsried, Germany
Hebel Hall

ISV26 **17:30** **J.O. Ortiz*[1], F. Brandt[1], V. Matias[1], S. Etchells[2], F.U. Hartl[2] and W. Baumeister[1]**
[1] Department of Structural Biology, Max-Planck Institute of Biochemistry, Martinsried, Germany
[2] Department of Cellular Biochemistry, Max-Planck Institute of Biochemistry, Martinsried, Germany
"From isolated molecules to intact cells: Structure of ribosomal arrangements in vitro and in situ"

ISV27 **18:00** **J. Elf**
Department for Cell and Molecular Biology Program for Computational and Systems Biology, Uppsala, Sweden
"Fast tracking of individual protein in the bacterial cytoplasm"

ISV28 **18:30** **Z. Luthey-Schulten**
Department of Chemistry, University of Illinois, Urbana, USA
"Protein and RNA dynamics in living cells"

NTV001 **19:00** **V. Sourjik**
Center for Molecular Biology (ZMBH), University of Heidelberg, Heidelberg, Germany
"Protein mobility in bacterial cytoplasm"

NTV002 **19:15** **H. Cypionka**
Institute for Chemistry and Biology of the Marine Environment, University of Oldenburg, Oldenburg, Germany
"Hologram stacking with PICOLAY: How to get confocal microscopy for free"

ACTIVITIES OF THE SPECIAL GROUPS

Mini-Symposia of the Special Groups: Monday, April 4, 17:30–19:30

Special Group Identifizierung und Systematik
Topic: Lipids as Part of Cellular Processes

Organisation: B. Tindall, German Collection of Microorganisms and Cell Cultures (DSMZ), Braunschweig, Germany; H.J. Busse, Institute for Bacteriology, Mycology and Hygiene, University of Veterinary Medicine, Vienna, Austria
Forum 1 & 2

OTV007 17:30 **B. Tindall**
German Collection of Microorganisms and Cell Cultures (DSMZ), Braunschweig, Germany
"Lipids – The fourth cornerstone in biological chemistry"

ISV29 17:45 **O. Geiger*, C. Sohlenkamp, I. López-Lara**
Center for Genomic Sciences, National Autonomous University of Mexico, Cuernavaca, Morelos, Mexico
"Biosynthesis and remodeling of bacterial membrane lipids"

ISV30 18:15 **Y.M. Zhang**
Department of Biochemistry & Molecular Biology, Medical University of South Carolina, Charleston, USA
"Regulation of membrane homeostasis in *Pseudomonas aeruginosa*"

ISV31 18:45 **M. Grininger**
Department of Membrane Biochemistry, Project Group "Biological Chemistry", Max Planck Institute of Biochemistry, Martinsried, Germany
"Fatty acid synthesis in fungal type I protein complexes"

OTV008 19:15 **B. Tindall**
German Collection of Microorganisms and Cell Cultures (DSMZ), Braunschweig, Germany
"Structural analysis of the polar lipids of Sphingobacterium spiritivorum and Pedobacter heparinus"

Mini-Symposia of the Special Groups: Wednesday, April 6, 09:00–11:30

Special Group Biotransformation
Topic: Hydroxylation, Oxygenation and Oxidation Reactions

Organisation: C. Syldatk, Karlsruhe Institute of Technology (KIT), Department of Chemical Engineering, Technical Biology, Karlsruhe, Germany, J. Eck, BRAIN AG, Zwingenberg, Germany
Clubroom

ISV32 09:00 **W. Aehle*, F. Niehaus**
BRAIN AG, Zwingenberg, Germany
"Universal high throughput FACS based screening systems for the discovery and optimization of biocatalysts from enzyme libraries"

GWV017 09:30 **D. Scheps*, S. Honda Malca, B. Nestl, B. Hauer**
University of Stuttgart, Institute of Technical Biochemistry, Stuttgart, Germany
"Regioselective hydroxylation of medium-chain ***n***-alkanes and primary alcohols by CYP153 enzymes"

GWV018 09:45 **A. Meffert*, E.A. Galinski**
Rheinische Friedrich-Wilhelms-Universität, Institut für Mikrobiologie und Biotechnologie, Bonn, Germany
"Whole-cell biotransformation for the stereospecific hydroxylation of the incompatible solute guanidinoectoine"

GWV019 10:00 **B. Halan*, A. Schmid, K. Buehler**
Technical University Dortmund, Laboratory of Chemical Biotechnology, Department of Biochemical and Chemical Engineering, Dortmund, Germany
"Catalytic biofilms: Real time solvent tolerance analysis of *Pseudomonas* sp. strain VLB120?C and profiling of EPS matrix"

GWV020 10:15 **M. Kluge[1]*, R. Ullrich[1], K. Scheibner[2], M. Hofrichter[1]**
[1]Internationales Hochschulinstitut Zittau (IHI), Bio- and Environmental Sciences, Zittau, Germany
[2]Lausitz University of Applied Sciences, Biology, Chemistry and Process Technology, Senftenberg, Germany
"Asymmetric benzylic hydroxylation and epoxidation of alkylbenzenes and styrene derivatives by *Agrocybe aegerita* aromatic peroxygenase"

Monday, April 4, 08:30–10:30

Environmental Microbiology I

Brahms Hall

Chair: Michael Friedrich
Co-Chair: Harold Drake

EMV001
08:30
S. HUNGER*, O. SCHMIDT, M. HILGARTH, M.A. HORN, S. KOLB, H.L. DRAKE
Anaerobic formate- and CO_2-assimilating prokaryotic taxa in a methane-emitting fen soil

EMV002
08:45
S. THIELE*, B. FUCHS, V. SMETACEK, K. ALTENDORF, R. AMANN
LOHAFEX – Investigation of the bacterial community in an iron fertilization experiment

EMV003
09:00
S. KLEINDIENST*, F. MUSAT, T. LUEDERS, F. VON NETZER, R. AMANN, K. KNITTEL
Active hydrocarbon-degrading sulfate-reducing bacteria at marine gas and oil seeps

EMV004
09:15
C. ALGORA*, K. WASMUND, C. RIDLEY, J. MÜLLER, T.G. FERDELMAN, L. ADRIAN
Enrichment of *Dehalococcoides*-related *Chloroflexi* from marine subsurface sediments

EMV005
09:30
J. DEUTZMANN*, B. SCHINK
Anaerobic oxidation of methane in Lake Constance sediments

EMV006
09:45
A. REIM*, P. FRENZEL
Half a millimeter makes a difference: a microscale study on distribution and specific activity of methanotrophs at an oxic-anoxic interface

EMV007
10:00
M. HERRMANN*, K. BUROW, A. HÄDRICH, K. KÜSEL
Archaea dominate the ammonia-oxidizing microbial community in an acidic fen

EMV008
10:15
K. GLASER*, J. JOHNKE, H. HARMS, A. CHATZINOTAS
How does land use influence bacterivorous protists in soils?

Pathogen Metabolism and Physiology

Mombert Hall

Chair: Jörg Vogel
Co-Chair: Sven Krappmann

MPV001
08:30
J. TOLLER*, B. ROSCHITZKI, C. FORTES, M. GIVSKOV, L. EBERL, K. RIEDEL
A metaproteomics approach to study host-pathogen interactions between *Pseudomonas aeruginosa* and *Caenorhabditis elegans*

MPV002
08:45
J. GLEICHENHAGEN*, M. WESSEL, M. AKTAS, S. KLÜSENER, S. HACKER, C. FRITZ, F. NARBERHAUS
A typical eukaryotic lipid in prokaryotic membranes: Synthesis and necessity of phosphatidylcholine in *Agrobacterium tumefaciens*

MPV003
09:00
C. LANG*, E. RASTEW, B. HERMES, E. SIEGBRECHT, S. BANERJI, A. FLIEGER
Virulence properties of *Legionella pneumophila* GDSL lipolytic enzymes: Proteolytic activation of PlaC acyltransferase activity

MPV004
09:15
N. GÖHRING*, I. FEDTKE, D. MADER, S. HEINRICH, D. KÜHNER, U. BERTSCHE, A. PESCHEL
A *yjbH*-homologue in *S. aureus*: a new role of a thioredoxin-like protein in ß-lactam resistance

MPV005
09:30
P. TIELEN*, N. ROSIN, L. JÄNSCH, M. SCHOBERT, D. JAHN
Iron-limitation triggers the virulence of *Pseudomonas aeruginosa* in urinary tract infections

MPV006
09:45
A. DJAMEI*, K. SCHIPPER, R. KAHMANN
Metabolomic priming by a secreted fungal effector

MPV007
10:00
M. BISCHOFF*, T. HARTMANN, R. BERTRAM, W. EISENREICH, B. SCHULTHESS, C. WOLZ, M. HERRMANN
SACOL0731, a new regulatory link between central carbon metabolism and virulence determinant production in *Staphylococcus aureus*

MPV008
10:15
A. WESCHE*, S. THOMA, M. HOGARDT, E. JORDAN, D. SCHOMBURG, M. SCHOBERT
Characterization of methionine auxotrophic clinical *Pseudomonas aeruginosa* isolates

Cellular Systems in Biotechnology

Clubroom

Chair: Ursel Kües
Co-Chair: Bernhard Hauer

GWV001
08:30
N. STOEVEKEN*, M. PITTELKOW, T. SINNER, J. HEIDER, E. BREMER
Paralogues aspartokinases from *Pseudomonas stutzeri* A1501: synthesis of the precursor for the compatible solutes ectoine and hydroxyectoine

GWV002
08:45
M. OELSCHLÄGEL*, J.A.D. GRÖNING, D. TISCHLER, S.R. KASCHABEK, M. SCHLÖMANN
A remarkable stable and active styrene oxide isomerase from *Rhodococcus opacus* 1CP with high biotechnological potential

GWV003
09:00
V. HAHN*, A. MIKOLASCH, F. SCHAUER
The enzyme laccase as biocatalyst for the synthesis of various novel organic compounds with potent bioactive properties

GWV004
09:15
V. KALLNIK*, C. SCHULZ, P. SCHWEIGER, U. DEPPENMEIER
The first hyperthermophilic D-arabitol dehydrogenase catalyzes the regiospecific oxidation of D-arabitol to D-ribulose

Monday, April 4, 08:30–10:30

GWV005
09:30
L. LAUTERBACH*, Z. IDRIS, J. LIU, K.A. VINCENT, O. LENZ
Cofactor regeneration: understanding the catalytic properties of the NAD$^+$-reducing [NiFe]-hydrogenase from *Ralstonia eutropha* by investigating its subcomplexes

GWV006
09:45
J. BECKER*, H. SCHRÖDER, O. ZELDER, S. HAEFNER, A. HEROLD, C. KLOPPROGGE, C. WITTMANN
Design-based construction of a lysine hyper-producing strain by *Systems Metabolic Engineering*

GWV007
10:00
F. JANKOWITSCH*, M. MACK
The gene rosA encoding N,N-8-amino-8-demethyl-D-riboflavin dimethyltransferase is located within a gene cluster possibly involved in biosynthesis of roseoflavin in Streptomyces davawensis

GWV008
10:15
S. REICH*, B. M. NESTL, B. HAUER
Enzyme Engineering of an Enoate Reductase from *Zymomonas mobilis* Affecting the Enzyme Activity and Enantioselectivity

NTV005
10:30
N. MUSTAFI*, M. BOTT, J. FRUNZKE
Development of a novel biosensor for the intracellular detection of L-methionine and branched-chain amino acids

Plant-Microbe Interactions

Room 2.05

Chair: Anke Becker
Co-Chair: Philipp Franken

FBV001
08:30
C. PLESKEN
Grey mould isolates from German strawberry fields reveal a new type of multidrug resistance and evidence for a novel taxon next to *Botrytis cinerea*

FBV002
08:45
D. MERNKE
Mutations and migration of *Botrytis cinerea* field strains with multidrug resistance phenotypes in French and German vineyards

FBV003
09:00
E.M. NIEHAUS*, B. TUDZYNSKI
Molecular and chemical characterization of secondary metabolite gene clusters in *Fusarium fujikuroi*

FBV004
09:15
A.L. MARTINEZ-ROCHA*, M. WORIEDH, W. SCHÄFER
Preventing fusarium head blight of wheat and cob rot of maize by inhibition of fungal deoxyhypusine synthase

FBV005
09:30
T. NGUYEN*, J. BORMANN, B. HADELER, C. KRÖGER, W. SCHÄFER
The mitogen-activated protein kinase HOG1 in *Fusarium graminearum* is involved in osmoregulation, sexual reproduction and virulence

MPV009
09:45
K. HEIMEL*, M. SCHERER, S. HASSINGER, J. KAEMPER
Connecting cell cycle to pathogenic development-regulatory cascades during pathogenesis of *Ustilago maydis*

SIV001
10:00
C. VOGT*, H. KUHN, N. REQUENA
A screen to identify fungal and plant signals during arbuscule formation in AM symbiosis

OTV001
10:15
J. SCHIRRMEISTER*, S. ZEHNER, M. WENZEL, L. FRIEDRICH, M. HOPPE, M. GÖTTFERT
The C-terminal domain DUF1521 of the *Bradyrhizobium japonicum* protein and its functional stability

Fungal Biology and Development

Hebel Hall

Chair: Erika Kothe
Co-Chair: Matthias Hahn

FBV025
08:30
D. FREIHORST*, E. KOTHE
Orphan GPCRs in *Schizophyllum commune*

FBV007
08:45
K. BACKHAUS*, J. HEINISCH
Cell wall thickness and composition in the yeasts *Saccharomyces cerevisiae* and *Kluyveromyces lactis* adapt to growth conditions

FBV006 will be presented as poster with the ID FBP045

FBV008
09:00
E. VOLLMEISTER*, C. HAAG, S. BAUMANN, M. FELDBRÜGGE
The AUACCC-binding protein Khd4 regulates cell morphology and pathogenicity in *Ustilago maydis*

FBV009
09:15
A. HERRERA*, U. ESQUIVEL-NARANJO, M. HERNÁNDEZ-OÑATE, E. IBARRA-LACLETTE
Molecular basis of photoconidiation in the filamentous fungus *Trichoderma atroviride*

FBV010
09:30
M. LEROCH
Using codon-improved GFP for imaging gene expression during germination and host penetration of *Botrytis cinerea* conidia

FBV011
09:45
A. HERRMANN*, B. TILLMANN, M. BÖLKER, P. TUDZYNSKI
Characterization of small GTPase complexes and their effects on polar growth during infection of *Claviceps purpurea*

FBV012
10:00
H.W. NÜTZMANN*, V. SCHROECKH, K. SCHERLACH, K. MARTIN, C. HERTWECK, A. BRAKHAGE
Interaction between *Streptomycetes* and *Aspergillus nidulans*

FBV013
10:15
S. PÖGGELER*, C.M. O'GORMAN, B. HOFF, U. KÜCK
Analysis of the mating-type loci from the homothallic Ascomycete *Eupenicillium crustaceum*

Monday, April 4, 08:30–10:30

Oxidative Stress Responses

Forum 1

Chair: Torsten Mascher
Co-Chair: Gerhard H. Braus

SRV001
08:30
D. ORTIZ DE ORUÉ LUCANA*, M. ROSCHER, H. SCHREMPF
Molecular mechanisms governing the three-component system HbpS-SenS-SenR from *Streptomyces reticuli*

SRV002
08:45
J. GLAESER*, B. BERGHOFF, A. NUSS, M. ZOBAWA, F. LOTTSPREICH, G. KLUG
Anoxygenic photosynthesis and photooxidative stress: A particular challenge for *Roseobacter*

SRV003
09:00
F. MARX*, A. EIGENTLER, B. DE CASTRO PIMENTEL FIGUEIREDO, T. MAGNANI DINAMARCO, G.H. GOLDMAN
The apoptosis inducing factor (AIF)-like mitochondrial oxidoreductase (*aifA*) mediates reistance towards the *Penicillium chryosgenum* antifungal protein PAF in *Aspergillus fumigatus*

SRV004
09:15
I. OBERPICHLER*, J. WESSLOWSKI, R. POKORNY, R. ROSEN, F. ZHANG, O. NEUBAUER, A. BATSCHAUER, E. RON, T. LAMPARTER
A novel type of DNA photolyase containing an iron sulfur cluster

SRV005
09:30
H. ANTELMANN*, B.K. CHI, G. PALM, K. GRONAU, U. MÄDER, D. BECHER, W. HINRICHS, M. HECKER
Specific control of hypochlorite resistance by the redox-sensing MarR/DUF24-type regulator HypR in *Bacillus subtilis*

SRV006
09:45
C. HERNANDEZ*, A. PEREIRA, P. TAVARES, S. ANDRADE
Structural studies on the Iron core formation in *Marinobacter hydrocarbonoclasticus* Dps

SRV007
10:00
K. MARIN*, C. LANGE, C. TRÖTSCHEL, D. SEIFERLING, I. OCHROMBEL, A. POETSCH, R. KRÄMER
Characterisation of the oxidative stress response in *C. glutamicum*

SRV008
10:15
G. STURM*, J. GESCHER
On the multitude of mechanisms that establish high-level heavy metal resistance in an aggregate forming bacterium

Special Session

Science and Infrastructure

Forum 2

Chair: Dagmar Fritze
Co-Chair: Philippe Desmeth

OTV002
08:30
D. FRITZE
Culture collections' provision of continuity for academic and industrial research – meeting the emerging challenges

OTV003
08:50
B. TINDALL
Authenticity of microbiological material – the impact in the research environment

OTV004
09:10
D. SMITH
Capacity building, transnational access and encouragement for the deposit of microbiological material – the EU project EMbaRC

OTV005
09:30
P. DESMETH
The ABS Protocol

OTV006
09:50
P. DESMETH
Tools to implement the Nagoya Protocol on ABS in microbiology

17:30–19:30

Enzymatic Systems in Biotechnology

Mombert Hall

Chair: Alexander Steinbüchel
Co-Chair: Michael Bott

GWV009
17:30
B. BLOMBACH*, T. RIESTER, S. WIESCHALKA, C. ZIERT, J.-W. YOUN, V.F. WENDISCH, B.J. EIKMANNS
***Corynebacterium glutamicum* engineered for efficient isobutanol production**

GWV010
17:45
S. LINDNER*, G.M. SEIBOLD, A. HENRICH, R. KRÄMER, V.F. WENDISCH
Phosphotransferase system (PTS) independent glucose utilization in *Corynebacterium glutamicum* by inositol permeases and glucokinases and application for improved L-lysine production

GWV011
18:00
B. ANDREEßEN*, A. STEINBÜCHEL
Biotechnological conversion of glycerol to 2-amino-1,3-propanediol (serinol) in recombinant *Escherichia coli*

GWV012
18:15
S. LÜTTE*, A. POHLMANN, H. HEUMANN, A. STEINBÜCHEL, B. FRIEDRICH
Autotrophic Production of Stable Isotope-labelled Amino Acids

GWV013
18:30
J. HANGEBRAUK*, R. STELLMACHER, R. SCHÄFER, J. BECKER, G. VON ABENDROTH, H. SCHRÖDER, S. HAEFNER, C. WITTMANN
Systems Metabolic Engineering of *Basfia succiniciproducens* for Biobased Production of Succinic Acid

GWV014
18:45
U. ENGEL*, B. BRUCHER, C. SYLDATK, *J. RUDAT
Chemoenzymatic synthesis and microbial degradation of enantiopure aromatic beta-amino acids

Monday, April 4, 17:30–19:30

GWV015
19:00
M. SEITZ, J. KLEBENSBERGER, M. BREUER, B. HAUER*
Natural Product Synthesis by Squalene-Hopene Cyclases (SHCs)

GWV016
19:15
M.M. MÜLLER*, B. HÖRMANN, C. SYLDATK, R. HAUSMANN
Rhamnolipids- Green Surfactants Based on Renewables

Anaerobic Metabolism

Room 2.08

Chair: Matthias Boll
Co-Chair: Gabriele Diekert

AMV001
17:30
A. REINHOLD*, M. WESTERMANN, T. FUTAGAMI, K. FURUKAWA, J. SEIFERT, M. VAN BERGEN, T. SCHUBERT, G. DIEKERT
Subcellular localization of *pce* gene products: implications for the biogenesis of physiologically active tetrachloroethene (PCE) reductive dehalogenase

AMV002
17:45
A. M. MOWAFY*, T. KURIHARA, W. BUCKEL, N. ESAKI
Evidence for the involvement of one electron transfer chemistry in 2-haloacrylate hydratase reaction

AMV003
18:00
K. SCHÜHLE*, J. HEIDER
***Acetophenone carboxylase* and *Acetone carboxylase*, enzymes employing new biochemical principles for carboxylation reactions**

AMV004
18:15
C. LÖFFLER*, J.W. KUNG, T. WEINERT, U. ERMLER, M. BOLL
The W-/Se-containing class II benzoyl-CoA reductase complex in obligately anaerobic bacteria

AMV005
18:30
J. ZEDELIUS*, R. RABUS, M.M.M. KUYPERS, F. SCHREIBER, F. WIDDEL
Nitrogen oxides involved in anaerobic alkane activation by strain HdN1

AMV006
18:45
W. J. MAALCKE*, C. FEROUSI, T.R. BARENDS, W.J. GEERTS, J.T. KELTJENS, M.S.M. JETTEN, B. KARTAL
The biochemistry of anaerobic ammonium oxidation

AMV007
19:00
C. PINSKE*, S. KRÜGER, M. BÖNN, G. SAWERS
The explanation for the hydrogenase-negative Phenotype of *Escherichia coli* B strain BL21(DE3)

AMV008
19:15
G. LAYER*, S. STORBECK
Structure and function of the SAM-dependent uroporphyrinogen III methyltransferase NirE involved in heme d_1 biosynthesis in *Pseudomonas aeruginosa*

Tuesday, April 5, 08:30–10:30

Cell Biology I

Brahms Hall

Chair: Reinhard Rachel
Co-Chair: Gero Steinberg

CBV001
08:30
S. FRIESER
Dynamic regulation of the Cdc24/Rac1/Cla4 signalling module during dimorphic switching of the phytopathogenic fungus *Ustilago maydis*

CBV002
08:45
A. TREUNER-LANGE*, K. AGUILUZ, L. SOGAARD-ANDERSEN
The ParA-like protein PomZ positively regulates positioning of the cell division site

CBV003
09:00
R. MOURIÑO-PÉREZ*, D.L. CALLEJAS-NEGRETE, R. DELGADO-ALVAREZ
Actin and actin binding proteins during polarized growth and septum formation in *Neurospora crassa*

CBV004
09:15
F. DEMPWOLFF*, C. REIMOLD, P.L. GRAUMANN
Interaction of bacterial cytoskeletal elements in a heterologous system

CBV005
09:30
N. ZEKERT*, C. SEIDEL, R. FISCHER
The Kinesin-3 Motor Protein UncA Reveals Different Microtubule Populations in *Aspergillus nidulans*

CBV006
09:45
S. SCHLIMPERT*, A. BRIEGEL, K. BOLTE, U.G. MAIER, J. KAHNT, G.J. JENSEN, M. THANBICHLER
Physical compartmentalization by a protein diffusion barrier in stalked alphaproteobacteria

CBV007
10:00
T. POHLMANN*, S. BAUMANN, M. JUNGBLUTH, M. FELDBRÜGGE
Microtubule-dependent co-transport of mRNPs and vesicles in *Ustilago maydis*

Tuesday, April 5, 08:30–10:30

CBV008
10:15
F. D. MÜLLER*, M. MESSERER, K. EMANUEL, C. LANG, J. PLITZKO, D. SCHÜLER
Functional analysis of cytoskeletal proteins implicated in magnetosome formation and cell division in *Magnetospirillum gryphiswaldense*

Virulence Factors

Mombert Hall

Chair: Petra Dersch
Co-Chair: Andreas Peschel

MPV010
08:30
Y. ZHAO*, H. GHAREEB, M.T. HABIB, J. SCHIRAWSKI
The molecular basis of symptom formation in *Sporisorium reilianum*

MPV011
08:45
C. HEDDERGOTT*, O. KNIEMEYER, A.A. BRAKHAGE
Secreted proteins of the dermatophytic fungus *Arthroderma benhamiae* and their contribution to pathogenicity

MPV012
09:00
P. KAISER*, D. LINKE, H. SCHWARZ, V. KEMPF*
Generation and functional characterization of truncated *Bartonella henselae* BadA mutants

MPV013
09:15
T. FUCHS
Interaction of *Yersinia* spp. with invertebrates

MPV014
09:30
J. OVERHAGE*, A. ZIMMERMANN, B. NUORI, A. NEIDIG, S. HÄUSSLER, C. MATZ
***Pseudomonas aeruginosa* virulence analyzed in a *Dictyostelium discoideum* model of infection**

MPV020
09:45
L. POPPINGA*, A. FÜNFHAUS, E. GARCIA-GONZALEZ, B. JANESCH, C. SCHÄFFER, E. GENERSCH
Functional analysis of the S-layer protein of *Paenibacillus* larvae

MPV015 will be presented as poster with the ID MPP066

MPV016
10:00
M. KOLBE*, M. LUNELLI
Structure/Function Analysis of the Type 3 Secretion System from *Salmonella typhimurium*

MPV017
10:15
C. WEIDENMAIER
The zwitterionic cell wall teichoic acid of *Staphylococcus aureus* provokes skin abscesses in mice by a novel CD4+ T-Cell-dependent mechanism

Food Microbiology

Clubroom

Chair: Christian Hertel
Co-Chair: Herbert Schmidt

FMV001
08:30
E. GRAF*, M. SCHMIDT-HEYDT, R. GEISEN
Influence of osmotic and pH stress on the alternariol biosynthesis in *Alternaria alternata*

FMV002
08:45
S. POLZIN*, I. ELSENHANS, H. SCHMIDT
Differential proteomic expression of enterohaemorrhagic *E. coli* O157:H7 EDL933 grown in intestinal simulating media

FMV003
09:00
M. NOLL*, C. WEILER, A. IFLAND, S. SIGIEL, A. NAUMANN
Characterisation of the incorporation of *Listeria monocytogenes* in a raw milk-biofilm

FMV004
09:15
A. WEISS*, S. WILD, H. SCHMIDT
Survival of *Listeria monocytogenes* in lubricants applied in the food industry

FMV005
09:30
M. SCHUPPLER*, L. AMATO, J. RITSCHARD, E. ROTH, L. MEILE
The impact of vacuum foil packaging on the quality characteristics of the surface smear microflora of semi-hard smear cheese

FMV006
09:45
M. WASSERMANN*, S. WEINHOLZ, C. CORDES, M. PEGLOW, W. PERGANDE
Granulation of lactic acid bacteria using the fluidized bed technology

FMV007
10:00
M. WIESCHEBROCK*, F. SCHILLING, C. HERTEL
Generation of new flavours in wheat doughs supplemented with by-products and fermented with non-*Saccharomyces cerevisiae* yeasts

FMV008
10:15
P. SEBASTIAN*, V. BLÄTTEL, E. GASSER, H. CLAUS, P. PFEIFFER, H. KÖNIG
A novel enzymatic approach for growth inhibition of undesired wine related microorganisms

Fungal Biotechnology

Room 2.05

Chair: Paul Tudzynski
Co-Chair: Bettina Tudzynski

FBV017
08:30
R. LEHNECK*, S. PÖGGELER
Characterization of a putative a-carbonic anhydrase from the filamentous ascomycete *Sordaria macrospora*

FBV018
08:45
A. NEUMANN*, K. BRZONKALIK, C. SYLDATK
Characterization, purification and cloning of the O-Methyltransferase of *Alternaria alternata*

FBV019
09:00
S. ALBERMANN*, B. TUDZYNSKI
Approaches for directed strain improvement targeting enhanced biosynthesis of gibberellic acid in *Fusarium fujikuroi*

FBV020
09:15
J. BORMANN*, P. ILGEN, C. KRÖGER, B. HADELER, W. SCHÄFER
New insights in the regulation of mycotoxin production in the plant pathogen *F. graminearum*

Tuesday, April 5, 08:30–10:30

FBV021
09:30
D. SAHA*, R. FISCHER
Molecular analysis of polyketide synthase genes involved in secondary metabolism of *Alternaria alternata*

FBV022
09:45
O. KNIEMEYER*, M. VÖDISCH, K. SCHERLACH, R. WINKLER, C. HERTWECK, H.-P. BRAUN, M. ROTH, H. HAAS, E.R. WERNER, A.A. BRAKHAGE
Analysis of the *Aspergillus fumigatus* proteome reveals metabolic changes and the activation of the pseurotin A biosynthesis gene cluster in response to hypoxia

FBV023
10:00
M. HOFFMANN*, J. ZIMMERLING, S.R. KASCHABEK, G. SCHÜÜRMANN, M. SCHLÖMANN
Fungal systems – Tools for the milligram-to gram-scale preparation of an environmentally relevant metabolite of fenoprofen

FBV024
10:15
P. WEIßHAUPT*, W. PRITZKOW, M. NOLL
Nitrogen metabolism of wood decomposing basidiomycetes and their interaction with diazotrophs as revealed by IRMS

Environmental Microbiology – ISME

Room 2.08

Chair: Meinhard Simon
Co-Chair: Jörg Overmann

EMV013
08:30
L. RIEMANN*, K. HOLMFELDT, M. MIDDELBOE, D. ODIC
Complex interactions between marine phages and their *Flavobacterium* hosts

EMV029
09:00
M. BIZIC-IONESCU*, B. FUCHS, R. AMANN, H.-P. GROSSART
Aggregate-colonizing microbial communities - a comparison of marine vs. freshwater systems

EMV014 will not be presented!

EMV015
09:15
H. FREESE*, B. SCHINK
The bacterial community in the digestive tract of the small aquatic crustacean *Daphnia magna*

EMV016
09:30
S.U. GERBERSDORF*, H.V. LUBARSKY, D.M. PATERSON, S. WIEPRECHT, W. MANZ
Microbial engineers control sediment dynamics in aquatic habitats

EMV017
09:45
A. RUSZNYAK*, D.M. AKOB, S. NIETZSCHE, T.R. NEU, K. KÜSEL
Calcite biomineralization in a karstic cave – bacteria hidden in the dark

EMV018
10:00
S. TÖWE*, A. ALBERT, K. KLEINEIDAM, R. BRANKATSCHK, J.C. MUNCH, J. ZEYER, M. SCHLOTER
Abundance of microbes involved in nitrogen transformation in the rhizosphere of *Leucanthemopsis alpina* (L.) Heywood grown in soils from different sites of the Damma glacier forefield

EMV019
10:15
C. JOGLER*, G. WANNER, S. KOLINKO, M. NIEBLER, W. LIN, Y. PAN, P. STIEF, A. BECK, D. DE BEER, R. AMANN, N. PETERSEN, M. KUBE, R. REINHARDT, D. SCHÜLER
Ultrastructural, genomic and ecological analysis of "*Candidatus* Magnetobacterium bavaricum" reveals a mechanism homologous to proteobacterial magnetosome formation

Experimental Progress and Molecular Tools

Hebel Hall

Chair: Ulrich Kück

FGV002
08:30
D. ZHURINA*, C. RIEDEL
Genome mining of anti-inflammatory *B. bifidum* S 17 reveals multiple loci potentially involved in host-microbe interactions

NTV003
08:45
I. TEICHERT*, M. NOWROUSIAN, U. KÜCK
Studying fungal development: Utilization of laser capture microdissection and next-generation sequencing techniques

OTV009
09:00
S. RUDEN, R. MIKUT, K. HILPERT*
Short cationic antimicrobial peptides versus multidrug resistant bacteria

OTV010
09:15
A. RIEDER*, T. LADNORG, C. WÖLL, U. OBST, R. FISCHER, T. SCHWARTZ
Recombinant hydrophobin coated surfaces and their influence on microbial biofilm formation

EMV020
09:30
M. PATEL, S. POLSON, U. HERBER*
Comparison of Genotypic, Proteotypic and Phenotypic Methods for the Identification of Bacteria

OTV011
09:45
H. ENGELHARDT*, M. EIBAUER, C. HOFFMANN
Investigating membrane proteins *in situ* by cryo-electron tomography

CBV009
10:00
D. DELGADO-ÁLVAREZ*, O. CALLEJAS-NEGRETE, R. MOURIÑO-PÉREZ
Imaging of the *Neurospora crassa* actin cytoskeleton with Lifeact

NTV004
10:15
T. GÜNTHER*, J. RAFF, K. POLLMANN
A simple method to prepare microorganisms for AFM analysis

Archaea

Forum 1 & 2

Chair: Jörg Soppa
Co-Chair: Felicitas Pfeifer

ARV001
08:30
M. ROTHER*, T. STOCK, S. GOETZ
Replacing the archaeal path of selenocysteine biosynthesis with the bacterial

Tuesday, April 5, 08:30–10:30

ARV002
08:45
B. MOLITOR*, N. FRANKENBERG-DINKEL
A heme-based redox sensor in the methanogenic archaeon *Methanosarcina acetivorans*

ARV003
09:00
B. MEYER*, S.-V. ALBERS
Elucidation of the *N*-glycosylation pathway in the thermoacidophilic crenarchaeon *Sulfolobus acidocaldarius*

ARV004
09:15
L. KREUTER*, U. KÜPER, T. HEIMERL, A. RÖHL, F. MAYER, V. MÜLLER, R. RACHEL, H. HUBER
Subcellular organization and energy conservation of *Ignicoccus hospitalis*

ARV005
09:30
K. LASSAK*, A. GHOSH, R. WIRTH, S.-V. ALBERS
Full speed ahead: analysis of the assembly of the archaeal flagellum

ARV006
09:45
R. WIRTH*, B. HERZOG
Microorganisms, peregrine falcons and cheetahs: Who is the fastest?

ARV007
10:00
S. FRÖLS*, F. PFEIFER
Screening and characterization of biofilm formation in halophilic Archaea

ARV008
10:15
M. NIKOLAUSZ*, R.F.H. WALTER, H. STRÄUBER, J. LIEBETRAU, T. SCHMIDT, S. KLEINSTEUBER, F. BRATFISCH, U. GÜNTHER, H.H. RICHNOW
Assessment of the predominant methanogenic pathways in anaerobic digesters by the combination of molecular techniques with the isotopic fingerprinting of the produced biogas

Wednesday, April 6, 09:00–11:00

Environmental Microbiology II

Brahms Hall

Chair: Andreas Brune
Co-Chair: Bodo Philipp

EMV021
09:00
D. IONESCU*, C. HEIM, M. BIZIC-IONESCU, V. THIEL, J. REITNER, L. POLERECKY, D. DE BEER
Insights into the community structure and activity of the iron oxidizing bacteria in the Äspö-Hard Rock Laboratory

EMV022
09:15
A. MÜLLER, M. FRIEDRICH*
Adaptation of the iron-reducing bacterial community to iron oxide availability in anoxic soil

EMV023
09:30
K. KUNTZE*, C. VOGT, H.-H. RICHNOW, M. BOLL
Insights in microbial communities – Functional marker genes in the anaerobic degradation of aromatic compound

EMV024
09:45
S. SCHELLENBERGER*, H.L. DRAKE, S. KOLB
Response of cellulose- and cellobiose-degrading soil bacteria to different redox potentials in an aerated soil

EMV025
10:00
B. DAM*, W. LIESACK
Genome analysis and transcriptome profiles of *Methylocystis* sp. strain SC2 grown at different methane concentrations

EMV026
10:15
H. WEINGART*, A. WENSING, S.D. BRAUN, D. SPITELLER, M.S. ULLRICH, B. VÖLKSCH
Suppression mechanisms involved in the antagonistic activity of *Pseudomonas syringae* strain 22d/93 against the soybean pathogen *Pseudomonas syringae* pv. *glycinea*

EMV027
10:30
S. JECHALKE*, C. KOPMANN, H. HEUER, K. KLEINEDAM, M. SCHLOTER, K. SMALLA
Effects of sulfadiazine entering via manure into soil on the abundance of antibiotic resistance genes and their transferability

EMV028
10:45
T. ENGELHARDT*, M. SAHLBERG, H. CYPIONKA, B. ENGELEN
Viral infections as controlling factor for the deep biosphere?

Microbial Diversity

Mombert Hall

Chair: Rudolf Amann
Co-Chair: Werner Liesack

MDV001
09:00
S. KOLINKO*, C. JOGLER, G. WANNER, E. KATZMANN, D. SCHÜLER
Single-cell analysis reveals unexpected phylogenetic and ultrastructural diversity of uncultivated magnetotactic bacteria

MDV002
09:15
K. RÖSKE*, I. RÖSKE
Complexity of the bacterial community in the sediment of the drinking water reservoir Saidenbach obtained by pyrosequencing

MDV003
09:30
C. KARWAUTZ*, K. HÖRMANN, T. LUEDERS
A close look at the diversity and dynamics of ultra-oligotrophic groundwater microbial communities during the restoration of a drinking water well

MDV004
09:45
A. HO*, C. LÜKE, P. FRENZEL
Recovery of methanotrophs from disturbance: population dynamics, evenness, and functioning

MDV005
10:00
S. E. RUFF*, J. ARNDS, K. KNITTEL, R. AMANN, G. WEGENER, A. RAMETTE, A. BOETIUS
Life in the cold, dark south – Microbial communities of marine methane seeps at Hikurangi margin (New Zealand)

MDV006
10:15
J. PETERSEN*, H. BRINKMANN, S. PRADELLA
Compatibility and phylogeny – Plasmid classification in the genomics era

Wednesday, April 6, 09:00–11:00

MDV007
10:30
M. JOGLER*, H. SIEMENS, H. CHEN, J. OVERMANN
Bacterial speciation – aquatic *Alphaproteobacteria* as a model system

MDV008
10:45
S. P. GLAESER*, V. STRATMANN, H.-P. GROSSAR[2], J. GLAESER
Singlet oxygen and hydrogen peroxide have different effects on Bacterioplankton community composition in a humic lake

Cell Biology II

Room 2.05

Chair: Martin Thanbichler
Co-Chair: Michael Feldbrügge

CBV010
09:00
R. RACHEL*, H. HUBER, U. KÜPER, C. MEYER, L. KREUTER, T. HEIMERL, R. WIRTH
Cell biology of *Ignicoccus hospitalis* – a unique crenarchaeon

CBV011
09:15
M. RIQUELME*, R.W. ROBERSON, S. BARTNICKI-GARCIA, M. FREITAG
Apical growth in *Neurospora crassa*

CBV012
09:30
E. SOMMER*, A. VAKNIN, A. MÜLLER, V. SOURJIK
Physical organization and interactions between sensory histidine kinases in *E. coli*

CBV013
09:45
G. STEINBERG*, N.J. SEVERS, P. ASHWIN, C. LIN, S. KILARU, M. SCHUSTER
Understanding long-range endosome trafficking: From measuring to modeling

CBV014
10:00
C. DONOVAN*, R. KRÄMER, M. BRAMKAMP
A synthetic in vivo system identifies a chromosome tethering factor in *Corynebacterium glutamicum*

CBV015
10:15
N. KELLNER*, K. HEIMEL, J. KÄMPER
The Num1 protein of *Ustilago maydis* is required for polar and filamentous growth

CBV016
10:30
H. STRAHL*, L. HAMOEN
Membrane potential plays a fundamental role in regulation and maintenance of bacterial morphology

CBV017
10:45
J. PÖHLMANN*, U. FLEIG
Vip1-like 1/3 inositol polyphosphate kinases regulate the dimorphic switch in yeasts

Other Stress Responses

Room 2.08

Chair: Hans-Ulrich Mösch
Co-Chair: Gottfried Unden

SRV009
09:00
M. KOHLSTEDT*, J. BECKER, C. KORNELI, P.K. SAPPA, H. MEYER, S. MAASS, M. LALK, U. MÄDER, E. BREMER, M. HECKER, U. VÖLKER, C. WITTMANN
Osmotic stress response in *Bacillus subtilis* – integration of the fluxome with the regulatory networks

SRV010
09:15
K. PAPENFORT*, D. PODKAMINSKI, C.K. VANDERPOOL, J. VOGEL
Post-transcriptional activation of the SacP phosphatase counteracts phosphosugar stress in enterobacteria

SRV011
09:30
D. WOLF*, M. REINECK, B. VOIGT, T. MASCHER
The phage-shock protein LiaH of *Bacillus subtilis*

SRV012
09:45
D. WARTENBERG*, M. VOEDISCH, O. KNIEMEYER, R. WINKLER, A.A. BRAKHAGE
Characterization of the farnesol-induced stress response in *Aspergillus nidulans*

SRV013
10:00
S. HUNKE*, P. SCHEERER, N. KRAUSS
Structural und functional insight into pilus sensing by the Cpx envelope stress system

SRV014
10:15
C. EYMANN*, S. SCHULZ, K. GRONAU, D. BECHER, M. HECKER, C.W. PRICE
***In vivo* phosphorylation patterns of key stressosome proteins define a second feedback loop that limits activation of *Bacillus subtilis* sB**

SRV015
10:30
I. HANEBURGER*, S. BUCHNER, A. EICHINGER, C. KOLLER, A. SKERRA, K. JUNG
Signal perception and transduction by the transcriptional activator CadC of *Escherichia coli*

SRV016
10:45
J. KLEBENSBERGER*, B. COLLEY, S. KJELLEBERG
Signal transduction and gene regulation in response to surfactant stress in *Pseudomonas aeruginosa*

Symbiotic Interactions

Hebel Hall

Chair: Ute Hentschel

SIV003
09:00
H.B. BODE
Drugs from bugs that kill bugs: Biosynthesis and function of natural products from entompathogenic bacteria

SIV004
09:15
C. THOMPSON*, C. SCHAUER, A. BRUNE
Host selection shapes microbial community structure in cockroach guts

SIV005
09:30
R. GROSS*, H. FELDHAAR, C. RATZKA
Immune response of the ant *Camponotus floridanus* against pathogens and its obligate mutualistic endosymbiont

SIV006
09:45
A. WILKENING*, S. DATTAGUPTA
The gut symbionts of *Niphargus* amphipods

SIV007
10:00
D. FINK*, C. BOROWSKI, N. DUBILIER
Symbiont response of deep-sea hydrothermal vent mussels to energy source removal

Wednesday, April 6, 09:00–11:00

SIV008
10:15
K. BAYER*, U. HENTSCHEL-HUMEIDA
Nitrogen fluxes in the Mediterranean sponge *Aplysina aerophoba* and its symbiotic microbial consortia

SIV009
10:30
H.P. GROSSART*, C. DZIALLAS
Interactions between the ciliate *Stentor amethystinus*, green algae and prokaryotes in Lake Stechlin

SIV010
10:45
P. FRANKEN*, S. KRESSNER, E. NEUMANN, E. GEORGE
Regulation of nutrient transporter genes in the extraradical mycelium of the arbuscular mycorrhizal fungus *Glomus intraradices*

Physiology and Regulation

Forum 1 & 2

Chair: Volker Müller
Co-Chair: Bernhard Eikmanns, Franz Narberhaus, Ruth Schmitz-Streit

PSV001
09:00
C. MAYER
Do Gram positives recycle their cell wall?

PSV002
09:15
T. J. ERB*, J.A. GERLT
A RubisCO-like protein links SAM-metabolism with isoprenoid biosynthesis

PSV003
09:30
A. PAULICK*, K. THORMANN
Flagellar motor tuning – a novel hybrid motor in *Shewanella oneidensis* MR-1

RGV002
09:45
S. SCHULMEISTER*, V. SOURJIK
Protein exchange dynamics and chemotaxis cluster stability in *Escherichia coli*

RGV003
10:00
C. M. SHARMA*, S. PERNITZSCH, G. GOLFIERI
***Helicobacter pylori* as a new model organism for riboregulation in bacteria lacking the RNA chaperone Hfq**

RGV004
10:15
T. WACKER*, T. PFLÜGER, S.L.A. ANDRADE, C. HERNÁNDEZ, S. MAIER, S. HELFMANN
Regulation of ammonium uptake and complex formation between Amt and GlnK proteins

RGV005
10:302
P. DEGREIF-DÜNNWALD*, G. UNDEN
The role of the cytoplasmic PAS domain of the *Escherichia coli* histidine kinase DcuS in signal transduction

RGV006
10:45
Ö. SARIKAYA BAYRAM*, O. VALERIUS, H.S. PARK, S. IRNIGER, J. GERKE, M. NI, K.-H. HAN, J.-H. YU, G. BRAUS
LaeA control of velvet family regulatory proteins for light-dependent development and fungal cell-type specificity

ISV01
The final meters to the tap

H.-C. Flemming
Faculty of Chemistry - Biofilm Center, University Duisburg-Essen, Duisburg, Germany

Household drinking water installations represent a sensitive, hygienically very relevant component of the supply chain. Public buildings are under surveillance; however, due to frequent capacity overload of authorities, not all of them have been visited. As private households are not under surveillance, household installations represent a somehow "grey zone" to which water works may deliver the best quality drinking water which may be compromised in the installation. In a joint research project, it was shown that in public buildings about 12 % of warm water samples contained legionella and 3 % contained Pseudomonas aeruginosa. Both were found in cold water much more frequently than expected. A central aspect is the choice of plumbing materials. If they leach biodegradable substances, they support biofilm growth and, thus, possible incorporation of hygienically relevant microorganisms with the potential of subsequent contamination of the drinking water. In principle, the admission of materials is regulated by passing of the test according to DVGW Working Sheet 270, but there are many materials on the market not meeting these requirements. Disinfection may improve the situation temporarily but not fundamentally. It was shown that fast growing organisms could be encouraged by disinfection. Furthermore, it was found that target organisms can transform into a viable-but-non-cultivable (VBNC) state and, thus, evade standard detection methods. This was shown in drinking water test systems and, in detail, for copper and subsequent resuscitation with a copper chelator which restored both viability and infectivity. Entering the VBNC state is considered a stress response which eliminates the organisms from the "detection radar" and the return from this state may explain cases of persevering contamination problems. In such situations, the additional employment of molecular biological methods is strongly recommended.
This project was funded by the BundesministeriumfürBildung und Forschung and carried out by 5 research institutions and 17 industrial partners.

ISV02
Microbial consumption of hydrocarbons in the deep sea: From methane seeps to oil spills

A. Boetius*[1], A. Wegener[2]
[1] *HGF-MPG Group for Deep Sea Ecology and Technology, Max Planck Institut for Marine Microbiology, Bremen, Germany*
[2] *Institute for Polar and Marine Research and Max Planck Institute for Marine Microbiology, Bremen, Germany*

Natural gas and oil are currently the most important sources of energy to mankind. The ocean floor contains large quantities of these hydrocarbons. But although they are constantly escaping from natural seeps, neither oil nor gas collect in the sea or on beaches. This can be attributed to the activity of hydrocarbon-degrading microorganisms, comprising specialists for consuming the simplest hydrocarbon – methane – as well as those oxidizing complex substrates contained in petroleum and tar. The ability of marine hydrocarbon degraders to clean the ocean from oil and gas spills has been recently stressed in the aftermath of the catastrophic explosion of the Deep Horizon drilling platform in the Gulf of Mexico. But still surprisingly little is known on the development and activity of environmental microorganisms responsible for oil and gas degradation. This presentation makes a journey from some of the hot spots of microbial methanotrophy in the deep sea such as methane hydrate deposits and erupting mud volcanoes, to natural asphalt seeps and its fascinating tar-degrading microbial consortia, which form the basis of a chemosynthetic food web. All of these extreme environments host the anaerobic methanotrophic archaea (ANME), which may be the most relevant group in controlling methane fluxes from the seafloor to the hydro- and atmosphere. The ANME represent special lines of descent within the Euryarchaeota and appear to gain energy exclusively from the anaerobic oxidation of methane (AOM), with sulfate as the final electron acceptor. They are widely distributed in the marine seafloor, and can form the densest biomasses of microorganisms known on Earth if both methane and sulfate are available as energy sources. The presentation will summarize the current knowledge on AOM habitats and limitations.

ISV03

No abstract submitted!

ISV04
Spatial regulation in *Caulobacter crescentus*

M. Thanbichler
Max Planck Institute for Terrestrial Microbiology and Laboratory for Microbiology, Philipps-University Marburg, Germany

In recent years, advances in microbial cell biology have led to a fundamental change in the perception of bacteria. While previously envisioned as membrane-bounded conglomerates of enzymes, bacterial cells have now emerged to be highly organized entities that tightly regulate cell shape and polarity, actively segregate plasmids and chromosomal DNA, and frequently undergo complex differentiation processes. As in eukaryotes, their temporal and spatial organization is controlled by multi-component regulatory networks, involving localized protein complexes and dynamic cytoskeletal structures.
Owing to its asymmetric design and unique developmental cycle, the Gram-negative species *Caulobacter crescentus* has developed into an important model system for the study of cellular organization in bacteria. This talk will highlight recent findings on the regulatory mechanisms that target cytoskeletal structures and enzyme complexes to distinct locations within the *C. crescentus* cell, thus controlling cytokinesis and executing the cellular morphogenetic program. The results presented will illustrate how one-dimensional genetic information is translated into the defined temporal and spatial regulatory patterns that underlie many aspects of cellular function.

ISV05
Intrinsic properties guide the function of bacterial cytoskeletal elements

P. L. Graumann*, J. Defeu-Soufo, F. Dempwolff, C. Reimold, B. Waidner
Faculty of Biology, Institut of Microbiology, University Freiburg, Germany

Bacteria contain actin-like MreB proteins and intermediate filament-like coiled coil-rich proteins (Ccrps) that give bacteria their typical rod or helical cell shape. These cytoskeletal elements assemble into defined structures underneath the cell membrane. How they gain the positional information and structure is unclear. It has been speculated that MreB filaments direct the incorporation of new cell wall material, or mechanically bend the cells in case of Ccrps, thus influencing the shape of the cell. Using heterologous cell systems, we show that several properties of cytoskeletal elements are based on intrinsic properties of the proteins. MreB and its orthologs Mbl and MreBH self-assemble into membrane-associated straight filaments, even in eukaryotic cells, and can exert force against the cell membrane, suggesting that they also mechanically stabilize cells. MreB paralogs have different filament architectures that can be drastically altered by single amino acid exchanges, and affect each other's architecture through direct interactions. Ccrps assemble into helical structures that are highly rigid in the absence of any additional bacterial cofactor. Filaments of tublin ortholog FtsZ can also have a helical structure as basic structure, which is seen as intermediate form in various bacteria including *Bacillus subtilis* and *Helicobacter pylori*. Finally, we show that translation elongation factor EF-Tu influences the positioning and dynamics of MreB filaments *in vivo* and *in vitro*, revealing a dual role in protein synthesis and cell shape maintenance of this highly conserved protein. Apparently, a variety of bacterial enzymes possess dual functions in metabolism and the generation of defined cell morphology, which will be discussed.

ISV06
Mitotic restructuring of the nucleus in the filamentous fungus *Aspergillus nidulous*

S. Osmani
Department of Molecular Genetics, Ohio State University, Columbus, USA

Mitosis is under the control of mitotic protein kinases which function to regulate the massive changes in nuclear structure involved in segregating replicated DNA equally to daughter nuclei. Studies using the model filamentous fungus Aspergillus nidulans have demonstrated that the NIMA kinase (the founding member the NIMA related NEK kinases of humans) initiates mitosis by promoting partial disassembly of nuclear pore complexes (NPCs). This allows diffusion of proteins between the cytoplasm and nucleoplasm to help facilitate mitosis. During mitotic exit those NPC proteins that are dispersed from the core structure return to reengage the nuclear-cytoplasm barrier and allow regulated nuclear transport to ensue during G1. Our current studies are aimed at understanding how protein-

protein interactions are changed by phosphorylation to cause NPC disassembly. Additionally we are interested in defining how certain NPC proteins play roles at the NPC during interphase and at chromatin during mitosis. We are employing single-step affinity purification and Mass Spectrometry analysis of NPC sub-complexes from G2 and mitotic cells to identify NPC proteins and to define how these complexes change during mitosis. Using this approach we have defined previously undefined NPC and nuclear envelope proteins and have established that some of these newly defined proteins help facilitate mitotic progression. Importantly we find that the mitotic behaviour of the NPC can be mimicked by ectopic induction of NIMA kinase activity which promotes the relocation of specific NPC proteins from the NPC onto chromatin. These data provide direct evidence that protein phosphorylation driven by the NIMA kinase regulates many aspects of mitotic nuclear structure.

(Supported by the National Institutes of Health)

ISV07

The general stress response, biofilm formation and cyclic-di-GMP signaling in *Escherichia coli*

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The ubiquitous bacterial signaling molecule cyclic-di-GMP, which is produced and degraded by diguanylate cyclases (carrying GGDEF domains) and specific phosphodiesterases (EAL domains), respectively, regulates transitions between the motile-planktonic and sedentary biofilm "life-styles" [1]. c-di-GMP controls a variety of targets, including transcription and the activities of enzymes and complex cellular structures. Many bacterial species possess many GGDEF/EAL proteins (29 in *E.coli*), which has lead to the concept of temporal and functional sequestration of c-di-GMP control modules [1]. Some GGDEF/EAL domain proteins (four in *E.coli*) have degenerate GGDEF/EAL motifs, are enzymatically inactive and can act by direct macromolecular interactions.
In *E.coli*, c-di-GMP signaling is tightly integrated with the general stress response, as many GGDEF/EAL genes are regulated by RpoS [3]. Moreover, c-di-GMP-dependent down-regulation of motility and induction of biofilm-associated functions such as the production of (auto)adhesive curli fimbriae occur during entry into stationary phase and require RpoS [2]. The talk will cover (i) the molecular mechanism of switching from motility to adhesion, which is based on a mutual inhibition of the FlhDC/motility and RpoS/CsgD/curli control cascades involving c-di-GMP signaling, and (ii) the molecular function of a locally acting c-di-GMP control module that regulates the transcription of the curli control gene *csgD*. Taken together, these and other studies [4] have also generated a novel general concept of the evolution of complex bacterial second messenger signaling [1].

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[2] Pesavento, C. et al (2008): Inverse regulatory coordination of motility and curli-mediated adhesion in *Escherichia coli*. Genes Dev. 22:2434-2446.
[3] Sommerfeldt, N. et al (2009): Gene expression patterns and differential input into curli fimbriae regulation of all GGDEF/EAL domain proteins in *Escherichia coli*. Microbiology 155:1318-1331.
[4] Tschowri, N. et al (2009): The BLUF-EAL protein YcgF acts as a direct anti-repressor in a blue light response of *E.coli*. Genes Dev. 23:522-534.

ISV08

Driving up the pressure: genetic and cellular responses of *Bacillus subtilis* to osmotic stress

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The soil-dwelling bacterium *Bacillus subtilis* inhabits an ecological niche subjected to frequent changes in osmotic and saline conditions that are caused by rainfall and desiccation. Such changes elicit water fluxes across the cytoplasmic membrane and can drive up turgor under hypo-osmotic conditions to such an extent that the cell will rupture, or under hyper-osmotic conditions cause the dehydration of the cytoplasm, a reduction in turgor and eventually growth arrest and cell death. Proteome and genome-wide transcriptional profiling studies have highlighted the complexity and multifaceted nature of the osmotic stress response systems of *B. subtilis*. However, it is beyond doubt that an effective water management by the cell is the cornerstone of its acclimatization to either sudden or sustained rises in the environmental osmolarity and the osmotic downshift that inevitably will follow hyperosmotic growth conditions [1]. The accumulation and expulsion of ions and compatible solutes play key roles in these cellular osmotic adjustment processes. I will discuss the nature of the systems responsible for ion fluxes in osmotically challenged *B. subtilis* cells and highlight the central role of the compatible solutes proline and glycine betaine in the acclimatization of the *B. subtilis* cell to sustained high salinity growth conditions.
Funding for our studies on cellular stress responses to changes osmolarity in *B. subtilis* is provided by the LOEWE program of the State of Hesse (SynMicro; Marburg) and a grant from the BMBF through the BaCell-SysMO2 consortium.

[1] Bremer, E. and R. Krämer (2010): The BCCT family of carriers: from physiology to crystal structure. 78:13-34.

ISV09

Tailor-made cell factories for a sustainable bio-economy

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The shortage of fossil resources and global warming are major drivers for a bio-based economy, basing the production of bulk and fine chemicals, bio-polymers as innovative plastics and biofuels on renewable resources. In the heart of this development are efficient biocatalysts, which provide the desired product at high yield and titer and open novel applications. The creation of such tailor-made cell factories requires the right combination of targeted genetic modifications, not an easy task taking the several thousands of genes into account which typically form a microbial genome. Novel concepts now open a design-based strain optimization on the basis of highly vital wild types. These combine systems wide omics analysis and computational modeling of metabolic networks as genome scale towards detailed understanding of the underlying metabolism as basis of knowledge based optimization. Key targets hereby comprise the utilization of alternative raw materials, the reduction of by-product formation as well as high titer, yield and productivity for the compound of interest. Design-based systems metabolic engineering will be demonstrated for the feed amino acid L-lysine with a world market of about 1.000.000 tons per year, the novel bio-polyamide building block diaminopentane as well as the platform chemical succinic acid. Integrated into the development of efficient fed-batch bioprocesses the created cell factories enable novel industrial applications.

Becker J, Zelder O, Häfner S, Schröder H, Wittmann C (2011) From zero to hero - design-based systems metabolic engineering of *Corynebacterium glutamicum* for L-lysine production. Metab. Eng. In press.

Buschke N, Schröder H, Wittmann C (2011) Metabolic engineering of *Corynebacterium glutamicum* for Production of 1,5-diaminopentane from hemicellulose. Biotechnol. J. In press.

Kohlstedt M, Becker J, Wittmann C (2010) Metabolic fluxes and beyond - systems biology understanding and engineering of microbial metabolism.Appl. Microbiol. Biotechnol. 88:1065-1075.

Kind S, Jeong WK, Schröder H, Wittmann C (2010) Systems-wide metabolic pathway engineering in *Corynebacterium glutamicum* for bio-based production of diaminopentane. Met Eng 12: 341-351.

ISV10

Genome-wide aspects of cellulase regulation in *Trichoderma reesei*

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Most of the industrial production of enzymes for plant biomass hydrolysis towards biofuel production is performed with mutants of the fungus *Trichoderma reesei* (the anamorph of the tropical ascomycete *Hypocrea jecorina*). Consequently, this fungus meanwhile serves as the model system for the molecular understanding of cellulase gene expression and secretion of the encoded cellulase proteins. The recent complete sequencing of the *T. reesei* genome (Martinez et al. 2008. Nature Biotechnol) enabled to study these biochemical events on a genome wide scale. Analysis of the *T. reesei* transcriptome during cellulase induction has led to the identification and functional characterization of new genes relevant to this process. In addition, I will demonstrate a regulation of cellulase and hemicellulase formation at the chromatin level. The results open new avenues for strain improvement towards further improvement of *T. reesei* strains.

ISV11
No abstract submitted!

ISV12
Monitoring of human pathogens and source identifiers in discharges across the United States: QMRA from source to bathing site
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This study has increased knowledge about relationships between pathogen indicators, source identifiers and pathogens to support Quantitative Microbial Risk Assessment (QMRA) efforts and the implementation of revised recreational water quality criteria. Data gaps for waterborne pathogens and indicators in fecally-impacted discharges to recreational waters were identified and filled by targeted monitoring campaigns. Simultaneous detection of pathogens in water samples used genetic-, culture-, and microscopy-based methodologies for *Salmonella*, *Campylobacter jejuni, Vibrio cholerae*, *Cryptosporidium, Giardia, Toxoplasma gondii*, adenoviruses, enteroviruses, noroviruses, rotaviruses, *Bacteroidales, Enterococcus,* and *Escherichia coli*.
The potential health risks associated with discharges-were estimated using QMRA and three scenarios: primary contact by adults, secondary contact by adults and/or inhalation by persons of any age, and primary contact by children. The differences in these three scenarios were driven by different water ingestion rates (adults ingest more water during primary than secondary contact; children ingest more water than adults; water inhalation rate is less than water ingestion rate for any age class). Norovirus posed the most dominant health risk followed by rotavirus. Norovirus and *Enterococcus* both had significant correlations with pathogens in discharges.
Using the same DNA extracts from discharge samples, microbial source tracking qPCR data on the fecal source identifier *Bacteroidales* was obtained and used in a new model to predict the true amount of human fecal contamination in a water sample by relating a human-associated genetic marker to a universal assay for fecal sources. The model output can be used to implement and evaluate management options intended to restore microbial water quality.

ISV13
No abstract submitted!

ISV14
No abstract submitted!

ISV15
Xanthomonas TALEs - from plant pathogen weapon to biotech hype
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Xanthomonas spp. are Gram-negative plant pathogenic bacteria with powerful molecular weapons to attack their plant hosts. Key for pathogenicity of *Xanthomonas* is a type III secretion system that injects a cocktail of effector proteins into plant cells to function as potent virulence factors. TALEs (transcription activator-like effectors) constitute a major family of *Xanthomonas* effectors that function as transcriptional activators of plant genes. The first TALE, AvrBs3, was identified 20 years ago. Today, more than 100 TALEs are known and all exhibit the same structural features: eukaryotic nuclear localisation sequences mediate import into the plant cell nucleus and a C-terminal activation domain is essential for target gene activation. A fascinating characteristic of TALEs is their central protein domain of tandem, near-identical 34 amino-acid repeats. Different TALEs differ in the number and order of repeats which can be classified via two adjacent hypervariable amino acids. Importantly, the repeats were shown to confer DNA-binding and only recently [1], the remarkably simple DNA-target specificity code was cracked. Each TALE repeat recognizes a single base pair in a contiguous DNA sequence and the variable diresidues specify the base that is bound. This uniquely modular DNA-binding code allows to straightforwardly read the specificity of any TALE from its repeat sequence. In addition, the modular architecture allows a simple reprogramming of DNA-binding specificity. First powerful tools for biotechnology have emerged.

[1] Boch, J. et al (2009): Breaking the code of DNA binding specificity of TAL-type III effectors. Science 326, 1509-1512.

ISV16
Toxin producing endofungal bacteria
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Pathogenic fungi generally exert their destructive effects through virulence factors. An important example is the macrocyclic polyketide rhizoxin, the causative agent of rice seedling blight, from the fungus *Rhizopus microsporus*. The phytotoxin efficiently binds to rice ß-tubulin, which results in inhibition of mitosis and cell cycle arrest.
By a series of experiments we could unequivocally demonstrate that rhizoxin is not biosynthesized by the fungus itself, but by endosymbiotic bacteria of the genus *Burkholderia*. Our unexpected findings unveil a remarkably complex symbiotic-pathogenic alliance that extends the fungus–plant interaction to a third, bacterial key player. In addition, we were able to culture the symbionts to produce antitumoral rhizoxin derivatives, and to elucidate the biosynthesis of the toxin. A second example for the formation of a 'mycotoxin' by endofungal bacteria is the cyclopeptide rhizonin.
Surprisingly, in the absence of bacterial endosymbionts the fungal host is not capable of vegetative reproduction. Formation of sporangia and spores is only restored upon re-introduction of endobacteria. The fungus has become totally dependent on endofungal bacteria, which in return provide a highly potent toxin for defending the habitat and accessing nutrients from decaying plants.
This talk highlights the significance of toxin-producing endofungal bacteria in the areas of ecology, medicine, and nutrition. Furthermore, progress in studying the molecular basis for the development and persistence of this rare microbial interaction is presented.

ISV17
Plants and arbuscular mycorrhizal fungi: born to be friends
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Arbuscular mycorrhizas (AMs) are symbiotic associations, which are commonly described as the result of co-evolution events between Glomeromycota fungi and plants where both partners benefit from the reciprocal nutrient exchange. Data from fossil records, recent characterizations of AM fungi in basal plant taxa, and live cell imaging of angiosperm colonization processes, indicate the ancient origin of AM interactions. Among the conserved cellular mechanisms, the presence of a symbiotic interface compartment which allows fungal development inside the cell lumen and maintains host cell integrity, is considered a landmark for AMs establishment.
The presentation will focus on mechanisms which are associated with the perception of the AM fungus and its accommodation inside the lumen of the host plant cell, leading to the assembly of the perifungal membrane and symbiotic interface. Our findings, based on an *in vivo* confocal microscopy approach, demonstrate that root cells perceive AM fungal signals and trigger calcium-mediated signaling in their nucleoplasm, both before and upon direct contact with the fungus. Nuclear calcium spiking seems to be a pre-requisite to the cellular reorganization that initiates after the adhesion of the fungal hyphopodium to the root and leads to the assembly of the so-called prepenetration apparatus (PPA) inside one or a few contacted epidermal cells. Lastly, and at least in *in vitro* conditions, PPA is instrumental for the assembly of the interface construction.
These features have been identified in legumes and in not-legume plants, but so far not in naturally collected plants or in lower taxa. Answers to these

questions may help to solve ongoing debate on plant-fungal co-evolution and on the functional role of AMF in natural systems.

ISV18
The genomic standards consortium: Bringing standards to Life

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The application of high-throughput sequencing technologies has transformed the way microbiologists approach questions in their field. The shift of sequencing capacity is now resulting in a dramatic increase in the amount of data available to a wider community, forming a rich stream of information. These data hold the promise of unparalleled insights into fundamental questions across a range of fields including evolution, ecology, environment biology, health and medicine. To fully exploit the promise of these data we need both scientific innovation and community agreement on how to provide appropriate stewardship of these resources for the benefit of all. One key insight into the function of a gene or organism is the environment where it occurs. Collection of contextual (meta) data, which delineates the source of a sequence in terms of the space, time, habitat, and characteristics of the environment, is thus essential in interpreting the unknown genes and species, as well as gaining new insights into the known fraction. At present, the valuable contextual data halo is often missing for sequences deposited in the International Nucleotide Sequence Database Collaboration (INSDC). In 2005, members of the community came together to form the Genomic Standards Consortium (GSC), an open-membership working body with the stated mission of working towards better descriptions of our genomes, metagenomes, and related data (www.gensc.org). Supported by the expertise of the members involved in many of the aforementioned mega-sequencing projects, the GSC has formalized the contextual data requirements for genomes and metagenomes as the Minimum Information about a Genome/Metagenome Sequence checklist (MIGS/MIMS) [1]. Furthermore, the extension of MIGS/MIMS to cover the description of phylogenetic and functional marker genes is in progress as the Minimum Information about an Environmental Sequence (MIENS) checklist (www.gensc.org/gc_wiki/index.php/MIENS) [2]. This family of minimum information checklists (MIxS) provides researchers with a condensed set of contextual data requirements, which range from description of the environment to sequencing procedures. Active participation to further develop the MIxS standards is highly appreciated. Requests for new features and reporting of bugs can be easily done via http://mixs.gensc.org.

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[2] Yilmaz, P. et al, for the Genomic Standards Consortium (under revision) The „Minimum Information about an ENvironmental Sequence" (MIENS) specification. Nat. Biotechnol.

ISV19
Standards in genomic sciences: A standards compliant open-access journal for the 'omics community

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Standards in Genomics Sciences (SIGS; www.standardsingenomics.org) is an Open Access eJournal that was created to promote the data standardization efforts of the Genomic Standards Consortium (GSC) and to provide a venue for publication of highly structured, MIGS compliant reports of genome and metagenome sequences, standard operating procedures, meeting reports, white papers and other articles that promote are in keeping with the objectives of the GSC. Whereas peer-reviewed companion publications of genomes were once commonplace in a number of journals, many general and discipline specific publications routinely decline such papers today. This leads to a loss of contextual information that is needed for analyzing and interpreting genome sequence data.

The GSC was founded in 2005 by an international community of like-minded scientists to work towards improving the descriptions of our growing collection of genomes and metagenomes. Without metadata standards, exchanging and integrating genomic data into analytical models and public knowledge bases increases while the overall value of each additional sequence diminishes. This is problematic because the ease and cost of producing sequence data have dropped sharply while the cost of annotation and documentation have increased.
At the time of writing, SIGS had already published over 100 articles, including more than 80 short genome reports that had been viewed by more than 25,000 readers in 130 countries. SIGS is listed in CrossRef, The Directory of Open Access Journals (DOAJ) and PubMed Central and has, within a period of less than two years, become one of top five journals publishing papers on new genome sequences.

ISV20
Reversing the paradigm -- The genome sequence of Candidatus Sulfuricurvum sp. derived from a complex short-read metagenome with more than 300 OTUs enables detailed studies of the novel epsilon-proteobacterium.

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Characterizing genomes of unculturable microbes (most species on earth) requires new approaches for genome assembly from environmental samples, e.g. communities involved in bioremediation at the Old Rifle uranium-contaminated site. Here we show we can reconstruct the complete genome (Candidatus Sulfuricurvum sp) via short-read metagenomics and novel approaches for assembly based on simple statistical principles. While previous examples of complete genome sequences from metagenomes stem from samples of very limited complexity (>10 OTUs), this sequence was obtained from a complex mix of over 300 OTUs. Traditional approaches fail because uneven numbers of sequence reads from common and rare species, and pan-genome variation, confuses traditional genome assemblers; species without close relatives sequenced cannot co-assemble: without our approach, additional sequence data hurts rather than helps assembly. In addition, the metabolic reconstruction of this genome permitted cultivation of this dominant organism from an ecosystem relevant to bioremediation. This novel approach will allow the assembly of genomes and cultivation of key species from diverse environments/enrichment cultures, thus providing new pangenomic insights into processes ranging from biofuel generation to identification of emerging pathogens.

ISV21
Translocation of Oomycete effectors into host cells

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The fungus-like oomycetes contain several species that are devastating pathogens of plants and animals. During infection several oomycetes translocate effector proteins into host cells where they interfere with host defence responses. Several oomycete effectors have a conserved Arg-Xaa-Leu-Arg (RxLR)-motif that is thought to be important for their delivery. We demonstrate that, whereas the RxLR-leader sequence of SpHtp1 from the fish pathogen Saprolegnia parasitica shows fish cell-specific translocation, the RXLR-leader of AVR3a from the potato-late-blight pathogen Phytophthora infestans promotes efficient binding of the C-terminal effector domain to several cell types. Our results demonstrate that the RxLR-leaders of SpHtp1 and AVR3a are dimerisation sites, able to form heteromers. We further demonstrate that cell surface binding of both RxLR-proteins is mediated by an interaction with modified cell surface molecules. These results reveal a novel effector translocation route based on effector dimerisation and receptor modification, which could be highly relevant for a wide range of host-microbe interactions.

ISV22
Applying ecological principles to microbial systems: Partitioning core and satellite taxa from within bacterial communities

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It is well known that microbial ecology is both driven and limited by the increasing plethora of techniques used to assess microorganisms and their communities. In many cases this has led to an almost unhealthy obsession for using the latest methodologies, typically at the expense of the research questions being asked. It has been previously argued that new technologies will increasingly lead us down 'blind non-generalist and expensive alleyways' and microbial ecology will remain in a state of 'accumulating situation-bound statements' of limited predictive ability if studies are not directed and driven by ecological theory. Given the central and global importance of microorganisms in natural and engineered ecosystems, progress requires the acceptance, development, and application of ecological theory and principles. However, the application of theory is still in its infancy in microbial ecology. The potential of exploiting theories, models and principles from general ecology, coupled with ever improving molecular methodologies, could well provide invaluable insights into how microbial communities organise and change in space and time. In time, this increased knowledge of microbial community ecology will help us better understand and predict changes in the natural environment, allow manipulation of agricultural and industrial processes and give improved protection of human health.
In general terms, I will outline the importance of developing microbial ecological theory. More specifically, I will discuss my recent and ongoing work that seeks to use ecological insights for clinical benefit, by partitioning bacterial communities involved in the lung infections of cystic fibrosis patients into core and satellite species groups. From a fundamental perspective this work also demonstrates that a community is comprised of core and satellite species, and that partitioning the two groups from a (spatial or temporal) metacommunity reveals important aspects of species abundance distributions, which would otherwise be neglected with without such a distinction.

ISV23
Effects of space and ecosystem type on the structuring of marine microbial communities at the global scale

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Despite the importance of marine microbes for global ecosystem functioning, still little is known about the factors that contribute to the structuring of their communities in ocean water and sediments worldwide. This presentation proposes a community ecology approach to characterizing the main patterns of microbial diversity over large spatial scales and to quantifying the respective effects of major factors of variation. By synthesizing, visualizing and testing hypotheses on large molecular datasets, novel insights about microbial ecology at various spatial, temporal and taxonomic scales may be obtained with respect to the comparison of benthic and pelagic communities, the scales at which ocean realms are structured, the taxonomic scales of relevance to describe microbial diversity patterns, and the types of abiotic and biotic processes being most likely at play.

ISV24
Mechanisms of c-di-GMP mediated cell cycle control in *Caulobacter crescentus*

U. Jenal
Biozentrum of the University of Basel, Switzerland

The development of all living organisms depends on the generation of specialized cells in appropriate numbers. This requires tight regulation of proliferation-differentiation decisions by integrating cell fate determination processes with replication and cell division. Many bacteria use complex developmental strategies to optimize their survival. Like their eukaryotic counterparts, bacteria tightly coordinate morphogenetic programs with growth and division, be this to facilitate the transition between a replicative and a terminally differentiated cell form or to couple obligate cell differentiation events to cell proliferation. The gram-negative bacterium Caulobacter crescentus divides asymmetrically to produce two polarized daughters with distinct morphologies, behavior, and replicative potential. This enables Caulobacter to periodically switch between a motile, planktonic and a sessile, surface adherent life style. Recent studies have identified cyclic di-GMP as a key regulator of cell polarity and cell cycle progression in this organism. In particular, c-di-GMP facilitates the dynamic assembly and disassembly of polar organelles and couples these developmental processes to the underlying cell cycle. The seminar will summarize these findings and will highlight molecular and cellular aspects of c-di-GMP signaling components that contribute to the temporal and spatial control of the C. crescentus life cycle.

ISV25
Dynamic cyclic di-GMP signaling in *Vibrio cholerae* during infection

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Vibrio cholerae cycles between aquatic environments and the human small intestine. Its success as a pathogen depends in large part on surviving the transitions between these two disparate environments. Successful transition requires changes in gene expression and phenotypic changes, which we find are regulated in part by the bacterial second messenger c-di-GMP. In aquatic environments, *V. cholerae* forms biofilms - a state that requires high c-di-GMP concentration. Upon entry into the small intestine through ingestion of contaminated water or food, the concentration of c-di-GMP is lowered through activation of specific phosphodiesterases. This results in the repression of biofilm formation genes, which interfere with infection, and the simultaneous activation of virulence genes, which are needed for colonizing the epithelial surface in the small intestine. Late in infection, in response to changing nutrient and oxygen concentrations as the density of bacteria becomes high, the situation reverses whereby the concentration of c-di-GMP is raised through activation of diguanylate cyclases. This serves to prepare *V. cholerae* for the transition to life outside the host.

ISV26
From isolated molecules to intact cells: Structure of ribosomal arrangements *in vitro* and *in situ*

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X-ray crystallography and EM single particle analysis (SPA) have provided unprecedented insights into the molecular architecture of ribosomes and have been instrumental in elucidating key events during translation. Cryoelectron tomography (CET) can complement these techniques in that it allows the visualization of flexible molecular structures both *in vitro* and *in situ*, i.e., in the functional environment of intact cells. We have used CET to study the native 3D organizations of *Escherichia coli* ribosomes in polysomes and hibernating ribosomes (100S).
The quantitative evaluation of cryoelectron tomograms is challenging due to the extremely low signal-to-noise of cryoelectron tomograms. 3D averaging is a way to overcome the problem of low contrast in CET. First, we pursue the identification of ribosomes with a known structure by template matching; the macromolecular structure is used as a template for a local correlation with the tomogram. Secondly, we align subtomograms containing single ribosomal particles to a common origin and average them to reveal details of the interaction between the identified complexes. An *in situ* implementation of this approach, i.e. in the functional environment of intact cells, allowed us to obtain ribosomal atlases of *Spiroplasma melliferum* cells [1].
Applying CET and template matching to *in vitro* translation systems, we showed that *E. coli* ribosomes adopt two preferential relative orientations in densely-packed polysomes. These alternative manners of ribosomal pairing result in variable 3D polysomal organizations, i.e, pseudo-planar or pseudo-helical polysomes. In polysomes, the 30S subunits point inwards, possibly protecting mRNA from degradation, and the 50S subunits outwards, positioning the nascent chain exit sites of adjacent ribosomes away from each other. We hypothesize that these organizations disfavor interaction between the non-folded nascent chains avoiding protein misfolding [2].

More recently, we have applied these methods to cytosolic fraction of 100S ribosomes, a dimerized form of 70S ribosomes associated with starvation in *E. coli* [3]. It was possible to purify *in silico* a particular ribosomal arrangement of the two 70S ribosomes that form a dimer. In contrast to the lateral contact of the 30S subunits observed in dense polysomes, the resolved 100S arrangement show that the contact of small subunits is frontal and implies a possible participation of the S9, S10 and S2 proteins as well as 16S rRNA. Moreover, this 100S ribosomal arrangement has been detected in tomograms of intact *E. coli* cells specifically in stationary phase, which reinforce the physiological role of 100S ribosomes as a storage form of ribosomes important for cell survival.

[1] Ortiz, J. O. et al. (2006): Struct Biol. 156:334.
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ISV27

No abstract submitted!

ISV28

Protein and RNA Dynamics in Living Cells

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Signaling pathways in RNA:protein complexes involved in translation are identified by community network analysis derived from molecular dynamics simulations. These complexes include the amino-acyl-tRNA synthetases, the elongation factor EF-Tu, and the ribosome. A dynamic contact map defines the edges connecting nodes (amino acids and nucleotides) in the physical network whose overall topology is presented as a network of communities, local substructures that are highly intraconnected, but loosely interconnected. While nodes within a single community can communicate through many alternate pathways, the communication between monomers in different communities has to take place through a smaller number of critical edges or interactions which are evolutionarily conserved. The time dependent variation of these networks during tRNA migration is consistent with kinetic data and reaction mechanisms suggested at each step of translation.
In bacterial cells, translation involves thousands of these RNA:protein complexes which occupy a large portion of the cell volume and make a major contribution to the extrinsic noise of gene expression. Using data from proteomics, cryo-electron tomography, and in vivo single molecule fluorescence experiments, we study the inducible lac genetic switch in a modeled E. coli cell. Compared to models in which the spatial heterogeneity is ignored, the in vivo model for fast-growing cells predicts an overall lowering of cellular noise, due to the influence of molecular crowding on repressor binding rates. The smaller slow-growing cells have a larger internal inducer concentration which lead to a significant decrease in the lifetime of the repressor-operator complex, an increase in the mean number of transcriptional bursts, and mRNA localization. The long time simulations of biochemical pathways under in vivo cellular conditions, were calculated with a lattice-based, reaction-diffusion model that runs on graphics processing units.

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[2] Trabuco, L. et al (2010): J. Mol. Biol. 402, 741-760.
[3] Alexander, R. et al (2010): FEBS Lett. 584, 376-386.
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[5] Roberts, E. et al (2009): Proc. 8th IEEE Intl. Meeting on High Performance Comp. Biol.

ISV29

Biosynthesis and remodeling of bacterial membrane lipids

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The model bacterium *Escherichia coli* contains the phospholipids phosphatidylglycerol, cardiolipin, and phosphatidylethanolamine (PE) as major membrane lipids and biosyntheses and functionalities of individual membrane lipids have mainly been studied in this organism. However, in other bacteria, additional and alternative membrane lipids are found and in many cases neither their biosyntheses nor their functionalities are understood. Some Gram-negative bacteria have phosphatidylcholine (PC) or sphingolipids in their standard repertoire, whereas many Gram-positives have glycosylated diacylglycerols and lysyl-phosphatidylglycerol in their membranes. Notably, phosphatidylinositol is an essential lipid for *Mycobacterium tuberculosis*. Steroid and hopanoid lipids only occur in some bacteria.
Bacterial membrane lipid composition should not be considered as an invariable constant, but rather as the result of a steady-state, characteristic for a given physiological condition. Under certain stress conditions, specific new membrane lipids can be formed in order to minimize the stress exerted. For example, challenge of proteobacteria with acid causes modifications of pre-existing membrane lipids, resulting in the formation of lysyl-phosphatidylglycerol or hydroxylations of ornithine-containing lipids. Under phosphorus-limiting conditions of growth, some bacteria form membrane lipids lacking phosphorus such as ornithine-containing lipids, or the diacylglycerol (DAG)-based glycolipids, sulfolipids, and betaine lipids.
In *Sinorhizobium meliloti,* a Gram-negative soil bacteria able to establish nitrogen-fixing root nodules with their respective legume host plants, the zwitterionic phospholipids PE and PC of its membrane are degraded upon phosphorus limitation by a specific phospholipase C to the respective phosphoalcohol and DAG [1]. DAG in turn is the lipid anchor from which biosyntheses are initiated during the formation of phosphorus-free, DAG-based membrane lipids. Inorganic phosphate (Pi) can be liberated from the phosphoalcohol. Obviously, in *S. meliloti* under phosphate-limiting conditions, membrane phospholipids provide a pool for metabolizable Pi, which in turn can be used for the synthesis of other essential phosphorus-containing biomolecules.

[1] Zavaleta-Pastor et al (2010): Proc. Natl. Acad. Sci. USA 107:302-307.

ISV30

Regulation of membrane homeostasis in *Pseudomonas aeruginosa*

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Membrane lipid biogenesis is a vital facet of bacterial physiology that is tightly regulated at both biochemical and genetic level. Bacterial survival depends on membrane lipid homeostasis and on the ability to adjust lipid composition to acclimatize the bacterial cell to optimize growth in diverse environments. The most energetically expensive membrane lipid components to produce are the fatty acids, which determine the viscosity of the membrane and, in turn, influence many crucial membrane-associated functions. Thus, bacteria have evolved sophisticated mechanisms to finely control the expression of the genes responsible for the metabolism of fatty acids. These regulatory mechanisms adjust the level and activity of biosynthetic enzymes to match the demand for new membrane. The versatile human pathogen *Pseudomonas aeruginosa* contains both saturated fatty acids (SFAs) and monounsaturated fatty acids (UFAs) in the membrane. In *P. aeruginosa*, the predominant UFA synthesis is carried out by the FabA-FabB pathway of the type II fatty acid synthase. The two key components for UFA production FabA and FabB are co-transcribed in a *fabAB* operon. Two oxygen-dependent desaturases, DesA and DesB, supplement the FabA-FabB pathway for UFA synthesis in *P. aeruginosa*, which is the first bacterium identified that has more than one pathway for UFA synthesis. These three complementary pathways for UFA formation allow the ubiquitous *P. aeruginosa* to survive in various environments. The FabA-FabB pathway is active under all growth conditions and produces the majority of the UFAs. Because DesA introduces double bonds into existing fatty acyl chain of phospholipids, it allows the bacterium to quickly modify the membrane properties to adapt to abrupt changes in growth conditions. DesB allows *P. aeruginosa* to modify the composition of exogenous fatty acids being transported into the cell. The FabA-FabB and DesB pathways for UFA synthesis are coordinately regulated by a TetR-family transcriptional factor DesT, which senses the composition of cellular acyl-CoA pool to fine tune the expression of the pathway enzymes. Saturated acyl-CoAs stabilize a conformation that cannot bind DNA, while unsaturated acyl-CoAs stabilize a conformation that binds DNA. Recently we found that the content of *cis*-vaccenate in the membrane plays a key role in the pathogenicity of *P. aeruginosa*. Reduced level of *cis*-vaccenate leads to decreased fluidity of the membrane and defects in the secretion of various extracellular virulence factors, biofilm formation, and motility. Therefore, membrane homeostasis is essential for both survival and virulence of *P. aeruginosa*, and may provide new strategies for the development of anti-*Pseudomonas* treatments.

ISV31
Fatty acid synthesis in fungal type I protein complexes

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Fatty acid synthesis in eukaryotes is performed by multienzyme proteins. These large catalytic machineries are assembled from long polypeptide chains to built multimeric arrangements. The Saccharomyces cerevisiae fatty acid synthase (FAS) is the archetypal fungal FAS. Six α- and six β-chains, each more than 200 kD in size, assemble to a barrel-shaped structure of 2.6 MDa. Recent structural insight give a detailed picture about the key elements in type I synthesis: arrangement of active sites in the macromolecular complex, substrate channelling by the acyl carrier protein, and chain length control in fatty acid production.

ISV32
Universal high throughput FACS based screening systems for the discovery and optimization of biocatalysts from enzyme libraries

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A key technology in enzyme discovery and in vitro evolution of enzymatic activity is the efficient high through-put screening for the discovery of new enzyme activities or improved enzymes from a library of enzyme variants.
The most efficient screens are based on selection methods, but their applicability is limited to screen-designs that allow for the selection of microbial clones, whose growth depends on the conversion of the substrate in question. The number of substrates, which are suitable for a selective screen is limited, which restricts the applicability of selection-based screens.
Fluorescent based cell sorters offer an interesting alternative for selective screens, because they have the potential to enrich up to 107 single cells/h. Such through-put would enable the enrichment of active clones for a targeted screen in a subsequent plate or liquid screening assay. An activity based cell-sorting of microbial requires performing an assay with a single cell and, for the sorting procedure, the isolation of one cell, enzyme and substrate in one compartment.
The separation of microbial cells by using double emulsions permits screening for enzymatic activity in high through-put. For this purpose microbial cells containing enzymes and soluble fluorogenic substrates are entrapped in the inner water phase of droplets of "water-in-oil-in-water" emulsions. Enzymatically released fluorescent products can be detected by flow cytometry and used for the selection and separation of a population of active droplets/cells.
We have developed this technology using the directed evolution of bacterial cytochrome P450 like oxygenases and fungal glucose oxydases as examples. It is however possible to use this technology as initial enrichment step during the screen of metagenomic expression libraries for an activity of interest.

AMV001
Subcellular localization of *pce* gene products: implications for the biogenesis of physiologically active tetrachloroethene (PCE) reductive dehalogenase

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The tetrachloroethene reductive dehalogenase (PceA) of the anaerobe *Desulfitobacterium hafniense* Y51 is a Fe/S-protein harboring a corrinoid cofactor. The enzyme catalyzes the reductive dechlorination of tetrachloroethene (PCE) to *cis*-1,2-dichloroethene as a part of the energy metabolism. The precursor form of the enzyme (prePceA) is a substrate of the twin-arginine translocation (Tat) pathway that exports folded and mostly cofactor-containing proteins across the cytoplasmic membrane. The gene encoding the enzyme, *pceA*, is organized in the *pce* operon that comprises four genes - *pceA*, *pceB*, *pceC*, and *pceT*. PceB is a hydrophobic protein proposed to serve as membrane anchor for the enzyme. PceC shows homology to transmembrane transcriptional regulators. PceT exhibited peptidyl-prolyl cis/trans isomerase and chaperone activity and was shown to interact with prePceA [1].
In this study, the subcellular localization of the PCE reductive dehalogenase and of the PceB and PceT proteins in *D. hafniense* Y51 cells subcultivated in the presence or absence of PCE was investigated using the freeze-fracture replica immunogold labeling (FRIL) technique. When PCE was present, the mature form of the enzyme (matPceA) was attached to the exoplasmic face of the cytoplasmic membrane. The interaction between the PCE reductive dehalogenase and the exoplasmic loop of PceB was shown by Far Western Blot analysis. When PCE was absent, a cytoplasmic but membrane-associated accumulation and aggregation of prePceA was observed. In such cells, the PceT protein showed almost the same localization pattern as the precursor of the enzyme, indicating its co-aggregation with prePceA. The prePceA aggregates were enriched via subcellular fractionation and purified by sucrose density gradient centrifugation. The composition of the protein aggregates was analyzed using tryptic digestion and subsequent liquid chromatography with detection of the peptides via tandem mass spectrometry (LC-MS/MS). Based on the results presented here, the biological role of the prePceA aggregates in PCE-depleted cells of *D. hafniense* Y51will be discussed with respect to the biosynthesis of the mature PCE reductive dehalogenase when PCE becomes available.

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AMV002
Evidence for the involvement of one electron transfer chemistry in 2-haloacrylate hydratase reaction

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2-Haloacrylate hydratase (CAA67_YL) is an $FADH_2$-dependent enzyme catalyzing the hydration of 2-chloroacrylic acid (2-CAA) to produce pyruvic acid and HCl, a reaction with no net change in the redox state of the coenzyme and substrate involved [1]. The strict requirement for a reduced flavin in this catalysis is puzzling. Herein, the UV-visible spectroscopic analysis of the CAA67_YL assay mixture revealed a 2-CAA-dependent generation of FAD semiquinone (FAD_{sq}). We also noted the generation of anionic form of FAD semiquinone during the photoreduction of CAA67_YL holoenzyme indicating that CAA67_YL active site has the necessary framework in place to bind FAD_{sq}. Additionally, CAA67_YL apoenzyme has restored its hydratase activity when reconstituted with 1-deazaFAD, but 5-deazaFAD did not support the catalysis. Taken together, these data support the involvement of one electron transfer chemistry in the hydration of 2-CAA where the reduced flavin plays as a radical catalyst that provides electron to the substrate to facilitate hydration.

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AMV003
Acetophenone Carboxylase and Acetone Carboxylase, enzymes employing new biochemical principles for carboxylation reactions

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The β-proteobacterium *Aromatoleum aromaticum* strain EbN1 degrades acetone or the aromatic ketone acetophenone as single substrates under aerobic and denitrifying conditions. The ketones are carboxylated to acetoacetate and benzoylacetate, respectively, by novel types of ATP-dependent carboxylases. Both enzymes, acetone carboxylase (Acx) and acetophenone carboxylase (Apc), have been purified and biochemically characterised. The enzymes show some sequence similarity although they differ in several crucial aspects of composition, cofactor dependence and

reaction mechanism. While Apc consists of 5 subunits in an $(\alpha\beta\beta'\gamma)_2\varepsilon_2$ composition, Acx is a $(\alpha\beta\gamma)_2$-heterohexamer. The catalytic properties of both enzymes and their respective reaction mechanisms were investigated and compared. Acetophenone carboxylase converts a variety of aromatic ketones, while acetone carboxylase shows a very narrow substrate spectrum and carboxylates only acetone and butanone. Also, the products of ATP-hydrolysis differ: per carboxylated substrate acetophenone carboxylase hydrolyses 2 ATP to 2 ADP, while acetone carboxylase hydrolyses 2 ATP to 2 AMP.
The observed reaction mechanisms of acetone carboxylase and acetophenone carboxylase represent novel ATP-dependent, biotin-independent carboxylation mechanisms in bacterial ketone catabolism, which likely involve the activation of both substrates via phosphorylation.

AMV004
The W-/Se-containing class II benzoyl-CoA reductase complex in obligately anaerobic bacteria

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Benzoyl-Coenzyme A (CoA) is a central intermediate in the anaerobic degradation of aromatic compounds which is dearomatized to cyclohexa-1,5-diene-1-carbonyl-CoA by benzoyl-CoA reductases (BCRs). There are two completely different classes of BCRs which both yield the identical product [1,2]. ATP-dependent class I BCRs, referred to as BcrABCD are [4Fe-4S] clusters containing enzymes that are present in facultative anaerobes. In contrast, obligately anaerobic bacteria are proposed to employ a W-/Zn-/FeS-/Flavin-/Se-containing, ATP-independent BamBCDEFGHI complex. The active site harbouring BamBC components were characterized from the aromatic compound degrading Deltaproteobacterium *Geobacter metallireducens* [1]. BamB is similar to aldehyde:ferredoxin oxidoreductases and is supposed to contain a W-pterin cofactor at the active site. We present kinetic and molecular properties of BamBC and provide evidence that class II BCRs are composed of the predicted high molecular BamBCDEFGHI complex. Initial data indicate that the exergonic electron transfer to the aromatic ring is driven by an electron bifurcation process.

AMV005
Nitrogen oxides involved in anaerobic alkane activation by strain HdN1

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Alkanes are naturally wide-spread hydrocarbons, originating from petroleum or synthesized by living organisms. Their degradation by microorganisms has been studied extensively in the past century. Only a small number of bacterial strains have been described so far with the ability to activate saturated alkanes under anaerobic conditions, employing unique biochemical reactions to overcome the inertia of C-H bonds. The *Gammaproteobacterium* strain HdN1 degrades linear alkanes between C_6H_{14} and $C_{30}H_{62}$ under denitrifying conditions. Genetic, proteomic and metabolic analyses did not yield any evidence for the well-described fumarate-addition mechanism for anaerobic alkane activation. Surprisingly for a denitrifier, N_2O did not sustain growth of strain HdN1 with alkanes, while it supported fast growth with fatty acids or long-chain alcohols [1]. Cultures that grew on tetradecane formed N_2O and N_2 in short-term experiments from nitrite or nitric oxide, as detected by membrane-inlet mass-spectrometry (MIMS). Monooxygenases presumably involved in alkane-activation were found to be expressed in cells grown on tetradecane and nitrate in anoxic medium, but not in cells grown with tetradecanoate and nitrate. A mechanism based on the dismutation of two NO molecules to O_2 and N_2 and the immediate use of the produced O_2 for „intra-aerobic" hydrocarbon-activation can be envisaged from these observations. A similar pathway has been suggested for the anaerobic methane oxidation by a denitrifying bacterium [2].

[1] Zedelius, J. et al: Env Microbiol Rep, DOI: 10.1111/j.1758-2229.2010.00198.x.
[2] Ettwig, K.F. et al. (2010): Nature 464: 543-548.

AMV006
The biochemistry of anaerobic ammonium oxidation

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Kuenenia stuttgartiensis is a planctomycete capable of the anaerobic oxidation of ammonium to dinitrogen gas, with nitrite as electron acceptor [1]. Anaerobic ammonium oxidation (anammox) is one of the latest additions to the nitrogen cycle, and found to play a major role in removing fixed nitrogen from oceanic oxygen minimum zones. In addition, the discovery of anammox led to innovative new ways of treating waste water [2].
Although the physiology of anaerobic ammonium oxidation is well understood, the biochemistry is less clear. Based on physiological studies and the genome sequence of *K. stuttgartiensis* [3], a metabolic pathway was predicted. This pathway involves the synthesis and subsequent oxidation of hydrazine, a toxic compound rarely found in biological systems. In the genome sequence, candidate gene clusters for these reactions were identified.
To provide biochemical evidence for this pathway, single cell anammox bacteria were cultivated in a membrane bioreactor. Several highly expressed haem-containing protein complexes were purified by FPLC and identified by MALDI-TOF spectroscopy. The activity of these enzymes was assayed using colorimetric assays, and gaseous end products were analyzed by using stable isotope labeled substrates and GC/MS.
Novel multihaem protein complexes were purified and their catalytic properties with respect to hydroxylamine and hydrazine conversion are investigated. Several of these had high sequence identity to hydroxylamine oxidoreductase. The detailed biochemical characterization and elucidation of the crystal structures of these complexes are currently in progress.

[1] Strous et al. (1999): Nature 400, 446-449.
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AMV007
The Explanation for the Hydrogenase-Negative Phenotype of *Escherichia coli* B Strain BL21(DE3)

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Under anaerobic conditions *Escherichia coli* K-12 synthesizes 3 membrane-associated [NiFe]-hydrogenases (Hyd). Hyd 1 and 2 are uptake hydrogenases that face the periplasm and transfer electrons from molecular hydrogen to the electron transport chain. Hyd 3, together with the formate dehydrogenase H, forms the hydrogen-evolving formate hydrogenlyase (FHL) complex, which uses formate as substrate. The *E. coli* B strain BL21(DE3) is phenotypically Hyd^- when grown anaerobically. Analysis of the genome sequence of BL21(DE3) revealed that all of the genes encoding structural and maturation proteins necessary for the synthesis of active [NiFe]-hydrogenases are present; however, many exhibit amino acid exchanges. In particular, the structural proteins of the FHL complex show multiple substitutions, which correlates with the strain's inability to produce hydrogen gas. Through a series of complementation analyses we could show that BL21(DE3) is able to produce active Hyd 1 and 2 when grown in the presence of high concentrations of nickel ions or when the *fnr* gene was introduced on a plasmid. Immunological evidence for an Fnr protein could not be found in strain BL21(DE3) consistent with the finding that the *fnr* gene of BL21(DE3) has an amber (UAG) mutation at codon 141. Nickel transport is known to be FNR-dependent [1]. Neither introduction of *fnr* nor addition of Ni^{2+} ions restored FHL activity, indicating that the amino acid exchanges in the structural proteins have inactivated at least one component of the complex. Surprisingly, introduction of the *fnr* gene into BL21(DE3) impaired anaerobic growth, suggesting that selective pressure for rapidly growing strains may have led to the inactivation of the *fnr* gene.

[1] Wu, L.-F. and Mandrand-Berthelot, M.-A. (1986): Genetic and physiological characterization of new *Escherichia coli* mutants impaired in hydrogenase activity. Biochimie 68:167-79.

AMV008
Structure and function of the SAM-dependent uroporphyrinogen III methyltransferase NirE involved in heme d_1 biosynthesis in *Pseudomonas aeruginosa*

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Anaerobic growth and survival of *Pseudomonas aeruginosa* is essential for biofilm formation and infection. Replacement of the electron acceptor oxygen by nitrate during denitrification is a powerful strategy for anaerobic energy generation and ecologically indispensable for the global nitrogen cycle. In the second step of the denitrification process the dissimilatory nitrite reductase (cytochrome cd_1) utilizes the prosthetic groups heme *c* and heme d_1 for the reduction of nitrite to nitric oxide. Heme d_1 is not a real heme, rather an isobacteriochlorin related to siroheme, vitamin B_{12} and coenzyme F_{430}. The multistep biosynthesis of this unique cofactor is only poorly understood. The SAM-dependent uroporphyrinogen III methyltransferase NirE catalyzes the key branchpoint step of heme d_1 biosynthesis, namely the methylation of uroporphyrinogen III to precorrin-2. We produced and purified recombinant NirE from *P. aeruginosa*. Purified NirE was biochemically characterized showing for the first time that this protein carries SAM-dependent uroporphyrinogen III methyltransferase activity. The crystal structure of NirE was solved in complex with its substrate uroporphyrinogen III and the reaction product *S*-adenosylhomocysteine. The role of conserved amino acid residues potentially involved in the catalytic mechanism was investigated by site directed mutagenesis. Based on the structure of the enzyme-substrate complex and the mutagenesis studies we propose a novel reaction mechanism for the NirE catalyzed reaction involving a highly conserved arginine residue as the catalytically essential base.

AMP001
The *ptx-ptd* locus from *Desulfotignum phosphitoxidans* has a dual function in phosphite metabolism of this strain

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Phosphorus in living systems typically exists in the [+5] oxidation state as phosphate, phosphate esters, or phosphate anhydrides. Several aerobic bacteria are able to oxidize phosphite [+3] to phosphate [+5] incorporating the latter into their biomass. The first proof of phosphite oxidation as a type of energy metabolism was found with the isolation of an anaerobic phosphite-oxidizing sulfate-reducing bacterium, *Desulfotignum phosphitoxidans* [1].
A genomic library of *D. phosphitoxidans* was screened for clones harboring a gene coding for a protein in the proteome of the strain that is induced by phosphite [2]. Sequence analysis of two positive clones revealed an operon of seven genes *ptxED-ptdFCGHI* predicted to be involved in phosphite oxidation. Four of these genes (*ptxD-ptdFCG*) were cloned and heterologously expressed in *Desulfotignum balticum*, a related strain that cannot use phosphite as either an electron donor, or as a phosphorus source. The four-gene cluster was sufficient to confer phosphite uptake and oxidation ability to the host strain [3]. Therefore the *ptx-ptd* cluster from *D. phosphitoxidans* plays a double role in phosphite metabolism in this strain, - once in the energy metabolism where phosphite serves as electron donor and second in the supplementation of the strain with phosphorus source for assimilation when needed.

[1] Schink, B. et al (2002): *Desulfotignum phosphitoxidans* sp. nov., a new marine sulfate reducer that oxidizes phosphite to phosphate. Arch Microbiol 177:381-391.
[2] Simeonova D.D. et al (2009): Unknown-genome-proteomics. A new NAD(P)-dependent epimerase/ dehydratase revealed by N-terminal sequencing, inverted PCR and high resolution mass spectrometry. Mol Cell Proteomics 8 (1): 122-131.
[3] Simeonova D.D. et al (2010): Identification and heterologous expression of genes involved in anaerobic dissimilatory phosphite oxidation by *Desulfotignum phosphitoxidans*. J Bacteriol, 192 (19): 5237-5244.

AMP002
Development of a Genetic System for *Geobacter metallireducens*

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Members of the obligately anaerobic, metal oxide respiring genus *Geobacter* play an important role in the bioremediation of organic compounds [1]. Growth substrates of *Geobacter* species include various aromatic compounds like benzoate, phenol, *p*-cresol and toluene. Recent studies revealed that obligately anaerobic bacteria such as *G. metallireducens* and facultative anaerobes use different key enzymes for the complete degradation of aromatic growth substrates [2]. To open the door for studying the role of unknown gene products in aromatic degradation pathways, a genetic system was established for *G. metallireducens*. The antibiotic sensitivity of this organism was characterized and conditions for efficient cultivation on solid medium were established. A procedure for introducing foreign DNA by electrotransformation was developed. The broad-host range vector pCD342 [3] was used for homologous expression of *bamY*, the only gene in the genome that was predicted to code for a benzoate-CoA ligase. This enzyme activates benzoate to benzoyl-CoA, the central intermediate of most anaerobic aromatic degradation pathways [4]. Mutants of *G. metallireducens* with a disrupted *bamY* gene were surprisingly still able to use benzoate as the sole carbon source. The presence of an unorthodox benzoate-CoA ligase or benzoyl-CoA:acceptor carboxylic acid CoA transferase is being studied.

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[2] Kung et al (2009): PNAS. 106(42):17687-176892.
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AMP003
Microbial reduction of Fe oxides at low ionic strength

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Microbial iron reduction is a major biogeochemical process in groundwater ecosystems and often associated with the degradation of organic contaminants. Iron reduction is limited by the high crystallinity and low solubility of iron oxides which can be overcome by the use of electron shuttles like humic substances [2]. Furthermore, a recent study showed that catalytic amounts of ferrihydrite colloids added to bulk ferrihydrite lead to the complete reduction of iron oxides by *Geobacter sulfurreducens* [1].
The objective of this work was to inquire if adsorbed organic molecules passivate the colloid surfaces or stimulate the catalytic effect of colloidal iron oxides. Microbial anaerobic reduction experiments with *G. sulfurreducens* were conducted with 260 nm ferrihydrite colloids in a 100-fold diluted freshwater medium. Acetate was used as model organic compound. Within the first 30 hours, the ferrihydrite was totally reduced. This high reactivity is attributed to the high spatial availability of the nanosized ferrihydrite colloids and therefore a higher bioavailability than bulk ferrihydrite. During sorption experiments with ferrihydrite colloids and fulvic acids from Gorleben the sorption capacity was determined.
In conclusion, nanosized iron oxides are supposed to play a significant role in electron transfer processes in anoxic ecosystems.

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AMP004
Function and Regulation of Carbon Monoxide Dehydrogenase/Acetyl-CoA Synthase in *Methanosarcina acetivorans*

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Methanosarcina species are among the most metabolically versatile methanogens, as they can use methylated compounds, H_2+CO_2 or acetate for growth as well. The model organism *Methanosarcina acetivorans*, a marine mesophile, is unable to utilize H_2+CO_2, but can use carbon monoxide (CO)

as the sole source of energy for growth. Carbon monoxide dehydrogenase/ acetyl-coenzyme A (acetyl-CoA) synthase (CODH/ACS) catalyzes CO oxidation as well as acetyl-CoA synthesis/cleavage, and is, therefore, the key enzyme for growth on CO or acetate. The *M. acetivorans* genome contains two copies of a six-gene operon encoding CODH/ACS-isoforms (designated Cdh1 and Cdh2), which share 95 % amino acid sequence-identity, and encodes a single stand-alone CdhA subunit, designated CdhA3. To address the role of these CODH/ACS-isoforms in *M. acetivorans,* the complete set of *cdh* disruption mutants was constructed and phenotypically analyzed. To address differential *cdh*-expression, reporter strains were constructed carrying fusions of the individual *cdhA* promoters and *uidA*, both in the wild-type strain background and in the single *cdh* mutants. Both analyses, of *cdh* gene expression and of the mutant phenotypes, will be presented and argue for a clear functional hierarchy and regulatory cross-talk of the CODH/ACS-isoforms.

AMP005
Thiosulfate dehydrogenase from *Allochromatium vinosum*: an unusual acidophilic *c*-type cytochrome

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Evidence is emerging that *c*-type cytochromes with an unusual axial His/Cys coordination of the heme iron play a pivotal role in sulfur-based energy metabolism [1]. We identified the acidophilic tetrathionate-forming thiosulfate dehydrogenase from the purple sulfur bacterium *Allochromatium vinosum* [2] as another probable member of this exciting group of proteins. The corresponding gene (*tsdA*, YP_003442093) was identified on the main *A. vinosum* chromosome (NC_013851) on the basis of the previously determined N-terminal amino acid sequence. The identity of the gene was confirmed by experiments with an *A. vinosum ΔtsdA in frame* deletion mutant. This strain completely lost the ability to produce tetrathionate from thiosulfate while the production of sulfate via the thiosulfate-oxidizing Sox multienzyme complex was unaffected. The *tsdA* gene starts with a sequence encoding a typical Sec-dependent signal peptide. The mature enzyme is a soluble periplasmic monomeric 25.8-kDa cytochrome *c*. Homolgous genes are present in a number of α-, β-, γ- and ε-proteobacteria including human pathogens like *Campylobacter jejuni*. The rather wide-spread occurrence of the gene agrees with reports of tetrathionate formation not only by specialized sulfur oxidizers but also by many chemoorganoheterotrophs that use thiosulfate as a supplemental but not as the sole energy source. The amino acid sequence deduced from the *A. vinosum tsdA* gene contains two possible Cys-X_2-Cys-His heme binding motifs. Comparative sequence analysis provides indication for axial coordination of the two heme irons by methionine (Met_{222} or Met_{236}) and cysteine (Cys_{123}). Recombinant TsdA produced in *E. coli* was indiscernible from the native *A. vinosum* protein regarding specific activity, pH optimum and UV-Vis spectrum. To investigate the role of conserved Cys_{123} for catalysis and heme coordination, mutant forms of the protein in which this residue was replaced by either glycine, histidine or serine were also produced. All these were essentially inactive, thereby proving the importance of Cys_{123} for catalysis. EPR spectroscopic characterization of the wild type protein yielded signals that can be provisionally attributed to a His/Cys-ligated heme.

[1] Grein et al (2010) Biochemistry 49, 8290-8299.
[2] Hensen et al (2006) Mol Microbiol 62, 794-810.

AMP006
Application of anaerobic fluorescence proteins for *in vivo* reporter systems in clostridia

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Fluorescent proteins such as the green fluorescence protein and its derivatives strictly require oxygen similar to luciferase-based reporter systems, which excludes these gentle *in vivo* reporters for applications in anaerobes. Recently, novel flavin mononucleotide (FMN)-based fluorescent proteins harboring light-oxygen-voltage domains were engineered for non-invasive reporter systems applicable for both aerobic and anaerobic conditions in *Escherichia coli* and *Rhodobacter capsulatus* (Drepper *et al.*, Nat. Biotechnol. 25:443-445). We have optimized these fluorescence-based reporters for *Clostridium acetobutylicum* and this study provides suitable applications for monitoring gene expression in members of the genus *Clostridium*. Since this group of anaerobic bacteria, which contains both important pathogenic strains and apathogenic species of biotechnological impact, severely lacks a good choice of genetic tools for modifying gene expression, we generated a basic plasmid portfolio to monitor gene expression in clostridia. For this, we constructed several *E. coli-Clostridium* shuttle vectors according to a new modular plasmid system comprising different origins of replication for the use in various clostridial species (Heap *et al.*, J. Microbiol. Methods 78:79-85). Furthermore, we provide a novel high-throughput application for analyzing and engineering gene expression in *C. acetobutylicum* in a 96-well microtiter plate scale.

AMP007
Studies on the interaction of the *O*-demethylase components of the anaerobe *Acetobacterium dehalogenans*

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The anaerobe homoacetogen *Acetobacterium dehalogenans* utilizes the methyl group of phenyl methyl ethers, which are products of lignin degradation, as a carbon and energy source. The *O*-demethylation reaction in which the methyl group of the substrate is transferred to tetrahydrofolate is mediated by the key enzymes, the *O*-demethylases, in the methylotrophic metabolism. Different *O*-demethylases are induced in response to different phenyl methyl ethers.
The *O*-demethylase complex consists of four enzymes: a methyltransferase I (MT I), a methyltransferase II (MT II), a corrinoid protein (CP) and an activating enzyme (AE). The methyl group is transferred from the phenyl methyl ether to the super-reduced corrinoid protein by MT I. The methylated corrinoid protein is subsequently demethylated and the methyl group is transferred to tetrahydrofolate by MT II. The inactivated form of the corrinoid protein, cob(II)alamin, which may be generated by inadvertent oxidation, is reduced to active cob(I)alamin by the activating enzyme in an ATP dependent reaction. To investigate the reaction mechanism of the enzyme system we purified and currently characterize the four protein components. The investigation also includes protein-protein interaction studies using biochemical methods and electron microscopy.

AMP008
Propionic acid metabolism during biowaste digestion-dominant degraders and their oxidation pathways

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Anaerobic digestion is known as a solution for biowaste utilization with biogas production and its potential is estimated to share at least 25 % of the bioenergy produced in European Union in the future [1]. It's complexity and sensitivity requires however an effort in maintaining the performance without any failure. The process is often disrupted by i.e. organic overload what leads to volatile fatty acids accumulation, especially propionic acid (PA), pH drop and digester upset [2]. The diversity of microorganism groups taking part in biowaste conversion into biogas makes it difficult to manage and describe. The need for analyzing microorganisms' communities in anaerobic digesters is essential to understand the process and facilitate stable ecosystem by finding optimal conditions [3]. However, there is still too little information about involved bacteria. Identification and description of PA degraders can be done by i.e. the metabolic degradation pathway identification and Fluorescence *in-situ* hybridization (FISH). A combination of a Dani 3950 headspace sampling unit (HS), a Varian 431 gas chromatograph (GC) and a Varian 210 mass spectrometer (MS) has been applied to quantify and specifically identify metabolites of PA oxidation. The use of 1-^{13}C-labeled PA as a carbon source for microorganisms allows differentiation between two known pathways (methyl-malonyl-CoA and C-6-dismutation) resulting in CO_2 and acetic acid (AC) production. Appearance of the ^{13}C-moiety either in the carboxyl and methyl moiety of AC can be detected by MS. The method was successfully applied for

pathway determination in digesters fed with market waste. Furthermore, the use of specific 16S rRNA oligonucleotide probes allowed pointing out the main species responsible for PA oxidation. The isolation of degraders by enriching inocula from several anaerobic digesters on PA as main carbon source and with addition of several electron acceptors is undertaken to find the suitable parameters for optimal growth of considered organisms. Finding the „fastest" ones should be crucial in sustaining CH_4 production without stagnation phase. The isolation of a species from *Syntrophobacter* group degrading PA with SO_4^{2-} as an electron acceptor resulted in obtaining the PA degradation rate of 4 mM/ day, what is promising in solving the problem of this acid accumulation.

[1] Chen, Y. et al (2008): Inhibition of anaerobic digestion process: A review. Biores Technol 99: 4044-4064.
[2] Schievano, A. (2010): Evaluating inhibition conditions in high-solids anaerobic digestion of organic fraction of municipal solid waste. Biores Technol 101: 5728-5732.
[3] Shin, S. (2010): Qualitative and quantitative assessment of microbial community in batch anaerobic digestion of secondary sludge. Biores Technol 101: 9461-9470.

AMP009
Switching *Clostridium acetobutylicum* to an ethanol producer by disruption of the butyrate/butanol (C4) fermentative pathway

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As a response to vastly decreasing fossil oil resources and increasing environmental problems, biotechnological routes for energy production become more and more important. *Clostridium acetobutylicum* naturally produces acetone, butanol and ethanol at a ratio of 3:6:1, and regained much interest recently for microbial biofuel production. However, the potential of the clostridial metabolic capacities have not been explored in much detail, because genetic inaccessibility of these bacteria prevented detailed research on the molecular level until today. In this study, the stable knock-out mutant *C. acetobutylicum* C4 was generated and validated by DNA/DNA (Southern) hybridization. Interestingly, the entire butyrate/butanol (C4) metabolic pathway of *C. acetobutylicum* could be inactivated without a severe growth limitation and indicated the general feasibility to manipulate the central fermentative metabolism for product pattern alteration. Cell extracts of *C. acetobutylicum* C4 revealed clearly reduced enzyme activities of the C4 biosynthetic pathway as compared to the wild-type strain. Neither butyrate nor butanol were detected in cultures of *C. acetobutylicum* C4, but instead up to 16 and 20 g/l ethanol were produced in glucose and xylose batch cultures, respectively. Further sugar addition in glucose fed-batch fermentations increased the ethanol production to a final titer of 33 g/l, resulting in an ethanol yield close to the theoretical maximum.

AMP010
Interaction of the Formate Channel FocA with Pyruvate Formate-Lyase in *Escherichia coli*

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Formate is one of the major products of mixed-acid fermentation in Enterobacteria such as *Escherichia coli* and is an important electron donor for many anaerobes. The accumulation of formate in the *E. coli* cell can lead to acidification of the cytoplasm and therefore a mechanism to regulate its level must be available. FocA is a formate channel protein that belongs to the family of formate-nitrite transporters (FNT). A mutant unable to synthesise FocA accumulates formate in the cytoplasm and has reduced ability to import exogenously supplied formate. FocA thus facilitates the bi-directional transport of formate across the cytoplasmic membrane. The aim of this study is to understand whether and how the direction of formate movement across the membrane is controlled. The *focA* gene is co-transcribed with *pflB,* which encodes pyruvate formate-lyase, the cytoplasmic enzyme responsible for formate generation. The strictly coordinated synthesis of FocA and PflB suggested that PflB might provide a means of controlling formate tranport. Indeed, a specific FocA-dependent interaction of PflB with the cytoplasmic membrane was demonstrated. Moreover, using a variety of experimental approaches including pull-down and two-hybrid methods, we could show that the inactive form of PflB interacts with FocA. Using a formate-dependent $fdhF_P$::*lacZ* transcriptional fusion as a reporter system to monitor changes in the intracellular formate concentration we measured the FocA activity in dependence of the presence of PflB and its activator PflA. Progress towards the identification of the mechanism underlying the control of formate transport by PflB will be discussed.

AMP011
The anaerobic metabolism of phenylalanine and tryptophan

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The genome of the sulphate-reducing hyperthermophile *Archaeoglobus fulgidus* contains putative genes for several interesting pathways [1]. Specific enzyme assays, NMR spectroscopy of labelled substrates and growth experiments, as well as genomic-wide searches for relevant genes, allowed us to propose a pathway of phenylalanine metabolism. The precedent for this is *Clostridium sporogenes, a* gut bacterium which ferments the aromatic amino acids phenylalanine [2] and tryptophan as electron acceptors in a Stickland reaction to the respective propionic acids. Indolepropionic acid (IPA) is produced in the gut from tryptophan and not by direct condensation of indole with acrylate or propionate. IPA is known as an oxygen radical scavenger in the human brain and is of great medical interest.

[1] Klenk, H.P. et al (1997): The complete genome sequence of the hyperthermophilic, sulphate-reducing archaeon *Archaeoglobus fulgidus*. Nature *390*, 364-370.
[2] Dickert, S. et al (2000): The involvement of coenzyme A esters in the dehydration of (*R*)-phenyllactate to (*E*)-cinnamate by *Clostridium sporogenes*. Eur J Biochem *267*, 3874-3884.

AMP012
Study of anoxic steroid metabolism by the denitrifying bacteria

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Steroid compounds have diverse and important physiological activities in higher organisms. Therefore, natural and man-made steroids have many pharmaceutical and clinical applications. On the other hand, in the environmental aspect, many steroids are also known endocrine disruptors for animals and aquatic species. Testosterone and estrone are difficult to be degraded by bacteria because of its complex chemical structure and low solubility in water, especially under anoxic conditions. The established oxic testosterone catabolic pathway involves several oxygenase-catalyzed reactions requiring molecular oxygen as co-substrate and thus is not available for anaerobes. Interestingly, almost nothing is known about microbial catabolism of estrone in the presence or absence of oxygen. *Steroidobacter denitrificans* DSMZ18526 shown to be able to degrade estrone or testosterone completely to CO_2 in the absence of oxygen was utilized as the model organism. We identified eight intermediates involved in anoxic catabolism of testosterone. According to our current data, a novel testosterone catabolic pathway has been proposed. We demonstrated that under anoxic conditions *S. denitrificans* use some common, but also some fundamentally different intermediates as compared to the established oxic pathway. In addition, two-dimensional gel electrophoresis has been applied to compare the soluble protein pattern of *S. denitrificans* grown anaerobically on glutamate with the pattern obtained for *S. denitrificans* cells grown on estrone or testosterone. Several protein spots corresponding to estrone or testosterone catabolism have been identified.

AMP013
Nitrogenase activity in *Wolinella succinogenes* and *Geobacter sulfurreducens*

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Nitrogen is an essential constituent of different biomolecules such as amino acids and nucleic acids. But only a small number of bacteria are able to reduce the inert form of atmospheric dinitrogen (N_2) to ammonia (NH_3). This reaction is catalyzed by the enzyme nitrogenase that contains two component metalloproteins, the Fe-protein and the MoFe-protein. The best known nitrogenase so far is the Mo-containing nitrogenase from *Azotobacter*

vinelandii. The proteins are encoded by the *nif*-genes (nitrogen-fixation). These *nif*-genes were also found in some other bacteria, for example *Geobacter sulfurreducens* and *Wolinella succinogenes*. We show that these bacteria expresses the *nif*-genes if there is only little or no ammonia availiable. The expression of nitrogenase could be followed by a reduction-assay of acetylene to ethylene using gas chromatography. The proteins were purified and identified by mass spektroscopy.

Baar, C. et al. (2003): Complete genome sequence and analysis of *Wolinella succinogenes*, *PNAS*, 100, 11690-11695.
Einsle, O. et al.(2002): Nitrogenase MoFe-Protein at 1.16 Å Resolution: A central ligand in the FeMo-Cofactor. *Science*, 297, 1696 - 1670.
Stubal, J. C. et al. (2009): Genome sequence of *Azotobacter vinelandii*, an obligate aerobe specialized to support diverse anaerobic metabolic processes, *Journal of Bacteriology*, 191, 4534-4545.

AMP014
Assessment of molybdenum oxidoreductases in *Wolinella succinogenes*: key enzymes for formate-dependent anaerobic respiration with nitrate, polysulfide and arsenate

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Molybdopterin-containing oxidoreductases are widely used in anaerobic microbial metabolism, for instance in the catabolic turnover of energy substrates such as formate, nitrate, polysulfide, thiosulfate, tetrathionate, dimethyl sulfoxide, trimethylamine N-oxide, chlorate, selenate and arsenate. In many cases, such proteins form complexes with an iron-sulphur cluster protein and a quinone/quinol-reactive membrane protein and typically this composition is reflected in the corresponding gene clusters. However, it is hard to predict the substrate specificity of a particular molybdoenzyme from its primary structure, especially in case of enzymes that catalyse the turnover of sulphur-containing compounds. The genome of the Epsilonproteobacterium *Wolinella succinogenes* encodes eleven distinct molybdopterin oxidoreductases comprising periplasmic nitrate reductase, polysulfide reductase, dimethyl sulfoxide reductase and at least two formate dehydrogenases (1). Three of the remaining enzymes are predicted to form membrane-bound complexes with an iron-sulphur protein and a membrane anchor of the widespread PsrC/NrfD family. The molybdopterin-containing subunit of these complexes is thought to be located in the periplasm due to the presence of Tat-type signal peptides. Corresponding gene deletion mutants were constructed and characterized concerning growth by anaerobic respiration and conversion of various typical molybdoenzyme substrates. It turned out that one of the so far uncharacterised enzymes sustained growth by arsenate respiration while another one served as an alternative polysulfide reductase.

[1] Baar et al (2003): Complete genome sequence and analysis of *Wolinella succinogenes*, Proc Natl Acad Sci USA. 100 (20): 11690-5.

AMP015
First steps into production of (*R*)-benzylsuccinate via an anaerobic microbial synthetic pathway

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The denitrifiying bacteria *Aromatoleum aromaticum*, *Thauera aromatica* and the iron(III)-reducing species *Geobacter metallireducens* degrade toluene under anaerobic conditions. The first intermediate of the pathway is (*R*)-benzylsuccinate, an aromatic compound of potential interest in the production of polymers, due to its two reactive carboxyl groups. We attempt to redesign the fermentation metabolism of standard bacteria such as *Escherichia coli* to establish the production of this intermediate as a biotechnological process. Synthesis of benzylsuccinate may either be started from the fermentation intermediate fumarate and exogenous toluene or from the fermentation product succinate and exogenous benzoate. We started our first efforts with the production of benzylsuccinate from benzoate, using the toluene pathway in reverse, since most steps can be catalyzed under aerobic conditions. To enter this reverse pathway, the precursor benzoate must be transported into the cytosol via a membrane-bound permease and activated to benzoyl-CoA. Subsequently, benzoylsuccinyl-CoA is formed from benzoyl-CoA and succinyl-CoA followed by several further steps to benzylsuccinate as end product. First results on establishing benzoyl-CoA formation and characterizing the benzoylsuccinyl-CoA thiolase of *Thauera aromatica* will be shown.

AMP016
Anaerobic metabolism of toluene in denitrifying, Fe(III) and sulfate-reducing bacteria

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Anaerobic degradation of toluene is initiated by an unusual addition reaction of the methyl group of toluene to the double bond of a fumarate cosubstrate to form the first intermediate (*R*)-benzylsuccinate. This reaction is catalyzed by the glycyl radical enzyme benzylsuccinate synthase, encoded in the toluene-inducible *bss*-operon. We produced all three subunit containing (*R*)-benzylsuccinate synthase (BssA, B and C) and the activating enzyme (BssD) of the denitrifying bacteria *Thauera aromatica* in *Escherichia coli* and present a complete cofactor characterization via Mössbauer-, EPR-, and UV/vis- spectroscopy.
(*R*)-benzylsuccinate is then further degraded via several steps to benzoyl-CoA and succinyl-CoA in a modified ß-oxidation pathway. In an early step of this pathway benzylsuccinyl-CoA is oxidized to phenylitaconyl-CoA by benzylsuccinyl-CoA dehydrogenase, which apparently interacts with electron transferring flavoprotein. Genetical and biochemical analysis of these enzymes in anaerobic toluene-degrading bacteria with different physiology gives insight into conservedness and differences of energy metabolism between denitrifying, Fe(III) and sulfate-reducing bacteria.

AMP017
Benzene degradation under anaerobic conditions

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The iron-reducing culture BF and the sulphate-reducing culture BPL were analyzed in order to identify the first step in benzene degradation under anaerobic conditions. Growth analysis of strain BPL showed that phenol and toluene, which would be intermediates during hydroxylation respectively methylation, are not used as substrates by this strain. Also by proteomic analysis of benzene grown cells no putative enzymes for these reactions could be identified [1]. Based on metabolite analysis with stable isotope-labelled benzene or bicarbonate buffer direct carboxylation to benzoate is proposed as initial reaction [2]. Combined proteomic and genomic analysis of strain BF led to the identification of a putative anaerobic benzene carboxylase (Abc) consisting of several subunits, which show 43% and 37% sequence identity to phenylphosphate carboxylase subunit PpcA and PpcD and 67% to 3-octaprenyl-4-hydroxybezoate carboxy-lyase (UbiD/ubiX) of *Aromatoleum aromaticum* strain EbN1 [2].

[1] Abu Laban *et al* (2009): FEMS Microbiol. Ecol. 68, 300-311.
[2] Abu Laban *et al* (2010): Environ. Microbiol. 12(10), 2783-2796.

AMP018
Metal reduction without outer membrane cytochromes in *Shewanella oneidensis*

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Respiratory metal reduction is a highly abundant form of microbial energy generation. Still, the solubility of metal oxides is often low under neutral conditions. Therefore, *Shewanella* species have established the formation of an extended respiratory chain delivering electrons through the periplasm and onto the cell surface to the insoluble electron acceptor. Surface localized outer membrane cytochromes (OMC) are believed to catalyze the final reduction step.
In previous work, we constructed a deletion mutant devoid of any OMC proteins [1]. This mutant contains an arabinose inducible promotor in front of known key genes for metal reduction: *mtrA* and *mtrB*.
Surprisingly, this mutant retained some low level metal reducing activity. We used this ability for a directed evolution approach selecting for faster growth with ferric citrate as terminal electron acceptor. After several generations, we could isolate a suppressor strain which shows reproducibly

nearly the same growth rate as the wild type during ferric citrate reduction although OMCs are not produced. This reduction is strictly dependent on arabinose induction which triggers the production of MtrA and MtrB.
We are currently investigating which proteins could have functionally replaced the OMCs. Candidates are the proteins of the DMSO reductase. This is the only other main protein complex which is also bound to the outer membrane of *S. oneidensis*. The results of an *in vitro* DMSO reductase measurement point in the same direction: The suppressor mutant showed an elevated DMSO reduction rate when the cells were pregrown on ferric citrate. We hypothesize that parts of this DMSO reductase complex could function as metal reductase module thereby interacting with MtrA and MtrB. This study displays the enormous respiratory versatility and genetic adaptability of *S. oneidensis*. It is furthermore the first evidence for an OMC independent electron transport chain to ferric iron which will most probably have implications in basic and applied sciences.

[1] Bücking, C. et al (2010): *FEMS Microbiol Lett* 306:144-51.

AMP019
Involvement of the *Shewanella oneidensis* decaheme cytochrome MtrA in periplasmic stability of the β-barrel protein MtrB.

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Shewanella oneidensis MR-1 is a model organism for the elucidation of molecular mechanisms involved in dissimilatory iron reduction. The outer membrane ß-barrel protein MtrB is an integral component of the respiratory chain to ferric iron due to its formation of a membrane spanning complex together with the periplasmic *c*-type cytochrome MtrA and the outer membrane *c*-type cytochrome MtrC [1]. We and others have found that MtrB is not detectable in a Δ*mtrA* mutant [2, 3]. In this study the reason for this MtrA dependence was investigated. An effect of *mtrA* expression on *mtrB* transcription was excluded using qPCR. Since heterologous expression experiments in *E. coli* also revealed an MtrA dependent MtrB production, we screened for periplasmic proteases in S. *oneidensis* MR-1 that are similar to ubiquitously distributed proteases in Gram-negative bacteria. A serine-protease (SO_3942) was detected in *S. oneidensis* MR-1 that is highly similar to *E. coli* DegP. Therefore, a conditional *degP E. coli* mutant was constructed and via western blot analysis, we showed that this mutant does not require MtrA for MtrB stability. It was possible to verify the detected DegP sensitivity of MtrB in the absence of MtrA via the construction of a Δ*SO_3942* mutant in *S. oneidensis*. To our knowledge, this is the first description of the necessity of an electron transfer protein (MtrA) for the periplasmic stability of an outer membrane ß-barrel protein (MtrB). Since moduls similar to *mtrA* and *mtrB* can be found in a multitude of proteobacteria it seems reasonable to assume that this novel mechanism of ß-barrel protein guidance through the periplasm is widely distributed as well.

[1] Ross et al (2007): Characterization of protein-protein interactions involved in iron reduction by *Shewanella oneidensis* MR-1. AEM.
[2] Hartshorne et al (2009): Characterization of an electron conduit between bacteria and the extracellular environment. PNAS.
[3] Schicklberger et al: Involvement of the *Shewanella oneidensis* decaheme cytochrome MtrA in periplasmic stability of the β-barrel protein MtrB. AEM accepted.

AMP020
Re-evaluation of the function of the F_{420} dehydrogenase in electron transport in *Methanosarcina mazei*

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Methanosarcina mazei is a methanogenic archaeon that is able to grow on H_2/CO_2, methanol, methylamines, or acetate. Electrons derived from the different substrates are utilized by both membrane-bound and cytoplasmic electron transport pathways before they finally enter the core methanogenic respiratory chain. A couple of redox-active proteins as well as small proteinaceous and non-proteinaceous electron donors are involved in electron transport and thus form the highly complex and branched respiratory chain of this methanogenic archaeon.
In this study, knockout mutants of one of the core proteins in methanogenic respiration were constructed: two genes encoding the membrane-bound F_{420} dehydrogenase were individually deleted (ΔfpoF and ΔfpoA-O) and the corresponding knockout mutants analyzed. Both mutants exhibited severe growth deficiencies with trimethylamine, but not with acetate or trimethylamine + H_2 as substrate. Cell lysates of the *fpo* mutants showed a strong reduction of the F_{420}: heterodisulfide oxidoreductase activity although a second enzyme involved in $F_{420}H_2$ oxidation, the soluble F_{420} hydrogenase, was still present. This led to the conclusion that the predominant part of cellular $F_{420}H_2$ oxidation in *Ms. mazei* is performed by F_{420} dehydrogenase and not by F_{420} hydrogenase.
Enzyme assays of cytoplasmic fractions of the two knockout mutants revealed that ferredoxin: F_{420} oxidoreductase activity was essentially absent in the ΔfpoF mutant, but was present in the other mutant and the wildtype. Subsequently, the single FpoF protein was overproduced in *Escherichia coli* and purified for further characterization. Purified FpoF catalyzed the ferredoxin: F_{420} oxidoreductase reaction with high specificity (K_m for reduced ferredoxin 0.5 µM) but low velocity (v_{max} 225 mU mg^{-1}) and was present in the *Ms. mazei* cytoplasm in considerable amounts. In summary, FpoF might have a dual function: first, to oxidize $F_{420}H_2$ as electron input module of the membrane-bound F_{420} dehydrogenase. Secondly, it might participate in electron transfer from reduced ferredoxin to coenzyme F_{420} in the cytoplasm. Consequently, it might facilitate survival of the *Ms. mazei* Δech mutant that lacks the membrane-bound ferredoxin-oxidizing Ech hydrogenase.

AMP021
Biosynthesis of the [Fe]- hydrogenase cofactor

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Hydrogenases catalyze the reversible activation of molecular hydrogen. The third type of hydrogenase, the [Fe]-hydrogenase, catalyzes the reversible hydrogenation of methenyltetrahydrometanopterin (methenyl-H_4MPT^+) with H_2 to methylene-H_4MPT. This enzyme harbours a unique iron-guanylylpyridinol (FeGP) cofactor in the active site, in which a low-spin iron(II) is coordinated with an acyl-carbon [C(O)-CH_2-pyridinol] and a sp^2-hybridized nitrogen of the pyridinol ring as well as by two carbon monoxide (CO) and the sulfur of cysteine 176 of the protein (Hiromoto et al 2009). In order to elucidate the biosynthetic pathway of the FeGP cofactor, the acetate auxotroph *Methanobrevibacter smithii* and the autotrophic *Methanothermobacter marburgensis* were grown in the presence of different stable isotopes. After cultivation, the FeGP cofactor was extracted and analyzed by mass spectrometry and NMR spectroscopy. These data indicated that six carbons are derived from C-1 of acetate, three carbons are from C-2 of acetate, five carbons are from C-1 of pyruvate and thus seven carbons are derived from CO_2 (not bound to pyruvate C-1). Based on the labeling patterns, the biosynthetic pathway of the FeGP-cofactor will be discussed.

Hiromoto T, Warkentin E, Moll J, Ermler U, Shima S. 2009. The crystal structure of an [Fe]-hydrogenase-substrate complex reveals the framework for H_2 activation. *Angew Chem Int Ed Engl* 48:6457-60

AMP022
In vitro reductive dearomatization of naphthoyl-Coenzyme A in a sulphate reducing enrichment culture

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Polyaromatic hydrocarbons (PAH) are harmful to the environment and human health; they are highly persistent due to the high resonance energy of the ring system and to the low bioavailability. Whereas the aerobic degradation pathways have been studied in great detail, only little is known about enzymes involved in the anaerobic metabolism of PAHs. The initial activation of naphthalene is considered to proceed either by carboxylation [2] or methylation [3]. In both cases 2-naphthoyl-CoA would be formed. Initial evidence was obtained that this key intermediate is dearomatized by a reduction yielding 5,6,7,8-tetrahydronaphthoyl-CoA (THNCoA) [1], which may be further dearomatized in another reduction step. In this work we demonstrate electron donor-dependent *in vitro* 2-naphthoyl-CoA reductase

and THNCoA reductase activities in extracts from the sulphate reducing enrichment culture N47 grown on naphthalene. The activity (0,7 nmol min^{-1} mg^{-1}) was sufficiently high for the growth rate of cells. Evidence was obtained that two different dearomatizing reductases were involved in anaerobic naphthalene degradation: while the first reduction step of the non-activated ring was independent of ATP hydrolysis, reduction of THNCoA was only observed in the presence of ATP.

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AMP023
Structure and function of the F_{420}-reducing [NiFe]-hydrogenases (Frh) from methanogens

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F_{420}-reducing [NiFe]-hydrogenase (Frh) is a cytoplasmic enzyme, which catalyzes the reversible reduction of coenzyme F_{420} with H_2. Coenzyme F_{420}, a 5-deazaflavin, structurally resembles a flavin. However, it functionally behaves more like the pyridine nucleotides $NAD(P)^+$ in transferring two electrons plus a proton (a hydride) rather than single electrons. F_{420} is involved as a hydride donor/acceptor in the central methanogenic pathway, in which F_{420} is used in the reversible redox reactions between methenyl- and methylene-H_4MPT and between methylene- and methyl-H_4MPT. Frh in the hydrogenotrophic methanogens regenerates the reduced form of F_{420}. Architecturally Frh forms a huge complex with a molecular mass of > 1200-kDa composed of 12 Frh protomers. Each protomer consists of the 47-kDa „large subunit" (FrhA) with the [NiFe]-center, the 26-kDa „small subunit" (FrhG) with three [4Fe4S]-clusters and the 31-kDa iron-sulfur flavoprotein (FrhB) with one [4Fe4S]-cluster and one FAD, which functions as one electron/two electron switch. The Frh-complex from *Methanothermobacter marburgensis* was purified under strictly anaerobic conditions to apparent homogeneity. The Frh complex forms unspecific aggregates with other proteins, which constrain the purification of this enzyme complex. To overcome this problem we used a detergent to solve this aggregates. Structure analysis of the purified enzyme by single particle electron cryo-microscopy and x-ray crystallography are in progress.

AMP024
Differential expression of reductive dehalogenase gene clusters in *Desulfitobacterium hafniense* DCB-2 during growth in the presence of different aromatic organohalides

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Lignin-degrading fungi of boreal forests show the ability to produce chlorinated organic compounds while growing on wood. The organohalides can be subsequently dechlorinated under anoxic conditions by a heterogeneous group of soil bacteria including *Desulfitobacterium* subspecies. Recently, a *Desulfitobacterium hafniense* strain was isolated from a soil sample, in which ligninolytic enzyme activities were detected [1].
D. hafniense strain DCB-2 harbors seven genes encoding reductive dehalogenases [2]. The organism was shown to degrade 3-chloro-4-hydroxyphenylacetate, a model compound for products of fungal lignin degradation, to 4-hydroxyphenylacetate with pyruvate as the electron donor. A 3-chloro-4-hydroxyphenylacetate reductive dehalogenase was purified from *D. hafniense* DCB-2 cells [3]. In the present study we tested the organism for the ability to dechlorinate different *ortho*- and *meta*-chlorinated phenols. Results will be presented that elucidate the effect of the different aromatic organohalides on the reductive dehalogenase (Rdh) gene expression in *D. hafniense* DCB-2. The transcript level of the different *rdh* genes was tested via RT-PCR and the formation of enzymes was examined via activity measurements. Experiments are underway to investigate the influence of fungal exudates and soil extracts on the set of reductive dehalogenases formed in resting cells of *D. hafniense* DCB-2.

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AMP025
The electron transport chain of nitrous oxide respiration in *Wolinella succinogenes*

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Laughing gas (nitrous oxide) is one of the most important greenhouse gases and accounts for about 10% of the global warming effect. It is commonly produced in the environment by denitrifying and nitrifying microbial species. In addition to denitrifiers, some respiratory nitrate-ammonifying Epsilonproteobacteria also reduce nitrous oxide to dinitrogen although these organisms probably do not produce substantial amounts of endogenous nitrous oxide in energy substrate turnover. The energy metabolism of one of these bacteria, *Wolinella succinogenes*, has been thoroughly characterized in the past. These cells use either hydrogen or formate as electron donor together with typical terminal electron acceptors of anaerobic respiration like fumarate, nitrate or polysulfide. Here, we show that *W. succinogenes* grows efficiently with formate and nitrous oxide as sole energy substrates to high optical densities. Nitrous oxide is reduced by an unconventional cytochrome *c* nitrous oxide reductase (*c*NosZ) whose presence seems to be largely restricted to Epsilonproteobacteria. The corresponding *nos* gene cluster predicts the presence of a unique electron transport system that is predicted to connect the menaquinone/menaquinol pool with *c*NosZ. The involved electron transport chain may comprise a menaquinol dehydrogenase of the unusual NapGH-type and one or two monohaem cytochromes *c*. Various *nos* gene cluster mutants were constructed and characterized with regard to growth behaviour and enzyme activity. Based on these data, a model of the respiratory *c*Nos system in *W. succinogenes* will be presented.

AMP026
Molybdo- and tungstoenzymes in the anaerobic metabolism of aromatics in *Aromatoleum aromaticum*: Ethylbenzene dehydrogenase and phenylacetaldehyde: ferredoxin oxidoreductase

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Aromatoleum aromaticum contains several molybdo- or tungstoenzymes of 3 different families: DMSO reductase, xanthin oxidase and aldehyde:ferredoxin oxidoreductase (AOR). Among these are enzymes involved in the degradation of different aromatics such as the DSMO reductase type enzyme ethylbenzene dehydrogenase and the AOR type enzyme phenylacetaldehyde:ferredoxin oxidoreductase. These two enzymes will be presented here.
Ethylbenzene dehydrogenase (EbDH) catalyzes the first step of anaerobic ethylbenzene degradation, namely the oxygen independent hydroxylation of ethylbenzene to (*S*)-phenylethanol. EbDH is a heterotrimeric ($\alpha\beta\gamma$) periplasmic enzyme of 160 kDa. The large α subunit contains a *bis*-molybdopterin cofactor as the active site of the enzyme (MoCo enzyme). The α- and β subunits contain 5 [Fe_4S_4] clusters which are involved in the transport of electrons. The smallest subunit (γ) contains a heme *b* which accepts the electrons from the iron-sulfur clusters of the β subunit. The basic biochemical and structural properties of the enzyme were investigated recently. New insights into the catalytic mechanism will be shown on our poster.
In contrast to EbDH, AOR enzymes contain a tungsten cofactor and most representatives described play important roles in peptide fermentation in hyperthermophlic archaea. However, more and more AOR type enzymes are also found in anaerobic mesophilic bacteria. When grown on phenylalanine as sole carbon source, *A. aromaticum* produces an enzyme homologous to these thermophilic tungsten enzymes. Simultaneously, an induced phenylacetaldehyde:ferredoxin oxidoreductase activity has been observed in

the corresponding cell extracts. The enzyme is currently purified and characterized for its biochemical features and the presence of metals. As indicated by the enzymes analyzed here, *A. aromaticum* may be a model system for the coexistence of molybdenum- and tungsten-enzymes in the same cell. In addition, detailed genome evaluation revealed hints for the existence of metal-specific isoenzymes for molybdate or tungstate transport and for molybdenum or tungsten insertion during molybdenum-cofactor biosynthesis.

AMP027
The Role of *adhE 2* in the solventogenic *Clostridium acetobutylicum*

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The large genus *Clostridium* encompasses species like *Clostridium acetobutylicum* that are able to ferment starch and sugars into solvents. Especially butanol is an important bulk chemical with a wide range of industrial applications for example as biofuel due to its superior properties compared to ethanol and biodiesel.
The aldehyde/alcohol dehydrogenase (AADH) is mainly involved in butanol formation and possibly plays an important role in the switch from acidogenesis to solventogenesis. During sequencing of the *C. acetobutylicum* ATCC 824 genome two open reading frames (ORFs) encoding for a bifunctional aldehyde/alcohol dehydrogenase (AADH) were identified. Both are carried by the pSOL1 megaplasmid. The 2,577-bp *adhE2* (CAP0035) is located about 47 kb away from the *adhE* (CAP0162) which has been shown to be the gene responsible for the two final steps of butanol production in solventogenic cultures. Because the *adhE2* is specifically expressed, as a monocistronic operon, under the condition of a high $NADH/NAD^+$ ratio, it is assumed to be responsible for the butanol production in alcohologenic cultures.
For further researches we generated a mutant of the gene *adhE2* using the ClosTron technique, a clostridial insertional inactivation system that based on the selective retargeting of a group II intron. Within batch fermentations of the mutant we took samples for quantitative analysis which include the determination of the substrate/product concentrations and transcriptome time series. Using an oligonucleotide-based microarray we obtained an overview of the transcript levels in the *adhE2*-mutant.

AMP028
Monitoring of a biogas producing microbial community by its metaproteome

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For the production of biogas, various agricultural wastes but also crops can be utilized by a complex microbial consortium consisting of fermentative bacteria and methanogenic archaea. Here, we present a metaproteomic approach to investigate the metabolic activities of methanogens within a biogas producing community.
A robust method for protein extraction and separation from biogas reactor samples was developed including (i) a phenol extraction step, (ii) a method for estimation of protein quantities in presence of interfering substances, and (iii) paper-bridge loading for first dimension isoelectric focusing leading to efficient removal of contaminants. After two-dimensional gel electrophoresis, major protein spots were analyzed by nanoHPLC online coupled to tandem mass spectrometry in order to identify major proteins.
Attention was directed to the extraction of intracellular proteins as this protein fraction promises to give further insight into the pathway of methanogenesis. From approximately sixty analyzed spots, almost a third could be mapped to the methanogenic pathway.
Key enzymes of methanogenesis like methyl-coenzymeM reductase were found to be expressed in high amounts by different members of the family of *Methanosarcinaceae*, which can produce methane from acetate as well as by the reduction of CO_2. Interestingly, also members of the *Methanomicrobiaceae*, which only use the hydrogenotrophic pathway of methanogenesis, were identified. These results suggest that methane is at least to a certain part produced from CO_2 and H_2. This claim is supported by the findings of several house-keeping enzymes of *Anaerobaculum hydrogeniformans*. This syntrophic organism produces H_2 and can only grow, if H_2 is removed by other organisms, for example by methanogenic archaea.
Our study proves the feasibility of the extraction and characterization of the metaproteome of complex biogas producing communities in principle and gives valuable insights into active metabolic pathways. In future, monitoring of protein expression patterns may act as a valuable tool for the estimation of the metabolic activity of a microbial community helpful for process optimization.

AMP029
Disproportionation and possible electron bifurcation reactions involved in crotonate fermentation by *Syntrophus aciditrophicus*

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The fermenting Deltaproteobacterium *Syntrophus aciditrophicus* is able to degrade crotonate in absence of a syntrophic partner with acetate and cyclohexanecarboxylate representing the main fermentation products [Mouttaki 2008]. The reducing equivalents formed during crotonate oxidation to acetate are recycled in reverse reactions of the benzoyl-CoA degradation pathway yielding cyclic mono-/dienoyl-CoA compounds, which might be further reduced to cyclohexanoyl-CoA. In this work we studied the unknown formation of cyclohexanecarboxylate from the proposed fermentation intermediates cyclohex-1-ene-1-carboxyl-CoA (monoenoyl-CoA)/cyclohexa-1,5-diene-1-carboxyl-CoA (dienoyl-CoA). Cell-free extracts from *S. aciditrophicus* grown on crotonate disproportionated both, monoenoyl-CoA to dienoyl-CoA plus benzoyl-CoA, and dienoyl-CoA to benzoyl-CoA plus monoenoyl-CoA. Such disproportionation reactions are assigned to activities of W-containing class II benzoyl-CoA reductases (BCR) as described previously for G*eobacter metallireducens* [2]. The cyclohexanoyl-CoA formed is then converted to cyclohexanecarboxylate either by a thioesterase or a CoA transferase. In the presence of external electron donors such as dithionite or NADH, the benzoyl-CoA formed during the disproportionation reactions was converted to reduced cyclic products. The endergonic reductive dearomatization of benzoyl-CoA to dienoyl-CoA by NADH ($\Delta G^{\circ\prime} = +58$ kJ mol^{-1}) can only be explained by an electron bifurcation mechanism. We propose that this reaction is driven by the concomitant reduction of dienoyl-CoA by NADH to monoenoyl-CoA ($\Delta G^{\circ\prime} < -50$ kJ mol^{-1}). The combined action of disproportionation and electron bifurcation reactions enables an extended recycling of reducing equivalents in *S. aciditrophicus* during growth on crotonate.

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[2] Kung, J.W. et al (2010): Reversible Biological Birch Reduction at an Extremely Low Redox Potential. Proc Nat Acad Sci 132:9850-9856.

AMP030
Anaerobic degradation of *p*-methylbenzoate by the denitrifying strain pMbN1 involves a novel type of benzoyl-CoA reductase

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In anaerobic bacteria a large variety of aromatic compounds is converted to the central intermediate benzoyl-CoA, which serves as substrate for dearomatizing benzoyl-CoA reductases (BCRs). However, common BCRs do not accept *p*-methylbenzoyl-CoA as a substrate, which is probably the reason why known aromatic compound degrading anaerobes cannot utilize *p*-methylbenzoate. The newly isolated denitrifying α-proteobacterium strain pMbN1, belonging to the genus *Magnetospirillum*, uses *p*-methylbenzoate or benzoate as sole carbon source, which enabled a first study of the unknown *p*-methylbenzoate degradation pathway.

Differential protein profiling (2D-DIGE) of *p*-methylbenzoate- in comparison to benzoate- or succinate-adapted cells revealed the specific up-regulation of several proteins. Their coding genes form two distinct clusters. The predicted functions of the gene products are in agreement with a degradation pathway analogous to the known benzoyl-CoA pathway. However, the putative *p*-methylbenzoyl-CoA reductase displays pronounced sequence disparity from the classical, *Thauera*-type benzoyl-CoA reductase, suggesting a specific adaptation for handling the methyl-group in *para*-position. This suggestion is supported by metabolite analysis of cultures grown with *p*-methylbenzoate, which identified methyldihydrobenzoate and methyltetrahydrobenzoate. In accordance, cell extracts of *p*-methylbenzoate-adapted cells transformed *p*-methylbenzoyl-CoA to the respective 4-methyl-dienoyl-CoA and 4-methyl-6-hydroxy-monoenoyl-CoA compounds. In addition, 3-methylglutarate was putatively identified in the culture medium, suggesting conservation of the methyl group after ring cleavage. This finding suggests that the further oxidation of the putative 3-methylglutaryl-CoA intermediate requires a C-skeleton rearrangement.

AMP031
Regulation of anaerobic aromatic hydrocarbons degradation in *Aromatoleum aromaticum* under anaerobic growth condition

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The denitrifying Betaproteobacterium *Aromatoleum aromaticum* utilizes a wide range of aromatic compounds under anoxic conditions i.e., ethylbenzene, acetophenone or toluene. The expression of the gene clusters coding for the enzymes of the respective metabolic pathways is induced in response to the presence of the specific substrates. The genome sequence of *A. aromaticum* allowed identifying the genes coding for the enzymes of anaerobic toluene or ethylbenzene metabolism. Moreover, three operons coding for two-component regulatory systems were found as possible candidates for affecting the coordinate induction of all toluene-catabolic genes (*tidSR*) and the sequential induction of ethylbenzene metabolism by ethylbenzene (*ediSR*) and the intermediate acetophenone (*adiSR*). We investigate here the operon *adiSR* which is probably involved in the regulation of acetophenone catabolic enzymes. The function of these genes was investigated by genetic and biochemical studies: a deletion mutant of *A. aromaticum* coding the *adiSR* operon was unable to grow on acetophenone and was complemented by adding the *adiSR* genes. Moreover, the predicted acetophenone sensing histidine kinase (AdiS) was overproduced in *E. coli* and its biochemical properties i.e. ligand binding or autophosphorylation were studied.

AMP032
Carbon isotope fractionation of homoacetogenic bacteria - taking the environment into account

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In biological systems the natural abundance of stable carbon isotopes (expressed as ratio $^{13}C/^{12}C$) can be used to track the metabolic interaction of organisms. It is generally believed, that every biological pathway has a certain isotopic selection, which can be summarized in the so called fractionation factor ε. Despite their physiological and genetic variance homoacetogenic bacteria have a rather uniform fractionation behavior, which however is governed by the environmental conditions. The apparent fractionation factor varies from -35 ‰ in a carbon limited phosphate medium and -60 ‰ in a carbon rich carbonate medium. When grown on H_2/CO_2 the isotopic signature of the initially formed acetate (around -60 ‰) is independent from the signature of the substrate. There is no intramolecular fractionation in the acetate formed. If formiate is added as additional substrate, the initially formed acetate still has the same signature (around -60 ‰). Therefore we speculate that the product release rather than the pathway itself may be the limiting fractionation step.

AMP033
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Genetic analysis revealed that the six *rnf*ABCDEG-genes from *Rhodobacter capsulatus* are responsible for the electron flow to nitrogenase (*rnf* = Rhodobacter nitrogen fixation). Homologous genes have been detected in *Clostridium tetanomorphum*, cloned and sequenced. The sequences are 40-45% identical to the deduced sequences of the Rnf-subunits from *R. capsulatus*. In this work, the membrane-bound, iron-sulfur and flavin-containing electron transport complex has been purified from *C. tetanomorphum* that catalyses the reduction of NAD^+ ($E°' = -320$ mV) with ferredoxin ($E°' \leq -420$ mV). The Rnf complex consists of six subunits (RnfABCDEG), of which four N-termini (RnfCDEG) could be sequenced. Here we present evidence that the Rnf complex is a Na^+-translocating enzyme involved in energy conservation using the difference in the redox potential of ≥ 100 mV between ferredoxin and NAD^+. To determine sodium ion transport, inverted membrane vesicles from *C. tetanomorphum* were prepared, reduced ferredoxin was generated by reduction with Ti(III)citrate and upon addition of NAD^+, transport was measured by using the radioisotope $^{22}Na^+$ [1]. Most likely *C. tetanomorphum* uses this Na^+-pump for additional energy conservation in the fermentation of glutamate to ammonia, CO_2, acetate, butyrate, and H_2 [2].

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AMP034
Real-Time Monitoring of Acetone-Butanol Fermentation by *Clostridium Acetobutylicum* using Reaction Calorimetry and Off-Gas Analysis

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Clostridium acetobutylicum has been used for production of bio-butanol for decades. However, despite being a well examined organism some fundamental metabolic processes are not fully understood yet. Calorimetric investigations are able to deliver valuable additional information.
This study was conducted to ascertain a correlation between heat production rate and gas emissions of *Clostridium acetobutylicum* in order to identify characteristic process states. Building hereupon it is investigated the possibility to use temperature measurement for fermentation process control. *Clostridium acetobutylicum* ATCC 824 was cultivated in a bench-scale reaction calorimeter *Mettler Toledo BioRC1* at 37°C on a synthetic minimal growth medium under anaerobic conditions. Heat production rate was analyzed and compared with conventionally derived growth kinetics and product formation during acetogenic and solventogenic metabolic phases. Data for pH, redox potential and gas production were logged online. For offline analysis of substrate consumption and product formation samples were taken from fermentation broth and exhaust gases. The experimental data show the connections between gas- and heat production rate. In particular for the solventogenic phase a strong correlation was determined. Indications for further interrelations between heat production rate and growth parameters are currently analysed in more detail and will be presented.

AMP035
Diversity and Distribution of Fe(II)-oxidizing and Fe(III)-reducing microorganisms in salt lake sediments of Southern Russia

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Hypersaline environments challenge their inhabiting (micro)fauna with their extreme hyperosmolaric conditions. These conditions result in the common observation that the metabolic diversity decreases with increasing salinity. Nonetheless, various microbial metabolisms have been found to occur at high salt concentration [1]. Currently, information about microbial Fe(III) and Fe(II) metabolism in hypersaline environments is very scarce. We studied Fe(II)-oxidizing and Fe(III)-reducing bacteria and archaea in five different salt lake sediments from the Kalmykien Steppe in Russia with a combination of culture-dependent and -independent techniques. Our goals were 1) to identify and quantify anaerobic Fe(II)-oxidizing and Fe(III)-reducing microorganisms in the salt lake sediments and 2) to measure up to which salinities microbial Fe(II) oxidation and Fe(III) reduction can be detected.
Results from enrichment and isolation experiments showed that Fe(III)-reducers were active and growing even at 5 M salinity while Fe(II)-oxidizing cultures only remained active for several transfers on 0.5 M NaCl. Results from most probable number (MPN) counts and quantitative PCR (qPCR) revealed that culturable Fe(III)-reducers and anaerobic Fe(II)-oxidizers represent <0.1% of all bacteria present in the different sediments. In a sediment core with an iron-oxide-rich layer total bacteria and archaea cell numbers were 10 times higher than in the other sediment layers. DGGE analysis also showed that microbial community diversity was highest in the Fe-mineral rich sediment layers. A 16S rRNA gene clone library was constructed from one of the salt lake sediments in order to study the bacterial and archaeal diversity. Currently, we are using qPCR to analyse the abundance and distribution of different groups of Fe-metabolizers in the salt lake sediments. Additionally, we use the Fe(III)-reducing enrichment cultures to study the effect of different salinities on Fe-transformation rates and iron mineral formation. In summary, this study demonstrates that Fe(II)-oxidizing and Fe(III)-reducing bacteria and archaea are abundant and active in hypersaline environments. In combination with geochemical data this suggests the presence of an active Fe cycle even at high salt concentrations.

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AMP036
Fitting *Methanosarcina* for conversion of carbohydrates to methane

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The anaerobic degradation of carbohydrates to methane and carbon dioxide is carried out by successive metabolic activities involving syntrophic interaction of a variety of microorganisms belonging to all three domains of life. This anaerobic food chain involves hydrolytic bacteria and protozoa, fermenting microorganisms, homoacetogens and methanogenic archaea. The last step in this process, production of methane, is exclusively catalyzed by methanogens. Up to now there is no organism known which is able to convert carbohydrates to methane, which could be due to evolutionary optimization of pathway lengths to maximize the rate of ATP synthesis for growth. Such optimized pathway lengths could also explain the observed division of metabolic labour in anaerobic biomass degradation. On the other hand, a pathway for methanogenesis from glucose would require a high number of enzymatic reactions. The respective organism would, therefore, be outcompeted in habitats with selection for faster growth but might be competitive in stable habitats, like e. g. biofilms, with selection for high ATP yield leading to slower growth.
We chose a synthetic microbiology approach to evaluate this hypothesis by fitting *Methanosarcina acetivorans* for conversion of carbohydrates to methane. From a genomic perspective *M. acetivorans* appears to encode most of the functions for glycolysis and/or gluconeogenesis, lacking only those for carbohydrate uptake and for carbohydrate activation. We have inserted heterologous genes into the genome of *M. acetivorans* to eventually establish conversion from glucose to methane in this organism. Preliminary results from these efforts will be presented and discussed.

AMP037
Clostridium ljungdahlii - insight in the ethanol metabolism of a homoacetogenic Clostridium

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Clostridium ljungdahlii is a mesophilic acetogenic bacterium that was isolated for its ability to produce ethanol autotrophically from synthesis gas. It is of industrial interest because syngas is an inexpensive substrate that can be easily generated by gasification of coal, biomass, or municipal waste.
During autotrophic growth no net-gain of ATP seems to be possible by substrate level phosphorylation as the ATP generated by acetate kinase has to be used for formate activation. This could be explained by the presence of genes for a Rnf-complex, as has been described for the Na^+-dependent homoacetogen *Acetobacterium woodii*. Therefore we propose that the Rnf-complex is involved in the generation of a proton gradient that could be used for ATP synthesis by electron transport phosphorylation. As the Rnf comlex is transcribed during autotrophic and heterotrophic growth, ethanol formation could be the consequence of energy generation from reduced ferredoxin by the Rnf-complex when resulting NADH has to be regenerated.
C. ljungdahlii can not only produce ethanol but can also use it as an electron donor with betaine as electron acceptor thereby producing trimethylamine and acetate. This mechanism could be seen as a new variation of a stickland reaction with an alcohol as electron donor and a glycine-derivative as electron acceptor. We determined the substrate/product levels during growth. Using DNA microarrays we compared the levels of mRNA in fructose grown cells with cells grown on ethanol and betaine and try to reconstruct the carbon and energy metabolism during growth on these substrates. *C. ljungdahlii* possesses two genes for betaine reductases of which only one is highly up-regulated when betaine is used as an electron acceptor.

AMP038
Functional and structural studies on the sulfite- and heterodisulfide reductase - essential steps in the energy metabolism of Archaea

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Dissimilatory sulfite reductase (dSir) catalyzes the reduction of sulfite to sulfide, a key step within the global biogeochemical sulfur cycle. The enzyme from various sulfate-reducing organisms has been described as a multimer, with a molecular mass of approximately 200 kDa. Dissimilatory sulfite reductase from the strict anaerobe *Archaeoglobus fulgidus* was purified and crystallized under the exclusion of dioxygen [1]. The structure of *A. fulgidus* dSir revealed a heterotetrameric organization, with a size of 125 Å x 80 Å x 60 Å that harbours four siroheme-[4Fe-4S] centers and four extra [4Fe-4S] clusters. To obtain further insight into the mechanism of the reaction we used a combination of biochemical, analytical and crystallographic studies [2].
Heterodisulfide reductase (Hdr) from methanogenic Archaea is an iron-sulfur protein that catalyzes one step of the methanogenic energy metabolism by reducing the heterodisulfide CoM-S-S-CoB to CoM-SH and CoB-SH. In methanogens containing cytochromes the reaction proceeds via two membrane associated enzyme complexes VhoACG and HdrDE building up an electrochemical proton potential. In methanogens without cytochromes CoM-S-S-CoB reduction with hydrogen is catalyzed via the cytoplasmic enzyme complex MvhADG-HdrABC, which is presumably the location of a electron bifurcation event [3]. Both enzyme types were purified and to characterize the structural and spectroscopic properties of the MvhADG-HdrABC complex from *Methanothermobacter marburgensis*

crystallographic studies, Mössbauer- and ENDOR spectroscopy were performed [4].

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[3] Thauer, R. K. et al (2008): Methanogenic archaea: ecologically relevant differences in energy conservation. *Nature Reviews Microbiology* 6, 579-591
[4] Fielding, A. J. et al: Multifrequency ENDOR and Mössbauer spectroscopy identifies a unique iron site on the iron-sulphur cluster involved in substrate reduction of heterodisulfide reductase, in preparation

AMP039

Temperature dependence of carbon isotope fractionation in methanogenic cultures

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In anaerobic environments the organic matter is degraded by a concerted action of fermenting, homoacetogenic, hydrolytic and methanogenic organisms. Due to the diversity of these microorganisms a mapping of the processes taking place in the microorganisms is difficult. To distinguish the metabolic pathways used by the different organisms, the isotopic composition of substrates and products can be used to determine an individual fractionation factor. The basic assumption is that each pathway has a characteristic discrimination of the two naturally occurring carbon isotopes ^{12}C and ^{13}C, which can be represented by the fractionation factor.
Conventionally the fractionation factors are calculated for pure cultures grown under standard laboratory conditions. It is however uncertain, if this data reflects the behavior of fractionation in natural environments. To obtain an impression about the robustness of the fractionation factor the isotope ratio of the substrate as well as of the product was measured by GC-IRMS for different methonaogenic archea (*M. acetivorans*, *M. barkeri*, *M. marburgensis*, *M. zinderi*) in pure cultures at various temperature and substrate conditions.
Irrespective of the used substrate, the impact of the temperature on the fractionation factor in all analyzed cultures was only minimal. This suggests that there is no mayor temperature effect on the fractionation of carbon isotopes.

AMP040

Chlorobenzene dehalogenation by *Dehalococcoides* sp. strain DCMB5 and reductive dehalogenase genes encoded in its genome

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The *Dehalococcoides* strains CBDB1 and DCMB5 are able to dehalogenate a multitude of halogenated aromatic compounds like dioxins or chlorobenzenes. Strain CBDB1 possesses 32 genes encoding homologues of reductive dehalogenases (Rdh) in its genome. The function of only one Rdh, CbrA, is known to date, which dechlorinates 1,2,3-tri- and 1,2,3,4-tetrachlorobenzene (Adrian *et al*. 2007). Screening of the recently sequenced genome of strain DCMB5 revealed the presence of 23 *rdh* genes. Thirteen of them have orthologs in strain CBDB1 with more than 97 % sequence identity at the amino acid level. These include one orthologue of *cbrA*. Nine are less than 50 % identical at the amino acid level to those of strain CBDB1 and they also show low similarities to known *rdh* genes of other cultivated *Dehalococcoides* strains.
Strain CBDB1 dechlorinates hexa-, penta-, all three tetra- and two trichlorobenzenes preferably at positions flanked by chlorines at both sides. Strain DCMB5 was enriched on chlorinated dioxins and finally isolated on 1,2,3-trichlorobenzene (TCB) (Bunge *et al*. 2008). In contrast to strain CBDB1, strain DCMB5 did not dechlorinate 1,2,4-TCB. RT-PCR showed that the *cbrA* orthologue was not induced by 1,2,4-TCB. The capacity of strain DCMB5 to dechlorinate higher chlorinated benzenes was analyzed in detail to correlate electron acceptor spectra and dechlorination pathways with genome-encoded *rdh* genes.

[1] Adrian et al (2007): Appl. Environ. Microbiol. 73, 7717-7724.
[2] Bunge et al (2008): Environ. Microbiol. 10, 2670-2683.

AMP041

Anaerobic degradation of propane and butane by sulfate-reducing bacteria from marine gas and oil seeps

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The short-chain, gaseous alkanes propane and butane are important constituents of natural gas and small quantities are also found dissolved in crude oil. They can enter the biosphere through natural oil and gas seeps. In deep-sea environments, such as the Gulf of Mexico, propane and butane are found in significant amounts in gas hydrates of structure Type II. The anaerobic degradation of these alkanes was recently reported with a pure culture (Strain BuS5) and several enrichment cultures of sulfate-reducing bacteria from diverse marine habitats (1). However, the diversity of anaerobic propane and butane degraders at deep-sea hydrocarbon seeps is largely unknown. In this study, enriched cultures of sulfate-reducing bacteria were obtained with propane and butane as substrates using marine sediments collected around gas and oil seeps in the Gulf of Mexico (PropS-GMe and BuS-GMe), and at Hydrate Ridge (BuS-HyR). Notably, the cultures PropS-GMe and BuS-HyR formed large, dark-red aggregates 1 - 2 mm in diameter. Substrate tests showed that the enrichment cultures were able to degrade propane and butane simultaneously. In temperature assays, propane- and butane-dependent sulfate-reduction peaked around 15°C, and completely ceased at temperatures higher than 25°C, reflecting the rather low *in situ* temperatures. Construction of 16S rRNA gene libraries revealed mostly phylotypes affiliated with the *Desulfosarcina/Desulfococcus* cluster of the *Deltaproteobacteria*. Whole-cell hybridization with newly developed sequence-specific probes showed that each enrichment culture was dominated ($\geq$ 70% of the total cell number) by a distinct phylotype most closely related to Strain BuS5. Short-term incubations of active cell suspensions with ^{13}C-labeled propane or butane followed by Halogen In Situ Hybridization-Secondary Ion Mass Spectroscopy (HISH-SIMS) analysis showed substantial ^{13}C-assimilation by the dominant phylotype in each enrichment culture. In contrast, the accompanying bacteria incorporated only negligible amounts of ^{13}C and became more enriched with increasing incubation time, probably by transfer of labeled metabolites from the dominant phylotype. These results demonstrate that in each enrichment culture the dominant phylotype was responsible for degradation of propane or butane, further expanding our knowledge on the diversity of such bacteria at hydrocarbon seeps. These bacteria may be actively involved in the in situ degradation of gaseous alkanes, offering thus an explanation for the high sulfate-reduction rates observed at marine gas or oil seeps.

[1] Kniemeyer, O. et al (2007): Anaerobic oxidation of short-chain hydrocarbons by marine sulphate-reducing bacteria. *Nature* 449, 898-901.

AMP042

Protein complexes involved in the electron transport chain of anammox bacteria

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Anaerobic ammonium oxidizing (anammox) bacteria conserve energy from the oxidation of ammonium to dinitrogen gas with nitrite as the electron acceptor.
This process involves a cyclic electron flow. Our current hypothesis is that electrons released from hydrazine oxidation by the hydrazine oxidase (HZO) are shuttled via the membrane-bound bc_1-complex to the nitrite reductase (NIR) and hydrazine synthase (HZS).
For carbon fixation, the low potential electrons from hydrazine oxidation are redirected towards the reductive acetyl-CoA pathway. In order to replenish the electrons, nitrite is assumed to be oxidized to nitrate by the nitrate reductase (NAR). As nitrite is a relatively poor reductant, the electrons have to be energized to enter the bc_1-complex or to feed a quinone pool, which implies reverse electron transport (RET).

The gene cluster in the genome of the anammox bacterium *Candidatus* Kuenenia stuttgartiensis that contains the catalytic subunits of nitrate reductase (*nar*GH) covers almost the full natural repertoire of electron carriers, apparently mediating electron flow and bifurcation associated with the RET. This includes genes encoding six putative heme-containing proteins and two putative blue-copper proteins and a putative anchor to the membrane showing homology to a cytochrome bd oxidase subunit (*cyd*A).
In order to understand the metabolic processes involved in energy conservation in anammox bacteria, respiratory membrane-bound enzyme complexes, including the NAR system, were separated by Blue Native - PAGE and identified by specific in-gel activity assays and LC-MS/MS analysis. The in-gel activity assays resulted in a single band showing NAR activity, when using reduced methyl viologen as artificial electron donor. Additionally, protein correlation profiling using LC-MS/MS data from consecutive Blue Native gel slices enabled the identification of many more protein complexes involved in energy conservation and RET of anammox bacteria.

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[3] Wessels, J.C.T. et al (2009): LC-MS/MS as an alternative for SDS-PAGE in blue native analysis of protein complexes. Proteomics 17:4221-4228.

AMP043
Monoterpene degradation in *Castellaniella defragrans*: Mutants, enantioselectivity and a first view on the genome

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Castellaniella defragrans is a betaproteobacterium metabolizing several monoterpenes by oxygen or nitrate respiration. After the establishment of a genetic system we have started tocreate a number of mutants lacking genes of the myrcene degradation pathway: the unique linalool dehydratase-isomerase (LDI, (1)), the geraniol dehydrogenase (GeDH) and bothgenes. Initial physiological investigations of *C. defragrans Δldi Δgedh* revealed a phenotype with growth on the monocyclic phellandrene (like the wild type), but no growth on the acyclicmyrcene. These observations indicated that the cyclic monoterpenes are not degraded via myrcene and that an independent activation reaction for the degradation of cyclicmonoterpenes exists. However, the analysis of our mutants suggested also that myrcene may be a byproduct of this unknown activation reaction. To disclose the proteins involved, wehave initiated a genomic and comparative proteomic study of the anaerobic monoterpene degradation pathway in *C. defragrans*. Initial results will be presented.
The stereospecificity of the linalool dehydratase-isomerase has been investigated with myrcene as educt. Product analyses by chiral GC revealed the formation of S-(+)-linalool. R-(-)-linalool was not detected. This may have potential applications in the white biotechnology.

AMP044
Thiosulfate reduction by thiosulfate reductase PhsABC of *Salmonella enterica* serovar Typhimurium is driven by the proton potential and reversible

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Thiosulfate is a common inorganic sulfur species in the biosphere in soils and marine environment. In the colon and cecum thiosulfate is formed from sulfide and from methanethiol that are produced in significant amounts by colonic bacteria. The enteric bacteria *Salmonella, Proteus* and *Citrobacter* have the capacity to utilise thiosulfate as a respiratory electron acceptor. The membrane-bound thiosulfate reductase PhsABC of *Salmonella enterica* catalyses the terminal step of thiosulfate respiration (menaquinol + thiosulfate -> menaquinone + sulfide + sulfite). Under standard conditions, this reaction is strongly endergonic (ΔE_0' = -328 mV). Thiosulfate reduction with hydrogen, formate or glycerol as electron donors is depended on the presence of a proton motive force (pmf) across the membrane. In thiosulfate respiration only the reaction catalyzed by PhsABC, and within PhsABC reaction only the menaquinol dependent reaction was sensitive to dissipation of pmf. Upon heterologous expression in *Escherichia coli* mutants, only menaquinone but not the more electro-positive demethylmenaquinone served as an efficient electron donor for thiosulfate reduction. Bioinformatic analysis suggests that the transmembrane protein PhsC of PhsABC contains four conserved His residues that are arranged in pairs typical for heme b binding, reminiscent of reverse redox-loop enzymes. The endergonic reaction, pmf dependence and presence of two putative heme b groups in transmembrane arrangement suggests that thiosulfate reduction by PhsABC is driven by pmf in a reverse redox-loop mechanism. PhsABC also catalysed the reverse reaction (oxidation of sulfide + sulfite to thiosulfate) when electron acceptors like TMAO or napthoquinone analogs were present. In contrast to thiosulfate respiration, sulfite/sulfide oxidation was pmf-independent and also took place with demethylmenaquinone.

AMP045
Induction of (1-methylalkyl)succinate synthase expression by *n*-alkanes and other hydrocarbons in strain HxN1

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The *Betaproteobacterium* strain HxN1 is able to degrade the *n*-alkanes hexane, heptane and octane under nitrate-reducing conditions. Due to the chemical stability of alkanes, a first activation step is necessary for the degradation. The enzyme (1-methylalkyl)succinate synthase (Mas) activates the *n*-alkanes by addition of a secondary alkyl radical to fumarate, analogous to the activation of toluene by benzylsuccinate synthase (Bss).
Based on enzymatic data from protein purification, the substrate range of HxN1 was reinvestigated, identifying that also pentane is a growth substrate, but with a significantly lower rate. On the other site western blot analysis was applied to examine expression of the large subunit MasD in relation to the presence of potential inductors. These experiments clearly demonstrated that the expression of (1-methylalkyl)succinate synthase is not the reason for the narrow substrate range. Interestingly, some additional hydrocarbons which cannot be metabolized by HxN1, as well as substituted hydrocarbons that do not require activation by (1-methylalkyl)succinate synthase, induced the expression. The only obvious similarity of all these hydrocarbons is a free methyl-group, suggesting a pivotal role of this group for expression of (1-methylalkyl)succinate synthase. However, caproate (*n*-hexanoate), which has a „free" methyl-group, strongly represses the (1-methylalkyl)succinate synthase expression. Additional specifications of the inductors and possible inhibitors are currently under investigation.

AMP046
Anaerobic degradation of naphthalene and 2-methylnaphthalene by marine sulfate-reducing bacteria

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Naphthalene and 2-methylnaphthalene as typical aromatic hydrocarbons are of great concerns due to their toxicity and recalcitrance. Anaerobic degradation of naphthalene and 2-methylnaphthalene were observed in anoxic habitats and microcosms under conditions of sulfate reduction, nitrate reduction and methanogenesis. 2-Methylnaphthalene degradation occurs in analogy to anaerobic toluene degradation by addition of fumarate to the methyl group. However, the activation mechanism of anaerobic naphthalene degradation is still unclear. In this study, anaerobic degradation of naphthalene and 2-methylnaphthalene was investigated with three marine sulfate-reducing bacteria, strains NaphS2, NaphS3 and NaphS6. These strains are able to utilize both naphthalene and 2-methylnaphthalene. Previous substrate tests showed that naphthalene-grown cells were not induced to utilize 2-methylnaphthalene, indicating that these strains do not activate naphthalene via methylation [1]. In order to examine whether 2-methylnaphthalene-grown cells were induced to utilize naphthalene,

naphthalene and 2-methylnaphthalene were directly dissolved in artificial sea water medium (ASW) and inoculated with dense cell suspension of 2-methylnaphthalene-grown cultures. Depletion of naphthalene and 2-methylnaphthalene were monitored by HPLC. Under this condition all three strains completely consumed 2-methylnaphthalene within 3-4 days; however, naphthalene degradation only started after about 10 days adaptation time and took around 30-40 days for complete depletion. On the other hand, under the same experimental conditions, NaphS2 cells grown with naphthalene were able to completely degrade naphthalene within 5 days of incubation. Results showed that the capacity to degrade naphthalene was not preserved in 2-methylnaphthalene-grown cultures; however, it could be induced. Based on these results proteins, specifically involved in anaerobic naphthalene degradation could be identified via differential display two-dimensional gel electrophoresis protein analysis.

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AMP047
A metabolomic view on the pathogenic bacterium *Staphylococcus aureus*

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Staphylococcus aureus as a facultative anaerobic bacterium is part of the mammalian commensal flora. Nevertheless under specific conditions *S. aureus* causes strong infections and is able to invade tissues and cells. With regard to its role as a leading nosocomial pathogen because of its increasing multidrug resistance, investigations on *S. aureus* are of great interest.
During host infection the bacterium has to cope with changing supply of carbon sources and varying oxygen availability up to anaerobic conditions. For a better understanding of its adaptive mechanisms and its regulatory processes, *S. aureus* COL cells were cultivated under different growth conditions. By using ^{1}H-NMR, GC-MS and LC-MS we investigated the extra- and intracellular metabolome and observed distinct differences between aerobically and anaerobically grown *S. aureus* COL cells.

ARV001
Replacing the Archaeal Path of Selenocysteine Biosynthesis with the Bacterial

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Biosynthesis of selenocysteine (sec), the 21st proteinogenic amino acid, occurs in a tRNA-bound fashion in all three domains of life. The sec-specific tRNA ($tRNA^{sec}$) is mis-aminoacylated with serine (ser), which is subsequently converted to sec. While in Bacteria this conversion involves a single step catalyzed by selenocysteine synthase (SelA), Archaea and Eukarya phosphorylate ser-$tRNA^{sec}$ to *O*-phosphoseryl-(sep)-$tRNA^{sec}$ (using sep-$tRNA^{sec}$ kinase, PSTK) which serves as substrate for sep-$tRNA^{sec}$:sec synthase (SepSecS) to generate sec-$tRNA^{sec}$. To investigate the physiological role of sep-$tRNA^{sec}$ in Archaea, mutant *Methanococcus maripaludis* strains lacking either PSTK or SepSecS were constructed and complemented with SelA from *Escherichia coli*. We could show that, both PSTK and SepSecS are indispensable for selenoprotein synthesis in *M. maripaludis*, but also that the archaeal sec-synthesis pathway can be „short-circuited" to the bacterial one. This finding rules out an essential role of this aminoacyl-tRNA species in Archaea. Potential functions of sep-$tRNA^{sec}$ other than as intermediate in sec synthesis are being addressed to eventually explain why Archaea (and Eukarya) have evolved a three-step mechanism for sec synthesis as compared to the two-step mechanism found in Bacteria.

ARV002
A heme-based redox sensor in the methanogenic archaeon *Methanosarcina acetivorans*

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The methanogenic archaeon *Methanosarcina acetivorans* C2A relies on methanogenesis as the energy conserving mechanism. Therefore, it is able to utilize common methanogenic growth substrates such as methanol, acetate and different methylated compounds, but not CO_2/H_2. In addition *M.acetivorans* can use CO as a growth substrate. In contrast to other CO utilizing organisms which produce H_2 during CO metabolism, *M.acetivorans* generates acetic acid, formic acid and methylated sulfides, besides methane, but not H_2.
It was shown that three methyltransferase/corrinoid fusion proteins are required for generating dimethylsulfide (DMS) from CO and CH_4 from DMS [1]. These proteins are each differentially regulated by a downstream regulator protein [2]. MA4560, one of these regulators, is a putative response regulator of a two component regulatory system together with the multi domain sensor histidine kinase MA4561. In order to learn more about the sensor function of MA4561, the full-length protein consisting of two consecutive PAS and GAF domains joint to a histidine kinase domain was heterologously produced in *Escherichia coli*. In addition, different truncated protein variants were produced and purified using metal affinity chromatography. UV-vis spectrometry identified a redox-active heme cofactor in the second GAF domain of this multi domain protein. In contrast to many other known heme-based sensor proteins which bind the cofactor non-covalently, covalent attachement of heme could be demonstrated. Interestingly, autophosphorylation of the protein is highly dependent on the redox state of the central heme iron. Due to the involvement of the corresponding response regulator MA4560 in regulating gene expression in response to CO and methylated sulfides, a potential role of the sensor kinase MA4561 in redox or CO sensing via the heme cofactor is postulated.

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ARV003
Elucidation of the *N*-glycosylation pathway in the thermoacidophilic crenarchaeon *Sulfolobus acidocaldarius*

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Historically it was long been believed that glycosylation is a unique phenomena restricted to Eukarya[1], however, when in 1976 Mescher and Strominger purified the S-Layer protein from *Halobacterium salinarium* which contained glycans covalently linked to asparagine residues[2], questions evoked how *N*-glycosylation occurs in *Bacteria* and *Archaea.* Today *N*-glycosylation is thought to be conserved across all three major domains of life. During the last years substantial progress in describing *N*-glycosylation pathways in three euryarchaeota[3-5] has been made. Although eukarya, bacteria, and archaea all seem to have certain characteristics of the *N*-glycosylation pathway in common, archaea displays a mosaic of features from the eukaryal and bacterial system. However, so far the *N*-glycosylation process in a crenarchaeota is still uncovered. Here we will report the first results elucidating the *N*-glycosylation pathway in the thermoacidophilic archaeon *Sulfolobus acidocaldarius.* The *N*-glycosylation in *S. acidocaldarius* show same significant differences compared to these of the other archaea, e. g. scattered gene localization of glycosyltransferases (GT), challenging in identification of GT involved in the glycosylation processes. In contrast to the non essential *N*-glycosylation pathway in the studied euryarchaeota, the *N*-glycosylation pathway is essential for the survival of *S. acidocaldarius*. Further *S. acidocaldarius* exhibited a unique composition and branched structure of the *N*-linked oligosaccharide, so far not found in other archaea.

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ARV004
Subcellular organization and energy conservation of *Ignicoccus hospitalis*

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Ignicoccus hospitalis is a chemolithoautotrophic Crenarchaeote that obtains energy from the reduction of elemental sulfur with molecular hydrogen as electron donor (1). It is able to carry out CO_2 fixation via a new pathway, named dicarboxylate/ 4-hydroxybutylate cycle. Acetyl-CoA is the primary acceptor molecule and is regenerated via the characteristic intermediate 4-hydroxybutyrate (2). *I. hospitalis*, like all identified *Ignicoccus* species, exhibits a unique cell architecture that differs from all other Archaea known so far. Its cell envelope consists of two membranes enclosing a huge intermembrane compartment (IMC) (3). In its lipid composition, the outer membrane of *I. hospitalis* significantly differs from the cytoplasmic membrane, as it comprises only archaeol and its derivatives, but no caldarchaeol. In addition, there are unique and abundant proteins only found in the outer membrane of *I. hospitalis*, like the pore-forming Ihomp1. Recently, it was shown that the outer membrane contains the H_2:sulphur oxidoreductase as well as the ATP synthase. Thus, *I. hospitalis* is the first organism with an energized outer membrane and ATP synthesis within the IMC. DAPI staining and EM analyses showed that DNA and ribosomes are localized in the cytoplasm, leading to the conclusion that in *I. hospitalis* energy conservation is separated from information processing and protein biosynthesis (4). In addition, we were able to demonstrate that the acetyl-CoA synthetase that activates acetate to acetyl-CoA in an ATP consuming process is associated to the outer membrane. This is the first energy-consuming process proven to take place in the intermembrane compartment.
These results raise questions on other metabolic reactions that are likely to occur in the IMC, e.g. the first steps in CO_2 fixation, and on the existence of transporters that convey ATP from the site of its synthesis to the cytoplasm where DNA replication and transcription take place. The findings may also shed light on the nature of the intimate association between *I. hospitalis* and *Nanoarchaeum equitans* (5). It is known that *N. equitans* receives amino acids and lipids from its host. However, it is still unclear at present if *N. equitans* is able to synthesize ATP or if it obtains this form of energy directly from *I. hospitalis*, too.

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ARV005
Full speed ahead: analysis of the assembly of the archaeal flagellum

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Microorganisms move towards optimum locations and escape from unfavorable conditions by use of motility structures like flagella.
The archaeal flagellum, which is distinct from the bacterial one, was studied intensively in Euryarchaeota. However, the crenarchaeal flagellar assembly system is not well understood. We study the thermoacidophilic crenarchaeon *Sulfolobus acidocaldarius* to analyse the assembly and function of their flagella system. Markerless in-frame deletion strains were constructed for all seven genes of the *fla* operon. To exclude polar effects, both at the transcriptional and translational level, we performed q-PCRs and Western Blots. Motility assays and electron microscopy analysis of all Δ*fla* strains revealed non-motile and non-flagellated *S. acidocaldarius* cells. Taken together, these results indicate the involvement of all seven genes of the *fla* operon in the correct flagellar assembly.
In a parallel approach pH, osmotic pressure, temperature and starvation were tested to stimulate flagellar biosynthesis and assembly. Interestingly, only starvation conditions induced the production of flagellar assembly proteins. Moreover, under these conditions thermo microscopy revealed highly motile cells, reaching swimming velocities up to 60 µm/s. Thus, we speculate that the crenarchaeal flagellum plays a role in escaping from nutrient limited environs.
Further experiments like pull-down assays and yeast two-hybrid experiments will elucidate Fla protein interactions. These findings will be the basis to understand the molecular mechanism of the crenarchaeal flagellar assembly system.

ARV006
Microorganisms, Peregrine Falcons and Cheetahs - Who is the fastest?

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An often asked question in Biology concerns velocity: who is the fastest? An answer to this question has to take into account the different body sizes of organisms to be compared. For this reason the term bps was introduced as 'bodies per second'; i. e. relative velocities are defined in movements of body size per second. By this definition man runs with ca. 5 bps, cheetahs run with a maximum speed of 30 bps; the peregrine falcon flies at ca. 100 bps and accelerates by dives into the air to a maximal 400 bps. This latter velocity often is referred to as maximum relative speed in nature (but is not reached by active movement).
Using a so-called thermomicroscope, allowing analyses at up to 95° C under anaerobic conditions we analyzed the swimming behavior of hyperthermophilic Archaea (some bacteria were used for controls). Our data clearly show that certain microorganisms are the fastest organisms on earth. E. coli swims with an average speed of ca. 45 µm/sec = ca. 30 bps. For Archaea we measured the following speeds: Halobacterium salinarum: 3 µm/sec = ca. 1 bps; Pyrococcus furiosus 60 µm/sec = 60 bps; Methanococcus voltae: 80 µm/sec = 80 bps; Methanocaldococcus villosus: 290 µm/sec = 290 bps; Methanocaldococcus jannaschii: 380 µm/sec = 380 bps. The latter two species did swim with maximal relative velocities of 470 and 590 bps, respectively - they for sure, thereby extend the maximum relative speed in nature by at least a factor of 5.
Very interestingly, the swimming behavior of hyperthermophilic Archaea differs from that of mesophilic Bacteria. Whilst the latter swim in more or less smooth runs, the former exhibit a 'seek and stay' behavior, which might be explained by the conditions they life in. Examples of those different swimming modes will be presented.

ARV007
Screening and characterization of biofilm formation in halophilic Archaea

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Biofilm formation is described for some hyperthermophilic and methanogenic Archaea, only one example of surface adhesion is reported for haloarchaea [1]. We developed a fluorescence-based adhesion assay to screen and quantify this property in haloarchaea. Eight extremely halophilic *Halobacterium salinarum* strains, four moderately halophilic *Haloferax* strains, one haloalkaliphilic strain and eight halopsychrophilic archaea were tested. Twelve of them showed adhesion, that were categorized in four groups. Members of group I did not adhere (0%-10%), group II exhibited a low to moderate (>10%-30%), group III a strong (>30%-70%), and group IV a very strong ability for adhesion (>70%). The latter group contains one Antarctic isolate and the gas vesicle producing *Hbt. salinarum* DSM3754, whereas the gas vesicle producing wildtype strains PHH1 and NRC-I did not adhere. Among the environmental isolates only half of them were adhesive and adhesion could also get lost after several rounds of incubation.
Biofilm producing strains cultivated on glass and plastic surfaces were analyzed by microscopy. Different growth parameters or variations of media supplements had almost no effect on biofilm formation. Biofilms of *Hbt. salinarum* DSM3754 and the Antarctic isolate were composed of flat cell layers with additional three-dimensional microcolonies. In contrast, biofilms of *Haloferax* and *Halorubrum* mainly consisted of large cellular aggregates that loosely attached to the surface. Extracellular polymeric substances (EPS) were composed of free nucleic acids and glycoconjugates. In the case of *Hbt. salinarum* DSM3754 attachment started one day after incubation. Electron microscopic studies showed that adherent cells were connected by a

network of different cellular appendices. The microcolonies were remarkably stable with almost 100% of viable cells after three month of incubation.
Our analysis demonstrated that the ability of adhesion is widely distributed in haloarchaea and the multicellular communities detected represent biofilm structures.

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ARV008
Assessment of the predominant methanogenic pathways in anaerobic digesters by the combination of molecular techniques with the isotopic fingerprinting of the produced biogas

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Laboratory scale continuously stirred tank reactors were run under various conditions using either cereal distillers grains, a by-product from bioethanol industry, maize silage or chicken manure as substrate. In addition to the standard process parameters the stable hydrogen and carbon isotopic composition of the produced biogas (methane and CO_2) was also analysed to estimate the predominant methanogenic pathways (acetotrophic vs. hydrogenotrophic). The methanogenic communities in the reactors were also investigated for their phylogenetic composition by terminal restriction fragment length polymorphism analysis and sequencing of the mcrA genes coding methyl-coenzyme M reductase. In addition, the expression of the gene was also studied as a better indicator of the metabolic activity. The carbon isotopic values ($\delta^{13}C$) of methane ranged between -68‰ and -35‰. This latter value of the maize silage reactor was probably influenced by the original high value (-12‰) of this C4 plant substrate. The hydrogen isotopic values (δD) of methane were very low (-369 to -347‰) except the samples from the maize silage reactor ranging from -292‰ to -281‰. Apparent fractionation factors ($\alpha_{CO2\text{-}CH4}$) suggested a hydrogenotrophic pathway in the chicken manure reactor, while probably both pathways influenced the isotopic signal of derived methane in the other reactors.
According to the molecular biological investigations the reactors were dominated by hydrogenotrophic *Methanomicrobiales* with *Methanoculleus* as the predominant genus. Sequences affiliated with acetotrophic *Methanosetaceae* were found only in one cereal distillers grains reactor, while sequences affiliated with *Methanosarcinaceae* were frequently found representing less abundant members of the methanogenic communities. At RNA level major changes in the relative abundance of the amplified sequences were observed compared to the results obtained from the isolated DNA.

ARP001
Toxicity of methylated Bismuth produced by intestinal microorganisms to *Bacteroides thetaiotaomicron*, a dominant member of human intestinal microbiota

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Bismuth compounds have significant application in medicine. Bismuth subcitrate is applied in a triple-therapy for the treatment of *Helicobacter pylori* which causes chronic inflammation of the stomach and is linked to the development of duodenal, gastric ulcers and stomach cancer. After ingestion of bismuth subcitrate, Bi^{3+} is methylated to volatile trimethylbismuth (TMBi) by the intestinal microbiota especially by methanoarchaea.
Here we investigate the influence of TMBi produced by *Methanobrevibacter smithii* on growth of *Bacterioides thetaiotaomicron,* an important member of the physiological intestinal microbiota. Transfer of TMBi from headspace of *Methanobrevibacter smithii* cultures to *B. thetaiotaomicron* cultures results in a significant growth inhibition of this organism. Closer investigations showed that the volatile TMBi rapidly decays into soluble dimethyl- and monomethylbismuth, which cause comparable growth inhibition effects, suggesting that these derivatives are the actual agents of growth inhibition. Analyses are presented, which give insight into possible mechanisms responsible for the toxic effects of the various methylbismuth derivatives.

ARP002
The fimbriae of *Methanothermobacter thermoautotrophicus*

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The fimbriae of the euryarchaeon *Methanothermobacter thermoautotrophicus* were among the first detailed characterized archaeal fimbriae. We have shown that these cell appendages with a diameter of 5 nm (mainly) consist of 16 kDa glycoprotein Mth60 and function as adhesins.
For further analyses Mth60 fimbriae were enriched from the supernatant of a 100 liter fermentor by PEG/NaCl induced precipitation, CsCl-gradient centrifugation and dialysis. Electron microscopy demonstrated that so prepared fimbriae are pure and well-structured. At some ends knob-like patterns could be detected. SDS-PAGE indicated four different proteins in the fimbriae fractions; the prominent band at about 16 kDa corresponds Mth60. The identification of the other proteins failed up to now.
Reverse transcription PCR and Northern Blots revealed the main fimbrin gene *mth60* to be part of two operons: it is co-transcribed with *mth58* and *mth59*, a further operon comprises *mth60* and *mth61*. It is well known from bacteria that all genes necessary for fimbriae formation are clustered. Bioinformatical investigations showed Mth59 to have a significant similarity to bacterial chaperone proteins. Chaperones play an important role in fimbriae assembly (chaperone-usher-pathway) of some bacteria. The homology of Mth59 to bacterial chaperones might indicate that archaeal and bacterial fimbriae have a related mode of assembly. The now available Mth59 antibodies are applied in co-immunoprecipitation experiments, thus analyzing the function of this protein. Immunolabeling of ultrathin sections of *M. thermoautotrophicus* will hopefully allow to clarify the localization of Mth59.

ARP003
Hot protein phosphorylation: carbon source dependent phospho-proteom mapping from *Sulfolobus solfataricus* P2

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Posttranslational modifications (PTMs) are of major interest for the regulation of cellular processes. Reversible protein phosphorylation is the main mechanism, which is applied to control the functional properties of proteins in response to environmental stimuli [1]. In the 80's protein phosphorylation has been demonstrated in the third domain of life, the Archaea. However, so far only few phospho proteins were identified and few protein kinases and protein phosphatases were investigated. A huge progress was achieved only recently by the determination of the complete phospho-proteom of the extremhalophilic Euryarchaeon *Halobacterim salinarium*, which was analyzed via MS with prior TiO_2 phospho-peptide enrichment [2].
Model organism of this study is the thermoacidophilic Creanarchaeon *Sulfolobus solfataricus*. Bioinformatic investigation revealed that *S. solfataricus* only harbors eukaryal protein kinases and classical two component systems are absent. Until now, only little is known about protein phosphorylation in this organism. So far, only six possible target proteins were reported. In addition three eukaryal type serine/threonine specific protein kinases as well as two protein phosphatases were characterized (PP1-arch1 and PTP) [1; 3]. In order to analyze the phospho-proteom of *S. solfataricus* in more detail, we applied a gel and enrichment free proteome approach by using the precursor acquisition independent from ion count (PAcIFIC) method [4]. The detailed pospho-proteom mapping with a special focus on the central carbohydrate metabolism (CCM) will be presented.

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ARP004
CRISPR/Cas in *Thermoproteus tenax*: A multifunctional stress system?

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Clustered regularly interspaced short palindromic repeats (CRISPR) are found in nearly all archaeal but only in 46% of all bacterial genomes. Studies of bacterial CRISPR/Cas systems suggest that these systems act as defence systems against mobile genetic elements (e.g. plasmids or phages) based on sequence similarities between spacer and foreign nucleic acids. It is assumed that CRISPR transcripts interact with Cas (CRISPR associated) proteins in a largely unknown interference reaction to inactivate the foreign genetic elements.
To gain insight into the function of archaeal CRISPR/Cas systems, we focused on the structure and function of the CRISPR arrays and *cas* genes of the hyperthermophilic Crenarchaeote *Thermoproteus tenax*. Small non-coding RNAs with a length of approx. 60 to 120 nt could be detected for five of the seven CRISPR loci. The core *cas* genes located between CRISPR array TTX_5 and 6 are organised in two operons (*casa1*, *casa2*). The recombinant proteins encoded by both operons form multimeric complexes with RNA nuclease activity (CasA1) or RNA/DNA binding capacity (CasA2) suggesting essential roles in processing CRISPR transcripts and interference reactions. Remarkably, transcription of the *cas* genes was induced by abiotic stress factors assuming a more complex role in stress response for this archaeal system.

ARP005
Incubation experiments of methanogenic archaea isolated from Siberian Permafrost with Mars analog minerals

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Due to its short persistence time in the atmosphere the detection of methane on Mars by *Mars Express* leads to the conclusion that it must have a recent origin. The correlation between the presence of water vapour and methane on the Martian surface [1] could be an indication of a potential biological source of the atmospheric methane on Mars. Methanogenic archaea from terrestrial permafrost are therefore one of the most suitable candidates for possible existing life on Mars. They have evolved under early Earth environment and are growing lithoautotorph under strictly anaerobic conditions. They are able to tolerate low temperatures and have survived in the extreme environments of permafrost for several millions of years.
This project focuses on experiments with methanogenic archaea isolated from the active layer of permafrost on Samoylov Island in the Siberian Lena Delta. Former studies with these strains revealed significantly higher survival rates compared to non-permafrost methanogens after the exposure to simulated Martian thermal conditions [2]. The aim of the current work is to determine the effects of the simulated Martian conditions on the metabolic activity of the permafrost strains not only in thermal aspects but also with regard on the impact of Mars analog minerals (MAM) and humidity.
As a first step growth tests were performed at different temperatures with adding increasing amounts of three distinct MAMs („JSC-Mars-1", „Early Acidic Mars" = EAM, „Late Basic Mars" = LBM) to the culture medium. Concentrations around 1% caused an increase of the methane production (e.g. 4.1 nmol CH_4 ml^{-1} h^{-1} with LBM) compared to the incubation without MAMs (e.g. 2.5 nmol CH_4 ml^{-1} h^{-1}). MAM amounts of more than 2.5% leaded to lower methane formation rates (e.g. 1.9 nmol CH_4 ml^{-1} h^{-1} with LBM) or showed no significant effects. Nonetheless, methanogenic archaea seemed to be capable to adapt to grow on MAM concentrations of 5% and higher, but this will need further verification through ongoing research activities.
Basically the tested Mars analog minerals had a positive effect on the activity of the strains which can be seen as a support to the idea of regarding methanogenic archaea from terrestrial permafrost as model organisms for possible life on Mars.

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ARP006
Hot transcription: Functional analysis of multiple general transcription factors in Crenarchaeota

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Archaea exhibit unique features as well as share characteristics with Bacteria and Eukarya. Archaea do not possess a nucleus, they have a relatively small circular chromosome like Bacteria and genes are organized in operon structures. In contrast to that, information processing (e.g. replication, transcription and translation) resembles respective eukaryal processes. The archaeal transcription machinery encompasses one multi-subunit RNA-Polymerase (RNAP), resembling the RNAP II of Eukarya, homologues of the TATA-binding protein (TBP) and Transcription Factor TFIIB (TFB). The current mechanistic understanding of transcription initiation is that TBP binds to the TATA-Box (~ 25 bp upstream of the transcription start site) whereupon TFB binds to the TBP:DNA complex, forming sequence specific contacts with a purine-rich TFB-responsive element (BRE). Subsequently the N-terminus of TFB recruits the RNAP to build the ternary pre-initiation complex. RNAP, TBP and TFB are solely sufficient for transcription of archaeal promoters *in vitro*. Therefore archaeal transcription is generally regarded as a simpler model of the more complex eukaryal processes.
Interestingly, Archaea possess multiple copies of general transcription factors (GTFs) however, the distribution is species-dependent. Whereas the function of multiple GTFs has been addressed in different Euryarchaeota (e.g. *P. furiosus*, *Halobacterium* NRC-1) the role in Crenarchaeota is still unclear.
The current studies focus on two Crenarchaeota the anaerobic hyperthermophile *Thermoproteus tenax* (86°C, pH 5) and the thermoacidophile *Sulfolobus acidocaldarius* (80°C, pH 2-3) which encode one TBP and four and three TFBs respectively. *S. acidocaldarius* is the emerging model organism within the Archaea, since it is easy to grow and one of the few genetically tractable Archaea. First insights into the function of multiple TFBs will be presented.

ARP007
Towards an archaeal expression host for metagenome analysis

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Many archaeal species occupy extreme habitats requiring effective adaptation and specialization strategies. Their proteins, so called „extremozymes", are active under harsh and unreal conditions which makes them very interesting for biotechnological applications. Unfortunately, the functional expression of many archaeal (hyper)thermophilic proteins in mesophilic expression hosts, such as *Escherichia coli*, or even thermophilic bacterial hosts, is limited. The missing archaeal post-translational machinery is supposed to be a major reason, just as well as the misfolding of proteins at low temperature for (hyper)thermophilic proteins. Therefore, in current metagenomic approaches only a fraction of the tremendous diversity can be accessed due to the pre-selection introduced by the choice of common bacterial expression systems. Thus, there is an urgent need for the establishment of alternative expression hosts and including an archaeal expression host is an important contribution to unravel and to use the biodiversity available in extreme habitats.
Sulfolobus acidocaldarius is a well characterized thermoacidophilic, obligate aerobic Crenarchaeon, that grows optimally at 78°C and pH 2-3. Most important, the organism is genetically tractable and a vector system for protein expression has been established [1]. First results about promotor selectivity in the archaeal expression host as well as the expression of archaeal (gluco)amylases, that failed to be expressed in common bacterial and eucaryal expression systems, will be presented.

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ARP008

ATP synthesis above 100°C: structure and function of the rotor of the A_1A_O ATP synthase from *Pyrococcus furiosus*

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ATP synthases are the most important enzymes in cellular bioenergetics and present in any life form. The ATP synthase of archaea (A_1A_O) is very unusual and shares properties with both the bacterial F_1F_O ATP synthases and the eukaryal V_1V_O ATPases. Most of the variation is in the membrane-embedded motor. Most archaea have an F-type like *c* subunit in their rotor rings, consisting of one hairpin with one ion binding site. In contrast, some archaea, e. g. *Pyrococcus furiosus*, have unusual *c* subunits [1]. The *c* subunit from the hyperthermophile *P. furiosus* is predicted to have four transmembrane helices, but only one ion (H^+/Na^+) binding site. It was isolated by chloroform/methanol extraction, purified and the molecular mass was determined with MALDI-TOF-MS. The mature *c* subunit of *P. furiosus* is indeed a 16 kDa protein. Labeling of the ion binding sites with the inhibitor N,N'-dicyclohexylcarbodiimide (DCCD) verified that the *c* subunit has indeed only one ion binding site. The influence of Na^+ on the labeling is object of current research. Based on these results and the recent finding that the A_1A_O ATP synthase of *P. furiosus* has a rotor with 10 *c* subunits [2], a homology model of the *c* ring is presented, giving first insights into the structure of an archaeal rotor ring with its ion binding site.

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ARP009

Interaction of transcription factor B with mutants of transcription activator GvpE of *Halobacterium salinarum*

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The GvpE protein of *Halobacterium salinarum* has been identified as transcriptional activator of the *gvp* gene cluster involved in gas vesicle formation. The two oppositely oriented promoters P_A and P_D driving the expression of the *gvp* genes are separated by 35 nt only. Both promoters are activated by GvpE. The putative binding sites of GvpE are adjacent to BRE (transcription factor B recognition element) and the TATA-Box (recognized by the transcription factor Tbp). The question arises whether GvpE is able to contact proteins of the basal transcription apparatus. Protein-protein interactions were found between GvpE and any of the five Tbp proteins [1]. In addition, we analyzed the ability of GvpE to interact with the seven different Tfb proteins. All of them were able to interact.
The putative secondary structure of GvpE indicates six α-helical regions including the amphiphilic helix AH6 resembling a leucin zipper preceded by a cluster of basic amino acids that could constitute DNA binding site (DNAB). The amphiphilic helix AH4 is highly conserved between the GvpE sequences of different organisms and also contains a cluster of basic amino acid. Various GvpE mutants are available in these regions [2] and additional mutants were constructed in the region between the amino acids 50 and 100 of GvpE. All of these mutants were unable to activate the P_A promoter.
The GvpE mutants were analyzed for their ability to interact with TfbC that appeared to be the strongest transcription factor. The His-tagged TfbC produced in *E. coli* was bond to Ni-NTA matrices and tested for interaction with the GvpE mutants present in lysates of *Hfx. volcanii*. All of these GvpE mutants interacted with TfbC, whereas the soluble gas vesicle protein $GvpH_{His}$ (used as control) did not bind these GvpE mutants. These results implied that the regions tested in GvpE are not involved in the binding of TfbC. However all these regions are required for the activating function of GvpE. Thus, the lack of activation of these mutants is not due to a lack of Tfb-GvpE binding, but rather due to the lack in DNA binding (DNAB, AH4) or dimerization (AH6).

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ARP010

Hot Trehalose: The Unusual Bifunctional TPSP Pathway of *Thermoproteus tenax*

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The multifunctional disaccharide trehalose is a widespread molecule, occurring in all three domains of life, where it plays a major role as storage compound and in stress protection (thermoadaptation, osmoregulation) [1]. The most common pathway for trehalose synthesis is the OtsA/OtsB pathway, where UDP-(ADP-)glucose and Glucose-6-phosphat is transformed into trehalose-6-phosphat by TPS and subsequently dephosphorylated by TPP into trehalose and P_i [2]. In the genome of the hyperthermopilic crenarchaeon *Thermoproteus tenax* a gene coding for a trehalose-6-phosphate synthase/phosphate (*tpsp*) was identified, encoding a protein with a C-terminal TPS- and N-terminal TPP-domain [3]. This gene is part of an operon harboring a putative glycosyl transferase (*gt*) and a putative small conductive mechanosensitve channel (*msc*). The two-domain TPSP structure has already been described for plants (e.g. *Selaginella leptophylla, Arabidopsis thaliana*) and for *Saccharomyces cerevisiae*, but the TPSPs only possess one activity, either TPS or TPP. Only recently a bifunctional TPSP activity has been reported from *Cytophaga hutchinsonii* [4]. For the archaeon *T. tenax,* biochemical studies of the recombinant protein revealed a bifunctional TPSP, however the enzyme posses only minor TPS and full TPP activity. Surprisingly, a significant increase of trehalose formation in *T. tenax* was observed in the presence of the putative GT. In our current model, we suggest that GT activates TPS by complex formation. The MCS might function as the emergency valve which allows the maintenance of the cell turgor in order to respond to environmental cues (e.g. osmotic stress).

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ARP011

The role of Trigger-loop in archaeal transcription

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The archaeal RNAP is closely related to eukaryotic RNAPII in terms of subunit composition and architecture, promoter elements and basal transcription factors required for the initiation and elongation phase of transcription. The possibility to reconstiute archaeal RNAP from single subunits expressed in *Escherichia coli* has been used for analyses of structure-function relationships in archaeal RNAP [1; 2]
Conformational changes in the active site of the RNAP are required for correct nucleotide incorporation during transcription elongation.The highly conserved trigger loop (TL) plays a key role at every distinct stage of the nucleotide addition cycle. Two residues of the TL, A´´ Leu83 (*S. cerevisae* Rpb1 Leu1081; *E.coli* β´ Met1238) and A´´ His87 (*S. cerevisae* Rpb1 His 1085; *E.coli* β´ His1242), interact, recognize and select the correct incoming NTP. Recent analyses of the bacterial TL showed that it is also required in different steps of transcription as selection of the correct nucleotide, intrinsic RNA cleavage and proofreading. Substitution of Leu83 and His87, and partial deletion of TL were introduced for this study.
Employing *in vitro* assays with reconstituted RNAP of *Pyroccocus furiosus* containing mutated subunit A´´, we investigated the role of TL in archaeal transcription. Mutations within the TL affect promoter-dependent transcription and nucleotide addition activity. Interestingly, substitution of His87 has only minor effects on RNA intrinsic cleavage, suggesting that the invariant His, which has a crucial role in bacterial intrinsic cleavage [3], is not required for intrinsic cleavage activity of the archaeal RNAP. Taken together, our results confirm the essential role of TL in transcription elongation.

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ARP012
In vivo analysis of archaeal transcription machinery by ChIP

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Gene expression in archaea is mediated by an eukaryotic-like transcriptional machinery and promoter elements. The minimal transcription apparatus of the hyperthermophilic euryarchaeon *Pyrococcus furiosus* consists of an 11 subunit RNA polymerase (RNAP) and the two general transcription factors TATA-binding protein (TBP) and transcription factor B (TFB). The presence of these two factors is sufficient for initiation and basal transcription *in vitro*. A third factor, transcription factor E (TFE), which is homologous to the N-terminus of the alpha subunit of the eukaryotic transcription factor IIE (TFIIE), seems to play an important role in open complex formation. Moreover, *in vitro* crosslinking data revealed an unexpected presence of archaeal TFE in elongation complexes, which was not observed for eukaryotic TFIIE.
To investigate the presence of archaeal TFE in elongation complexes *in vivo*, we applied the method of Chromatin Immunoprecipitation (ChIP) to various components of the archaeal transcription apparatus at selected genes (eg. *gdh, pfk* and *hsp20*). As expected, the presence of TBP and TFB is restricted to promoter regions and RNAP was located both in promoter and transcribed regions of these genes. Currently, we are applying this approach to study the occupancy and distribution of TFE at selected genes. These studies will contribute to a better understanding of the role of TFE in archaeal transcription elongation *in vivo*. Furthermore, combining ChIP with next-generation sequencing technologies (ChIP-Seq) will help to elucidate the general role of TFE in archaeal transcription.

ARP013
GvpD-mediated reduction of the transcription activator GvpE of *Halobacterium salinarum* using GFP fusions as reporter

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Fourteen *gvp* genes are involved in gas vesicle formation in *Halobacterium salinarum* that are arranged in two oppositely orientated gene clusters, *gvpACNO* and *gvpDEFGHIJKLM*. The products of *gvpE* and *gvpD* are involved in the regulation of gas vesicle formation. GvpE is a transcriptional activator enhancing the transcription at the two promoters P_A and P_D, whereas GvpD is involved in repression. The presence of GvpD leads to the absence of GvpE in *Haloferax volcanii* transformants [1, 2]. To investigate whether the reduction of the amount of GvpE in the presence of GvpD is due to proteolytic degradation of GvpE, N- and C-terminal fusions of GFP were constructed. The function of these fusion proteins was studied in P_A-*bgaH* transformants where the β-galactosidase activities were very similar using wild-type GvpE, GvpE-GFP or GFP-GvpE. The proteolytic reduction of GvpE was quantified in the respective *H. volcanii* transformants carrying GvpD in addition to the GvpE fusion proteins. GvpD wild-type and two GvpD mutants were used, $GvpD_{Mut6}$ lacking the repressing function and the superrepressor $GvpD_{3\text{-}AAA}$. Both GFP fusion proteins showed similar effects in the presence of different GvpD variants, i.e. the presence of defective $GvpD_{Mut6}$ did not alter the fluorescence, whereas wild-type GvpD and superrepressor $GvpD_{3\text{-}AAA}$ reduced fluorescence. The effect was much stronger when GFP-GvpE was used with an observed reduction of 70% for wild-type GvpD and below 10% for superrepressor $GvpD_{3\text{-}AAA}$. This GFP-GvpE fusion will be used as a valuable reporter system to study effects of further GvpE mutations and the interaction of GvpE and GvpD.

[1] Zimmermann and Pfeifer (2003): Mol. Microbiol. 49(3): 783-794.
[2] Scheuch et al (2008): Arch. Microbiol. 190(3):333-339.

ARP014
Functional expression of an archaeal A_1A_O ATP synthase in a bacterial host

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The class of archaeal A_1A_O ATP synthases is the least understood class of ATP synthases. Structural information was obtained in recent years for the A_1A_O ATP synthase purified from *Methanocaldococcus jannaschii* (1) and *Pyroccoccus furiosus* (2) and some subunits have been overproduced in *E. coli*, purified and their structure has been determined. In contrast, still little is known about their function. This is mainly due to the poor growth and the non-availability of a genetic system for most archaea. To overcome this shortcoming, we have cloned the A_1A_O ATP synthase operon from the mesophile *Methanosarcina mazei* in an expression vector and expressed the genes in a F_1F_O ATP synthase negative mutant of *E. coli*. This recombinant strain expresses a functional ATP synthase, as demonstrated by DpH-driven ATP synthesis in cell suspensions (3). Next, we prepared inverted vesicles from the recombinant strain. These coupled NADH oxidation to the synthesis of ATP that was inhibited by DCCD and DES. ATP synthesis was inhibited by protonophores as well as sodium ionophores, indicating DY as driving force for ATP synthesis. These data demonstrate, for the first time, the production of a functional archaeal A_1A_O ATP synthase in a bacterial host and pave the road for a molecular analysis of the class of archaeal A_1A_O ATP synthases.

(1) Ünal Coskün, Yuriy L. Chaban, Astrid Lingl, Volker Müller, Wilko Keegstra, Egbert J. Boekema and Gerhard Grüber (2004): Structure and Subunit Arrangement of the A-type ATP Synthase Complex from the Archaeon *Methanococcus jannaschii* Visualized by Electron Microscopy. J. Biol. Chem. 279, 38644-38648.
(2) Janet Vonck, Kim Y. Pisa, Nina Morgner, Bernhard Brutschy and Volker Müller (2009): Three-dimensional Structure of A_1A_o ATP Synthase from the Hyperthermophilic Archaeon *Pyrococcus furiosus* by Electron Microscopy. J. Biol. Chem. 284, 10110-10119.
(3) Kim Y. Pisa, Claudia Weidner, Heiko Maischak, Holger Kavermann, Volker Müller (2007): The coupling ion in the methanoarchaeal ATP synthases: H^+ vs. Na^+ in the A_1A_o ATP synthase from the archaeon *Methanosarcina mazei* Gö1. FEMS Microbiol. Lett. 227, 56-63.

ARP015
Presence of a Na^+-translocating ATP synthase in the methanogenic archaeon *Methanosarcina acetivorans*

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During metabolism methanogenic archaea can build up a proton- as well as a Na^+-gradient [1]. The ATP synthase of methanogenic archaea has a conserved sodium ion binding motif in the membrane-embedded rotor subunit c but evidence for Na^+ driven ATP synthesis in methanogens is lacking [2]. To address this question, we have established an inverted membrane vesicle (IMV) system of *Methanosarcina acetivorans*. IMVs catalyzed ATP hydrolysis with a rate of 35 nmol/min*mg. ATP hydrolysis was accompanied with the transport of $^{22}Na^+$ into the lumen of the IMVs. Na^+-transport was inhibited by sodium ionophores but not by protonophores indicating a direct coupling. Furthermore, ATP synthesis as well as Na^+-transport was inhibited by the ATP synthase directed inhibitor DCCD. These data demonstrate that the A_1A_O ATP synthase can use Na^+ as coupling ion.

[1] Deppenmeier, U. and V. Müller (2008): Life close to the thermodynamic limit: how methanogenic archaea conserve energy. Results Probl. Cell. Differ. 45: 123-152.
[2] Pisa, K.Y. et al (2007): The coupling ion in methanoarchaeal ATP synthases: H^+ versus Na^+ in the A_1A_O ATP synthase from the archaeon *Methanosarcina mazei* Gö1. FEMS Microbiol. Lett. 277(1):56-63.

ARP016
In vivo analysis of gas vesicle-coding proteins of *Halobacterium salinarum* PHH1

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Halobacterium salinarium PHH1 is a gas vesicle-producing organism. The gas vesicle formation is encoded by the p-vac region, consisting of 14 gas vesicle protein (*gvp*) genes, located in two clusters, p-*gvpACNO* and p-*gvpDEFGHIJKL*. GvpA and GvpC are structural proteins, whereas GvpE and GvpD are involved in regulation. The functions of other Gvp proteins

are not yet known. A single deletion of p-*gvpF, G, J, K, L, M, A* or *O* leads to the lack of gas vesicles in transformants, whereas a deletion of p-*gvpC, D, E, H* or *I* results in transformants still containing gas vesicles. The former 8 *gvp* genes are thus essential for gas vesicle formation [1].
Here we determined the effect of an overexpression of single *gvp* genes on the formation of gas vesicles by co-expressing the p-vac region and the respective additional *gvp* gene in *Hfx. volcanii* transformants. The presence of larger amounts of GvpK or GvpM resulted in a reduction of gas vesicles, whereas larger amounts of GvpG or GvpH led to a nearly complete inhibition of the gas vesicle formation. The few gas vesicles produced in p-vac plus *gvpM* transformants were twice as long as in the wild type. All other Gvp proteins did not affect the gas vesicle formation when produced in larger amounts. To determine the effect of these Gvp proteins in further detail, Gvp-GFP fusions were analyzed in *Hfx. volcanii* transformants in the presence or absence of the p-vac region. In *gvpM-gfp* transformants fluorescence dots could be seen suggesting a strong aggregation of GvpM proteins. Transformants habouring the p-vac region plus *gvpM-gfp* also showed fluorescence dots but only in a few cells. Transformants habouring *gvpH-gfp* exhibited the fluorescence throughout the cells but p-vac plus *gvpH-gfp* transformants contained fluorescence signals at certain locations suggesting an aggregation with additional Gvp proteins such as GvpM or GvpJ. In contrast *gvpL-gfp* or p-vac plus *gvpL-gfp* transformants showed an equal distribution of fluorescence in the cell.
The aggregation of GvpM and of GvpH with other Gvp proteins might be the reason for the reduction or lack of gas vesicles in the respective transformants.

[1] Offner et al (2000).

ARP017
Identification of functional autoinducer hydrolase genes in archaea

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Autoinducer I hydrolases have so far mostly been described from mesophilic bacteria [1]. Here we report on the search and identification of autoinducer I hydrolase genes in metagenome libraries containing archaeal DNA. The libraries originated from hydrothermal vent microbial communities and from the *Cand.* Nitrososphaera gargensis genome, which we have recently established by metagenomic reconstitution from an enrichment culture [2].
The hydrothermal vent metagenome library contained 8,256 clones and the *Cand.* Nitrososphaera gargensis enrichment culture library consisted of 6,720 clones. Functional searches using a previously published protocol based on the *Agrobacterium tumefaciens* reporter strain NTL4 [3].
Altogether nine fosmid clones were identified that repeatedly resulted in a degradation of added autoinducer I molecules (3-oxo-C(8)-HSL). Eight clones were derived from the hydrothermal vent library and one fosmid clone (pFos3C3) was mapped to the *Cand.* Nitrososphaera gargensis chromosome. Further 454 sequencing revealed that fosmid clones derived from the hydrothermal vent library contained DNA that was similar to DNA from known Thermococcales.
Current work focuses on an identification of the respective ORFs and a biochemical characterisation of the already identified autoinducer hydrolase genes.

[1] Williams et al (2007): Philos Trans R Soc Lond B Biol Sci 362(1483): 1119-34.
[2] Zumbrägel et al (2011): in preparation.
[3] Schipper et al (2009): Appl Environ Microbiol 75:224-233.

CBV001
Dynamic regulation of the Cdc24/Rac1/Cla4 signalling module during dimorphic switching of the phytopathogenic fungus *Ustilago maydis*

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The morphogenetic transition from yeast to filamentous growth is a characteristic feature of many pathogenic fungi. The corn pathogen *Ustilago maydis* serves as an excellent model system to study the molecular mechanism of polarized growth. Dimorphic switching is part of its sexual life and requires the small GTP-binding protein Rac1 and its downstream effector, the p21-activated kinase Cla4. Small GTP-binding proteins of the Rho-family are activated by guanine nucleotide exchange factors (GEFs) and inactivated by GTPase activating proteins (GAPs). Guanine nucleotide dissociation inhibitors (GDIs) extract the GTPases from the membrane and sequester the inactive form in the cytosol.
We could show that dimorphic switching involves *b* mating-type dependent stimulation of the Rac1-specific GEF Cdc24. During polarized growth active Cdc24 recruits Rac1 into a Bem1-scaffolded complex which is located at the hyphal tip. Remarkably, ternary complex formation triggers destruction of Cdc24, most presumably by Cla4 dependent phosphorylation of Cdc24. Expression of nondegradable Cdc24 mutants interfered with filamentous growth and plant infection indicating an important role for Cla4 induced destruction of Cdc24 during the maintenance of polarized growth. We propose that degradation of Cdc24 ensures dynamic localization of active Cla4 kinase at the apical growth zone.
This negative feedback regulation requires that Rac1 has the ability to pass through its GDP bound state. Therefore we analysed the distinct functions of Rac1-specific GAPs and the Rho-GDI Rdi1 during hyphal tip growth. We provide evidence that recycling of inactive Rac1 from the membrane depends on Rdi1 and endocytosis.

CBV002
The ParA-like protein PomZ positively regulates positioning of the cell division site

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In all cells, accurate positioning of the division site is essential for generating appropriately-sized daughter cells with a correct chromosome number. In bacteria selection of the site of cell division has been thought to rely on negative regulators only; however, we recently showed that the ParA homologue PomZ (*Po*sitioning at *m*idcell of Fts*Z*, MXAN0635) positively regulates Z-ring formation in *Myxococcus xanthus*. Lack of PomZ results in cell division defects with the formation of filamentous cells and chromosome-free minicells. Consistently, in a *pomZ* mutant FtsZ-ring formation is significantly reduced and the Z-rings that are formed are abnormally positioned. PomZ localizes in three distinct patterns, which correlate with cell length and cell cycle progression. In short cells, PomZ localizes in a patchy /diffuse pattern, as cell length increases PomZ localizes to a cluster slightly off mid-cell and in longer cells PomZ localizes at mid-cell. Co-localization studies demonstrated that PomZ and FtsZ co-localize at mid-cell. Importantly, PomZ arrives at the incipient division site before FtsZ, suggesting that it recruits FtsZ at mid-cell. In agreement with this idea, we found that PomZ and FtsZ interact *in vivo* and that PomZ stimulates FtsZ filament formation.
We hypothesized that additional proteins are involved in directing PomZ to the mid-cell. To identify such proteins, we focussed on genes flanking *pomZ*. *pomX* (MXAN0636) encodes a protein with a C-terminal coiled-coil region. A *pomX* mutant phenocopies a *pomZ* mutation, indicating that PomX is also involved in cell division. Consistently, in the absence of PomX, FtsZ-ring formation is significantly reduced and the Z-rings formed are abnormally localized. Notably, in the absence of PomX, PomZ localization to the off-center cluster and at midcell is also abolished. In wild type cells, a PomX-mcherry fusion mostly localize to mid-cell. Intriguingly, this localization pattern is abolished in the absence of PomZ. A *pomX* mutation in combination with a Δ*pomZ* mutation is lethal. Preliminary data sugest that PomX interacts with PomZ *in vitro*. Moreover, His_6-PomX forms filaments on its own. According to our current working hypothesis for PomX and PomZ in cell division these two proteins interact to form a complex with FtsZ in that way fulfilling two purposes, recruitment of FtsZ to mid-cell and stabilization of the Z-ring.

CBV003
Actin and actin binding proteins during polarized growth and septum formation in *Neurospora crassa*

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Actin plays essential role in filamentous fungi in a wide variety of cellular processes including cell growth, intracellular motility, and cytokinesis. We visualized F-actin organization and dynamics in different stages of development in living *Neurospora crassa* expressing GFP fusions with homologues of the actin-binding proteins fimbrin (FIM) and tropomyosin

(TPM-1), a subunit of the Arp2/3 complex (ARP-3) and coronin (COR1). FIM-GFP, ARP-3-GFP, and COR1-GFP associated with small patches in the cortical cytoplasm that were concentrated in a subapical ring. These cortical patches were short-lived, and a subset was mobile throughout the hypha, exhibiting both anterograde and retrograde motility. TPM-1-GFP and Lifeact-GFP co-localized within the Spitzenkörper core at the hyphal apex, and were also observed in actin cables throughout the hypha. All GFP fusion proteins studied were also transiently localized at septa: Lifeact-GFP first appeared as a broad ring during early stages of contractile ring formation and later coalesced into a sharper ring, TPM-1-GFP was observed in maturing septa, and FIM-GFP/ARP3-GFP-labeled cortical patches formed a double ring flanking the septa. Our observations suggest that each of the N. crassa F-actin-binding proteins analyzed associates with a different subset of F-actin structures, presumably reflecting distinct roles in F-actin organization and dynamics during all the stages of development and septation. Actin is present since early stages of septum formation, the contractile force of the actomyosin ring is related to the presence of tropomyosin and it seems that there is a need of plasma membrane remodeling regards the presence of endocytic patches labeled by fimbrin, coronin and Arp2/3 complex.

CBV004
Interaction of bacterial cytoskeletal elements in a heterologous system

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Bacterial cytoskeletal element MreB has been shown to be essential for the maintenance of rod cell shape in many bacteria. MreB forms rapidly remodelling helical filaments underneath the cell membrane in *Bacillus subtilis* and other bacterial cells, and co-localizes with its two paralogs, Mbl and MreBH. We show that MreB localizes as dynamic bundles of filaments underneath the cell membrane in *Drosophila* S2 Schneider cells, which become highly stable when the ATPase motif was modified. Extended induction of MreB resulted in the formation of membrane protrusions, showing that like actin, MreB can exert force against the cell membrane. Mbl also formed membrane associated filaments, while MreBH formed filaments within the cytosol. When co-expressed, MreB, Mbl and MreBH built up mixed filaments underneath the cell membrane. RodZ membrane protein localized to internal membranes in S2 cells, but localized to the the cell membrane when co-expressed with Mbl, showing that membrane associated structures can recruit a membrane protein. Thus, MreB paralogs form a self-organizing filamentous scaffold underneath the membrane that is able to recruit other proteins to the cell surface.

CBV005
The Kinesin-3 Motor Protein UncA Reveals Different Microtubule Populations in *Aspergillus nidulans*

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The MT cytoskeleton is not as rigid and uniform as the name implies, but is characterized by its dynamic instability. In addition, MTs can be made up of different tubulin isoforms and can be post-translationally modified. MT modifications are evolutionarily old „inventions" and occur in primitive eukaryotes such as *Giardia lamblia*, whereas detyrosination appeared later during evolution. Here we found that the *A. nidulans* kinesin-3, UncA, transports vesicles along microtubules (MTs) and is required for hyphal extension. Most surprisingly, UncA-dependent vesicle movement occurred along a subpopulation of MTs. GFP labelled UncA^{rigor} decorated a single MT bundle, which remained intact during mitosis, while other cytoplasmic MTs were depolymerised. Mitotic spindles were not labelled with GFP-UncA^{rigor} but reacted with a specific antibody against tyrosinated alpha-tubulin. Those results suggest that UncA binds preferentially to detyrosinated MTs [1] and that different MT populations exist in *A. nidulans*. To confirm this aim we searched for the MT modification enzyme - tubulin tyrosine ligase (TtlA) - and constructed a *ttlA*-deletion strain and a *ttlA*, alpha tubulin 2 (*tubB*) double deletion strain. Currently we are characterizing the MT cytoskeleton and its modification in the wild type strain and in the deletion strains using different assays and techniques.
To understand how UncA is able to distinguish between different MTs, deletion analyses revealed a specificity region in the tail of UncA between amino acid 1316 and 1402. A non-targeted Y2H approach was used to identify interaction partners of this region, which are most likely involved in recognition of MT subpopulations. Two candidates appeared to be associated with vesicles and currently different assay are performed to confirm their interaction with UncA.

[1] Zekert, N. and R. Fischer (2009): Mol. Biol. Cell 20, 673-684.

CBV006
Physical compartmentalization by a protein diffusion barrier in stalked alpha-proteobacteria

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Prosthecae, also known as stalks, are a widespread phenomenon among bacteria, but the biogenesis and function of these structures is still unclear. In the dimorphic alpha-proteobacterium *Caulobacter crescentus*, the stalk represents a thin extension of the cell envelope that is free of DNA, ribosomes and most cytoplasmic proteins. It is segmented at irregular intervals by so-called crossbands, disk-like structures that traverse the entire width of the stalk perpendicular to the long-axis of the cell. Crossbands have been observed in a variety of prosthecate species and are generally thought to have an architectural, stabilizing function.
Here, we report the identification and characterization of four novel stalk proteins, StpABCD, that are essential for crossband formation and stalk compartmentalization in *Caulobacter*. Synthesis of StpABCD is initiated at the onset of stalk outgrowth, an obligate and irreversible developmental checkpoint in the *Caulobacter* cell-cycle. We found that StpABCD are specifically targeted to the periplasmic space of the stalk, with StpA acting as a recruitment factor for StpBCD. The four proteins colocalize in distinct foci that display the same subcellular distribution as crossbands. Additionally, coimmunoprecipitation analysis supports the idea that StpABCD interact *in vivo* to form a multi-protein complex. Electron cryo-tomography revealed that cells deficient in StpAB consistently lack crossbands. We used fluorescence-recovery after photobleaching (FRAP) to test for the presence or absence of protein compartmentalization in wild-type and StpAB-deficient cells. Interestingly, our experiments demonstrated that crossbands act as diffusion barriers for periplasmic and inner membrane proteins. However, the mechanism by which cytosolic proteins are retained in the cell-body is still unclear. We are currently examining whether the function of crossbands is conserved among stalked alpha-proteobacteria.
Based on our findings, we hypothesize that StpABCD are required for synthesizing crossbands, which act as a protein diffusion barrier to compartmentalize the periplasmic space of the stalk and physically separate it from the cell-body. Crossband formation thus represents a novel mechanism to topologically restrict protein mobility within a cell.

CBV007
Microtubule-dependent co-transport of mRNPs and vesicles in *Ustilago maydis*

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Long-distance transport of mRNAs is important in determining polarity in eukaryotes. In *U. maydis* this process is mediated by the RNA binding protein Rrm4 which is a key component of large motile ribonucleoprotein complexes (mRNPs) shuttling along the microtubule cytoskeleton. Disruption of long-distant mRNP transport by deleting or mutating *rrm4* leads to defects in filamentous growth and a reduced virulence. In spite of identifying numerous transported mRNAs which encode upon others polarity and translation factors, the composition of the mRNPs and the motor proteins involved in their transport were not known. Here we show that the plus end-directed conventional kinesin Kin1, the UNC104/Kif1A-

like Kinesin 3 as well as the minus end-directed split dynein Dyn1/2 are involved in the shuttling of the Rrm4-containing mRNPs. Kin3 transports the mRNPs to the apical pole of the growing hyphae, whereas Dyn1/2 mediates the retrograde movement of the mRNPs from the tip to the basal pole. Kin1 is indirectly involved by transporting Dyn1/2 to the apical tip of the hyphae. Interestingly the same set of motors is involved in the long-distance transport of vesicles in the *U. maydis* cell. Indeed, Rrm4 co-localises with t-SNARE Yup1 positive vesicles, revealing a new mechanism of coupled microtubule-dependent transport of Rrm4-containing mRNPs and vesicles.

CBV008
Functional analysis of cytoskeletal proteins implicated in magnetosome formation and cell division in *Magnetospirillum gryphiswaldense*

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Magnetotactic bacteria use magnetosomes to move along magnetic field lines. Magnetosomes are organelles which consist of membrane-enclosed nanometer-sized magnetite crystals lined up along the cell axis. This magnetosome chain is located at midcell and split during cell division, whereas magnetosomes are segregated to daughter cells and re-localized from the new cell poles to the new Centers by an as yet unknown mechanism. Midcell information in other bacteria is ususally provided by the essential cell division protein FtsZ which also exerts constrictive forces onto lipid membranes. Intriguingly, *M. gryphiswaldense* has two *ftsZ*-like genes (*ftsZ*$_{Mgr}$ and *ftsZm). ftsZm* is co-loacted within the genomic magnetosome island with other magnetosome genes including *mamK*, which encodes a further, actin-like cytoskeletal protein that polymerizes into straight magnetosome filament structures. We have analyzed the function of several homologues of cytoskeletal elements likely implicated in the magnetosome chain division process.
An operon deletion including *ftsZm* had no effect on cell division but on magnetite crystal biomineralization in *M. gryphiswaldense*. Fluorescence microscopy in *E. coli* revealed that both FtsZ$_{Mgr}$ and FtsZm form filamentous structures distinct from MamK and interfere with the endogenous FtsZ function, resulting in division impaired elongated cells. Expression in *M. gryphiswaldense* however, suggests a different localization pattern and a distinct role in this organism. Transmission electron microscopy of septation-inhibited elongated *M. gryphiswaldense* cells demonstrated that magnetosome chains localize to division planes. As revealed by time lapse fluorescence microscopy, magnetosome localization during cell cycle is dynamic. Overall, our preliminary data suggest that magnetosome segregation during cell division occurs by an active mechanism that might be divisome-dependent.

CBV009
Imaging of the *Neurospora crassa* actin cytoskeleton with Lifeact

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Actin is the most abundant protein in eukaryotic cells. It forms a complex network of filaments that play pivotal roles in a wide variety of processes. It is a component of the Spitzenkörper (Spk), a typical structure of filamentous fungi, where it is present in the core. The Spk functions as a supply center for vesicles prior to exocitosis to the apical dome of growing hyphae. Actin is also present in the subapical region of hyphae forming patches that are highly mobile and are components of the endocytic machinery. Finally, actin is also essential for cytokinesis to occur; it forms the actomyosin ring responsible for the constriction of the membrane to achieve, in the case of filamentous fungi, the formation of septa. Lifeact had been used to visualize the actin cytoskeleton of the yeast fungus *S. cerevisiae* but had not been expressed in filamentous fungi. In this work we present the results from the labeling of the actin cytoskeleton of *Neurospora crassa* by means of a reporter named Lifeact, a 17 aminoacid peptide from the non-essential protein Abp140 of *Saccharomyces cerevisiae* fused to GFP (Riedl et al. 2008). The functionality of the Lifeact reporter was corroborated by disruption of the actin cytoskeleton and microtubular cytoskeleton by specific drugs. The fluorescence patterns revealed by confocal microscopy of Lifeact-GFP fluorescence match those of other full-length actin binding proteins (ABPs). Lifeact also labels filamentous structures close to the developing septa that had not been previously described for *N. crassa* or for other filamentous fungi. We conclude that Lifeact-GFP is an excellent reporter for the actin cytoskeleton in *N. crassa* and potentially for other filamentous fungi.

CBV010
Cell Biologie of *Ignicoccus hospitalis* - a unique Crenarchaeon

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The hyperthermophilic Crenarchaeon *Ignicoccus hospitalis* exhibits an in many aspects unique cell biology. The cells grow by sulfur-hydrogen chemolithoautotrophy, i.e. by oxidation of molecular hydrogen, using elemental sulfur as electron acceptor [1]. *I. hospitalis* cells can adhere to surfaces by extracellular appendages named 'fibers', which are not used for motility [2]. The cells are hosts for *Nanoarchaeum equitans,* by forming a special co-culture, the only known intimate association of two Archaea. *N. equitans* cells cannot thrive alone but depend on a direct cell-to-cell contact to *I. hospitalis* and obtain at least lipids and amino acids from their 'host' [3]. The ultrastructure of *I. hospitalis* cells is unique, as they have two distinct compartments: the central one is densely stained in electron micrographs, and contains ribosomes and many proteins. DAPI staining demonstrated that it also contains the DNA. Between the inner and the outer membrane is the intermembrane compartment (IMC), which is only lightly stained in electron micrographs, most likely due to a far lower density of biomolecules. In the IMC, many round or elongated vesicles are found, surrounded by a lipid bilayer; they are likely to function as carrier of lipids or proteins from in to out [4]. The outermost membrane was investigated in great detail: it contains huge amounts of a unique pore-forming protein, Ihomp1, and, much to our surprise, also two protein complexes which are key players in the energy metabolism of *I. hospitalis* cells: the H_2:S^0 oxidoreductase, acting as primary proton pump, and the A_1A_O ATP-synthase complex, possibly the exclusive ATP producing machinery in *I. hospitalis* cells [5]. Thus, among all prokaryotes possessing two membranes in their cell envelope (including Planctomycetes, Gram-negative Bacteria), *I. hospitalis* is the first organism with an energized outer membrane and ATP synthesis within the IMC. Accordingly, in *I. hospitalis*, energy conservation is located in the IMC, and is separated from information processing and protein biosynthesis (in the cytoplasm). Future research is directed to further analyze and explain e.g. the transport of ATP from the IMC to the cytoplasm; the molecules involved in formation of the vesicles inside the IMC; the subcellular distribution of the enzymes involved in CO_2 fixation; and the architecture of the fibers, their anchor in the cell, and their ultrastructure at high resolution.

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[2] Müller D et al 2009 J Bacteriol 191: 6465.
[3] Huber H et al. 2008 PNAS 105:7851.
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CBV011
Apical growth in *Neurospora crassa*

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Apical growth in filamentous fungi is supported by the constitutive exocytosis of secretory vesicles, which maintain the normal complement of plasma membrane proteins and lipids through „full fusion". In *Neurospora crassa* vesicles containing cell-wall building enzymes are transported along the hyphae and accumulate temporarily in the Spitzenkörper in a stratified manner. The Vesicle Supply Center (VSC) model for fungal morphogenesis predicted that these vesicles are distributed from the Spitzenkörper outwards

in all directions, generating a sharp gradient of exocytosis, with a maximum at the pole and vanishing gradually in the subapex. Those secretory vesicles reaching the plasma membrane, prior to SNAREs recognition, are presumably tethered to their target acceptor membrane in a process mediated by the exocyst complex. We endogenously tagged with GFP the exocyst components SEC-3, SEC-5, SEC-6, SEC-8, SEC-15, EXO-70 and EXO-84 in *N. crassa*. Some components accumulated surrounding the frontal part of the Spitzenkörper, while others were found in a delimited region of the apical plasma membrane that correlates with the place of intensive exocytosis during polarized growth. A more detailed analysis by TIRFM revealed that the fluorescently labeled exocyst components followed a pulsatile exocytotic process, suggesting anorderly mechanism for exocytosis of the vesicles constituting the Spitzenkörper. Our results show that the region of exocyst-mediated vesicle fusion at the hyphal apical plasma membrane has the same extension than the exocytosis gradient predicted earlier by the VSC model.

CBV012

Physical organization and interactions between sensory histidine kinases in *E. coli*

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Microorganisms commonly use 'two-component' signaling systems for sensing environmental conditions. Prototypical two-component systems are comprised of a sensory histidine kinase and a response regulator that is phosphorylated by the kinase and typically acts as a transcription factor. Apart from a few well-investigated cases, such as signaling in bacterial chemotaxis or asymmetric cell division in bacteria, intracellular organization of sensory kinases remains largely unclear. We characterize the spatial distribution and oligomeric state of these sensors in the model bacterium *Escherichia coli*, using fluorescence imaging, fluorescence resonance energy transfer (FRET) and fluorescence polarization microscopy. We find that at physiological expression levels most fluorescently tagged sensors show a uniform membrane distribution with no preference towards polar regions, a few kinases exhibit lateral localization patterns. Measurements of FRET confirmed that at physiological expression levels most sensors self-associate to form small complexes, presumably dimers, but not larger oligomers. We demonstrate that in some of the cases interactions between sensors are sensitive to specific stimulation, suggesting that changes in protein arrangement play a role in signal processing. We further observed several cases of mixed complex formation between different sensors, indicating interconnections between different signaling pathways. However, different from the signaling in chemotaxis, only few two-component sensors showed a distinct punctuate localization in the cell or low levels of fluorescence polarization that are indicative of higher-order complexes, suggesting that at low expression levels most of them function as isolated dimers.

CBV013

Understanding long-range endosome trafficking: From measuring to modelling

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In filamentous fungi microtubules form long tracks that are used by molecular motors to transport organelles, vesicles and RNA over long distances. Such membrane trafficking is essential for hyphal tip growth, and the underlying molecular machinery is conserved amongst filamentous fungi. The fungal pathogen *Ustilago maydis* is a genetically tractable system to investigate motor cooperation in trafficking of early endosomes. Imaging of native levels of motors and their cargo in living cells in combination with quantitative analysis and mathematical modelling revealed that a combination of stochastic motor behaviour and active retention concentrate dynein at microtubule ends. This is essential for efficient cargo loading and retrograde transport of early endosomes.

CBV014

A synthetic *in vivo* system identifies a chromosome tethering factor in *Corynebacterium glutamicum*.

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The chromosome partitioning system of the rod-shaped *actinomycete*, *Corynebacterium glutamicum* consists of the Walker-type ATPase ParA, the DNA-binding protein ParB and *parS* sites that are found near the chromosomal origin of replication. Once chromosome replication has been initiated, the *C. glutamicum* ParB protein specifically binds the *parS* sites of the newly replicated *oriC*. As the chromosome is replicated, ParA binds the ParB-*parS* nucleoprotein complex, and is thought to provide the driving force to relocalize the replicating chromosome to the opposite cell pole. The chromosome is then stably attached to the cell pole, where it remains and the cell divides in between the segregated chromosomes. We were interested in identifying and analyzing the chromosome polar targeting factor. One possible candidate for tethering the chromosome to the cell poles is the DivIVA protein, which influences apical growth and cell shape determination in *C. glutamicum*, similar to other organisms like *Streptomyces coelicolor* and *Mycobacterium smegmatis*. Indeed, bacterial-two-hybrid analysis showed an interaction between DivIVA and the Par proteins. However, to further analyse these interactions, a synthetic *in vivo* approach was developed. In this system, *E. coli* cells are used as a host for expression of the fluorescently labeled proteins. *E. coli* is advantageous for this purpose as it does not contain homologues of the Par system or DivIVA. When expressed individually, DivIVA-GFP localized to the curved polar membranes and division sites, while ParB-CFP showed no specific localization. However, upon co-expression, ParB-CFP was completely recruited to the polar and septal localized DivIVA. Using this system, along with mutational analysis the interaction sites between ParB and DivIVA could be mapped. Also, similar interaction studies were also carried out for the notorious pathogen *Mycobacterium tuberculosis*, showing that this system is a general mechanism within the *Corynebacterianae*.

CBV015

The Num1 Protein of *Ustilago maydis* is Required for Polar and Filamentous Growth

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In the corn smut fungus Ustilago maydis, sexual development is initiated by the fusion of two haploid sporidia, resulting in a filamentous growing dikaryon that is capable to infect the host plant. Growth of the dikaryon requires an elaborate regulation of the cell cycle, migration and distribution of the two nuclei and the polar growth of the hyphae.
We have identified the Num1 protein with a pivotal function during these processes. Num1 is a homologue of SPF27, one of the core components of the highly conserved Prp19/CDC5 splicing associated complex. Vegetative growth of sporidia is not altered in num1 deletion mutants; however, the hyphae show various polarity defects, delocalized septae and dislocalized nuclei. Using the Yeast Two-Hybrid system, we identified CDC5, another conserved component of the Prp19/CDC5 complex, as Num1 interactor. Interestingly, we also identified various proteins with functions during vesicle-mediated transport, in particular the kinesin 1 motor protein. The Num1/Kin1 interaction was verified by Co-Immunoprecipitation and Split-GFP analysis. Both num1 and kin1 deletion strains exhibit identical phenotypes with respect to vacuole morphology, filamentous and polar apical growth, corroborating the genetic interaction between Num1 and Kin1.
Our data connect the splicing machinery and long distant transport in U. maydis. We will present our current view whether (and how) these two disparate mechanisms may be matched.

CBV016
Membrane potential plays a fundamental role in regulation and maintenance of bacterial morphology

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The emerging knowledge about the distinct localisation of proteins and other cellular components has radically changed our view of bacterial cells. The organisation of different cellular functions to specific areas of the cell reflects the existence of a well-defined cellular architecture. However, the presence of a high level of organisation is fundamentally linked to the energy required for its maintenance. In addition, many cellular structures are dynamic in their localisation and macromolecular structure, further emphasizing the critical role of energy supply. The role of high energy phosphates like ATP and GTP in maintaining the cell architecture has been previously analysed in great detail. However, all living organisms also utilise another fundamental energy source, the transmembrane proton motive force (pmf). This second major cellular energy source is crucial for various processes including transport, signalling and ATP-synthesis across all domains of life. Although cell membranes and membrane proteins play a central role in bacterial morphology, nothing is known about the role of pmf in these processes. A detailed analysis of key morphological proteins in Bacillus subtilis revealed a drastic effect on their localisation when the pmf was dissipated. Based on these results, we propose a novel function of the membrane potential in regulation and maintenance of bacterial morphology.
Strahl H, Hamoen LW. (2010) Membrane potential is important for bacterial cell division. PNAS 107:12281-12286.

CBV017
Vip1-like 1/3 inositol polyphosphate kinases regulate the dimorphic switch in yeasts

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It has long been known that the environmentally induced transition of fungal growth forms is an essential initial requirement for pathogenesis. The ability to undergo a dramatic morphological change from a single cell form to a multi-cellular invasive form in response to extrinsic cues is conserved in fungi and also found in non-pathogenic model yeasts such as *S. pombe* and *S. cerevisiae*. Here we describe the identification and characterization of the *S. pombe* Asp1 protein as a key regulator of the dimorphic switch. Asp1 is a member of the highly conserved Vip1 family of 1/3 inositol polyphosphate kinases, which generate specific inositol pyrophosphates that have been shown to regulate cyclin-CDK complexes. Vip1-like proteins have a dual domain structure consisting of an N-terminal „rimK"/ATP-grasp superfamily domain and a C-terminal part with homology to histidine acid-phosphatases. Asp1, which acts downstream of the cAMP PKA pathway, is essential for the transition to the pseudohyphal invasive growth mode under nutrient limitation. Intriguingly, an increase in the cellular amounts of Asp1 generated inositol pyrophosphates increases the cellular response thus implying that these molecules might act as second messengers. Remarkably the Asp1 kinase activity is regulated negatively by its C-terminal domain. Thus the fine tuning of the cellular response to environmental cues is modulated by the same protein. Interestingly, the *S. cerevisiae* Vip1 family member is also required for the dimorphic switch in this yeast. Therefore we propose Vip1 family members have a general role in regulating fungal dimorphism and are presently testing this in a number of fungi.

CBP001
Coordinated separation - the late stage of bacterial cell division

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In the late stages of bacterial cell division, the remodelling of the cell wall requires a delicate balance between synthesis and degradation of peptidoglycan. Only few components of the protein network orchestrating this process have been identified, and the mode of their spatial and temporal regulation remains unclear. To address this issue, we investigate the function of cell division proteins in the gram-negative model organism *Caulobacter crescentus*.
Cell wall peptidoglycan is a structural element preserving cell integrity and contributing to cell shape. Additionally, it serves as a scaffold for anchoring proteins that are part of the cell envelope. To identify factors involved in the late stage of cell division, we focused on proteins containing predicted peptidoglycan-binding domains. Using fluorescence microscopy, we selected promising candidates that localized to midcell during cell division and subsequently examined them in more detail.
Based on this approach, we identified and characterized a structural homologue of the late essential cell division protein FtsN from *Escherichia coli* in *C. crescentus*. FtsN was previously thought to be poorly conserved outside the enteric bacteria. However, a database search based on the typical structural features shared by *E. coli* and *C. crescentus* FtsN showed that FtsN-like proteins are in fact widespread among all proteobacteria [1]. Building on these results, we identified an interaction partner of FtsN, named DipM, for *di*vision- and *p*olarity-related *m*etallopeptidase. DipM requires FtsN for midcell localization. Interestingly, in the absence of DipM, invagination of the cell wall and outer membrane is delayed, leading to severe division and polarity defects [2]. These results provide more evidence for a key role of FtsN in the regulation of cell wall remodelling during the final stage of cell division.

[1] Möll, A., and M. Thanbichler (2009): FtsN-like proteins are conserved components of the cell division machinery in proteobacteria. Mol Microbiol 72: 1037-1053.
[2] Möll, A. et al (2010): DipM, a new factor required for peptidoglycan remodelling during cell division in *Caulobacter crescentus*. Mol Microbiol 77: 90-107.

CBP002
Mechanism of Gradient Formation by the *Caulobacter* Cell Division Inhibitor MipZ

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Intracellular protein gradients play a critical role in the spatial organization of both prokaryotic and eukaryotic cells, but in many cases the mechanisms underlying their formation are still unclear. Recently, a bipolar gradient of the Walker ATPase MipZ was found to be required for proper division site placement in the differentiating bacterium *Caulobacter crescentus*. MipZ interacts with a kinetochore-like nucleoprotein complex formed by the DNA partitioning protein ParB in proximity of the chromosomal origin of replication. Upon entry into S-phase, the two newly duplicated origin regions are partitioned and sequestered to opposite cell poles, giving rise to a bipolar distribution of MipZ with a defined concentration minimum at the cell center. Acting as a direct inhibitor of divisome formation, MipZ thus effectively confines cytokinesis to the midcell region. Building on the crystal structures of the apo and ATP-bound protein, we have dissected the role of nucleotide binding and hydrolysis in MipZ function. Our findings indicate that gradient formation results from alternation of MipZ between a monomeric and dimeric form that display marked differences in their interaction networks and diffusion rates. As a consequence, MipZ undergoes an elaborate localization cycle, involving its oscillation between the polar ParB complexes and pole-distal regions of the nucleoid. The MipZ gradient thus represents the steady-state distribution of molecules in a highly dynamic system, providing a general mechanism for the establishment of protein gradients within the confined space of the bacterial cytoplasm.

CBP003
Functional analysis of SPFH domain-containing proteins, Flotillin and Stomatin, in *Aspergillus nidulans*

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Polarized growth of filamentous fungi depends on the microtubule and the actin cytoskeleton along with their associated motor proteins. Apical membrane-associated landmark proteins, so-called „cell end markers" link the two cytoskeletons. Our latest results indicate that apical sterol-rich membrane domains (SRDs) play important roles in polarized growth and

localization of cell end markers [1; 2]. Although the importance of SRDs is getting clearer, the roles and formation mechanism of SRDs remain almost unknown. To analyze the functional roles of SRDs, we investigate the mechanism of SRD (or raft cluster) formation and maintenance. There are numerous studies on raft formation in different organisms and some components are known. Flotillin/reggie proteins for instance were discovered in neurons and are known to form plasma membrane domains. The flotillin/reggie protein and a related microdomain scaffolding protein, stomatin, are conserved in filamentous fungi but have not yet been characterized. We have started the investigation of their functions by gene deletion and GFP-tagging. It was revealed that the flotillin/reggie protein FloA-GFP accumulated at hyphal tips. The deletion of *floA* showed smaller colony than that of wild-type strain and often exhibited irregular thickness of hyphae. Moreover, the stomatin related protein StoA-GFP localized only at young branch tips and subapical cortex in mature hyphal tips. The deletion of stoA also showed smaller colony than that of wild-type strain and exhibited irregular hyphae and increased branching. The localization of SRDs, cell end markers, and actin etc. are analyzed in the mutants.

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CBP004
Mode of action of a cell cycle arresting yeast killer toxin

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K28 is a heterodimeric A/B toxin secreted by virally infected killer strains of the yeast *Saccharomyces cerevisiae*. After binding to the cell wall of sensitive yeasts the a/b toxin enters cells via receptor-mediated endocytosis and is retrogradely transported to the cytosol where it dissociates into its subunit components. While β is polyubiquitinated and proteasomaly degraded, the α-subunit enters the nucleus and causes an irreversible cell cycle arrest at the transition from G1 to S phase. K28-treated cells typically arrest with a medium-sized bud, a single nucleus in the mother cell and show a pre-replicative DNA content (1n).
Since other cell cycle arresting killer toxins like zymocin from *Kluyveromyces lactis* or *Pichia acaciae* toxin PaT cause a similar „terminal phenotype", we tested the effect of K28 on *S. cerevisiae* mutants that are resistant against those toxins. Agar diffusion assays showed that deletion of *TRM9* or *ELP3* did not lead to toxin resistance, indicating that the arrest caused by K28 differs from zymocin or PaT induced cell cycle arrest. Interestingly, RNA polymerase II deletion mutants (*rpb4, rpb9)* show complete resistance against K28.
To gain deeper insight into the mechanism(s) of how K28α arrests the cell cycle, we further studied the influence of the toxin on transcription of cell cycle and G1-specific genes. Northern blot analyses showed that G1-specific *CLN1* and *CLN2* mRNA levels rapidly decrease after toxin treatment, though it is unclear if this decline is due to a direct effect. Potential toxin targets were found using the yeast two hybrid system and were verified biochemically by coIP and GST pulldown assays. To confirm that the nucleus represents the compartment where *in vivo* toxicity occurs we constructed protein fusions between K28α and mRFP and analysed their intracellular localisation.

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CBP005
Reverse SECretion or ERADication?

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K28 is a virus encoded A/B protein toxin secreted by the yeast *Saccharomyces cerevisiae* that enters susceptible target cells by receptor-mediated endocytosis. After retrograde transport from early endosomes through the secretory pathway, the α/β heterodimeric toxin reaches the cytosol where the cytotoxic α-subunit dissociates from β, subsequently enters the nucleus and causes cell death by blocking DNA synthesis and arresting cells at the G1/S boundary of the cell cycle [1].
Interestingly, K28 retrotranslocation from the ER into the cytosol is independent of ubiquitination and does not require cellular components of the ER-associated protein degradation machinery (ERAD). In contrast, ER exit of a cytotoxic α-variant expressed in the ER lumen depends on ubiquitination and ERAD, indicating (i) that α masks itself as ERAD substrate for proteasomal degradation and (ii) that ER retrotranslocation mechanistically differs under both scenarios [2]. To elucidate the molecular mechanism(s) of ER-to-cytosol toxin transport in yeast as well as in mammalian cells, the major focus of the present study is to identify cellular components (including the nature of the ER translocation channel) involved in this process. The requirement of proteasomal activity and ubiquitination to drive ER export, and the identification of cellular K28 interaction partners of both, the α/β toxin as well as K28α are being analysed *in vitro* on isolated microsomes and IP experiments.

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CBP006
Follow the light: Visualization of K28 cell entry and its receptor's mobility

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K28 toxin, secreted by virus-infected killer strains of the yeast *Saccharomyces cerevisiae*, is a α/β heterodimeric protein of the A/B toxin family. After initial toxin binding to the surface of sensitive target cells, K28 is taken up by receptor-mediated endocytosis and subsequently delivered to an early endosomal compartment from where it is transported backwards through the Golgi and the endoplasmic reticulum (ER) to the cytosol. Within the cytosol, the toxin′s β-subunit is polyubiquitinated and targeted for proteasomal degradation, while α enters the nucleus and causes a G1/S cell cycle arrest and cell death.
Both, toxin uptake and intracellular transport crucially depend on the cellular HDEL receptor Erd2p which ensures that the toxin is targeted from the plasma membrane to the secretory pathway of intoxicated cells. Thus K28 represents a powerful tool and substrate for general studies of endocytosis and endosomal trafficking in eukaryotic cells. To elucidate the trafficking route of the toxin, biologically active K28/mCherry fusion proteins as well as inactive controls were expressed in *Pichia pastoris* and used to track the toxin′s *in vivo* binding to the yeast cell and transit through the endocytic pathway. Another approach includes the investigation of the GFP-tagged toxin receptor Erd2p with the help of TIRF microscopy. Erd2p mobility in wild-type and endocytic mutants was compared quantitatively.

CBP007
A bacterial dynamin-like protein promotes magnesium assisted membrane fusion

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Membrane dynamics are of fundamental importance for all cells. Dysfunction of membrane remodeling in mitochondria plays a role at the onset of virtually all neurodegenerative diseases and hence detailed molecular understanding of membrane dynamics are of great importance. Mitochondria are dynamic organelles that undergo constant fusion and fission events which require membrane remodeling events catalyzed by a group of large GTPase, dynamin-related proteins (DRPs). However, the exact biochemical details as to how DRPs catalyze membrane remodeling remain largely elusive. The inner membrane of mitochondria is homologous to the cytoplasmic membrane of heterotrophic bacteria. Not surprisingly many homologous proteins involved in vital mitochondrial processes are also found in bacterial membranes. Strikingly, the dynamin superfamily is not restricted to eukaryotes, but has bacterial origin with many species containing an operon coding for two genes of the mitofusin class of dynamins. Our lab uses the bacterium Bacillus subtilis as a model system to study membrane dynamics. In this organism we identified a bacterial DRP, DynA that is homologous to the mitofusin branch of the DRPs. DynA of *Bacillus subtilis* is remarkable in that it arose from a gene fusion. Using purified, recombinant protein we were able to study dynamin-related functions such as membrane association and lipid-binding. We found that DynA exhibits cooperative GTP hydrolysis and that self-interaction is modulated by both dynamin subunits, which in turn only allow homotypic contacts. DynA is able to tether adjacent membranes via one of its dynamin subunits. Strikingly, DynA catalyzes fusion of synthetic vesicles *in vitro*,

requiring only magnesium as cofactor. Thus, we have identified a minimal set of factors essential for efficient membrane fusion.

CBP008

The MreB-like Mbl protein of *S. coelicolor* A3(2) requires MreB for proper localization during spore wall synthesis

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The majority of rod-shaped bacteria contain an actin-like cytoskeleton consisting of MreB polymers which form helical spirals underneath the cytoplasmic membrane to direct peptidoglycan synthesis for elongation of the cell wall. In contrast, MreB of *Streptomyces coelicolor* is not required for vegetative growth, but has a role in sporulation [1]. Beside MreB, *S. coelicolor* encodes two further MreB-homologous proteins, Mbl and SCO6166, whose function is unknown. Whereas MreB and Mbl are highly similar, SCO6166 is shorter, lacking subdomains IB and IIB of actin-like proteins.
We showed that MreB and Mbl are not functionally redundant but cooperate in spore wall synthesis. Expression analysis by semi-quantitative RT-PCR revealed distinct expression patterns. *mreB* and *mbl* are predominantly induced during morphological differentiation, whereas *sco6166* is strongly expressed during vegetative growth but switched off during sporulation.
In contrast to rod shaped bacteria, deletion of *mreB* and/or *mbl* is tolerated in *S. coelicolor*. Vegetative growth was not affected but parts of the aerial hyphae lysed, spores were swollen and germinated prematurely. The mutants were also more sensitive to high salt concentrations. Whereas *S. coelicolor* M145 was still able to grow on LB supplemented with 6% NaCl, growth of *ΔmreB* or *Δmbl* mutants was abolished. Deletion of *sco6166* had no effect on morphological differentiation and its role in sporulation is unclear up to now.
During aerial mycelium formation an Mbl-mCherry fusion protein colocalized with an MreB-eGFP fusion protein at the sporulation septa. Whereas MreB-eGFP localized properly in the *Δmbl* mutant, Mbl-mCherry localization depended on the presence of a functional MreB protein.
Our data suggest that *Streptomyces* requires *mreB* and *mbl* for morphological differentiation probably to build up a thickened peptidoglycan spore wall able to resist detrimental environmental conditions.

[1] Mazza, P. *et al.* Mol Microbiol. 2006. 60:838-852.

CBP009

Impact of membrane-perturbing antimicrobial peptides on bacteria visualized by electron microscopy

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The effect of membrane-perturbing antimicrobial peptides (AMPs) has been studied extensively in the last decades, but the exact mode of action is yet not fully understood. We therefore visualized the impact of two representative cationic amphiphilic AMPs on bacteria using transmission (TEM) and scanning electron microscopy (REM). The peptide PGLa is α-helical and carries 5 positive charges, while Gramicidin S has a cyclic β-stranded structure with two cationic side chains. Their minimal inhibition concentrations (MIC values) were determined in salt-free medium for two representative Gram-positive and Gram-negative bacterial strains, *E. coli* ATCC 25922 and *S. aureus* ATCC 25923. For the EM samples, bacteria were treated with sub- and supra-MIC concentrations, and fluorescence microscopy using SYTO9/propidium iodide confirmed that at supra-MIC the membrane integrity was disturbed, while at sub-MIC the cell membranes remained intact.
After AMP treatment with either type of peptide, SEM revealed increased turgidity of *E. coli* cells, and numerous bubbles and blisters formed on the cell surface. *S. aureus* cells were severely damaged, showing deep holes and burst cells. TEM revealed intracellular membranous structures in both bacterial strains, probably as a result of lateral membrane expansion due to peptide insertion into the lipid bilayer. Additionally, the DNA region of *S. aureus* seemed to be compacted after AMP incubation.
Treatment of *E. coli* in a medium with low ionic strength at sub- or supra-MIC led to highly turgid cells, compared to untreated controls. This observation suggests that enhanced osmosis is facilitated across the inner bacterial membrane, before the more pronounced cell damages occur. Comparing our fluorescence and electron microscopy data, it is clear that antimicrobial peptides render the bacterial membranes leaky even at sub-MIC concentrations, allowing small molecules like water to pass through, though not the larger propidium iodide. This means that even at low concentration the membrane permeabilizing effect of AMPs can result in a reduced ability of the cells to regulate their osmotic pressure.

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CBP010

Lipid Rafts in Bacteria

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Question: A feature common to all living cells is the presence of a lipid membrane that defines the boundary between the inside and the outside of the cell. Proteins that localize to the membrane serve a number of essential functions. In eukaryotic cells, membrane proteins that mediate signal transduction and protein secretion are often localized in membrane microdomains enriched in certain sterol lipids that are commonly referred to as „lipid rafts" (1, 2). Lipid rafts are required for the proper function of the harbored proteins. Thus, disruptions of lipid rafts are associated with a large variety of human diseases including Alzheimer's, Parkinson's, cardiovascular and prion diseases (3). Up to now, lipid rafts have been identified and characterized in eukaryotic cells. However, many bacterial membrane proteins involved in cell-cell signaling and signal transduction pathways are distributed heterogeneously across the cytoplasmic membrane (4), suggesting that specialized membrane microdomains are also a feature of bacterial cells.
Results: Our work shows that bacteria contain lipid rafts functionally similar to those found in eukaryotes They harbor and organize proteins involved in signal transduction, small molecule translocation and protein secretion. The lipids associated with the bacterial rafts are probably polyisoprenoids synthesized via pathways that involve squalene synthases because inhibitors of this enzyme interfere with the formation of lipid rafts. In addition, membrane microdomains from diverse bacteria harbor homologs of the protein Flotillin-1, a eukaryotic protein found exclusively in lipid rafts, responsible to orchestrate events occurring in lipid rafts. A mutant devoid of Flotillin-1 is defective in the signal transduction pathways whose sensor kinases are found in the rafts.
Conclusions: Organization of physiological processes into microdomains may be a widespread feature in living organisms. On a more practical note, it is possible that lipid rafts can be exploited as a new target to control bacterial infections because disrupting lipid rafts simultaneously affects several key physiological processes associated with pathogenesis in different bacteria.

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CBP011

A role for the membrane curvature sensor DivIVA in cell separation and virulence of *Listeria monocytogenes*

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DivIVA proteins are membrane binding proteins that are highly conserved among the *Firmicutes* and the *Actinomycetes*. They have the remarkable feature to accumulate at such areas where the membrane is most strongly bent and these are the invaginating septum at the site of cell division and the cell poles. Membrane binding is mediated via a unique dimeric lipid binding domain at the N-terminus that exposes two phenylalanine side chains to the solvent which insert into the hydrophobic phase of the phospholipid bilayer.

By the use of their C-terminal domains, DivIVA proteins are thought to recruit a number of binding partners to the septum and the poles that have various crucial functions in cell division, peptidoglycan biosynthesis or endospore formation. We decided to analyse the role of DivIVA in cell division and infectivity of the facultatively intracellular pathogen *Listeria monocytogenes* since cellular polarity has been reported to be important for its survival inside eukaryotic host tissues. We found that DivIVA is a crucial topogenetic factor required for the completion of cross wall formation at the site of cell division in *L. monocytogenes*. The severe morphological abnormities accompanying the loss of *divIVA* may explain why these cells are inable to swarm, severly impaired in biofilm formation at plastic surfaces and clearly attenuated in a cell culture infection assay. We can show that *L. monocytogenes* cells lacking *divIVA* are impaired in their ability to enter and to egress eukaryotic cells. Our results suggest that DivIVA proteins might represent a useful target structure for the development of new antibacterial drugs.

CBP012
Import and activation of the colicin M protein toxin requires the periplasmic FkpA prolyl *cis-trans* isomerase /chaperone in *E. coli*.

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Colicin M (Cma) is a protein toxin that is formed by *E. coli* strains that carry ColBM plasmids. It is imported into the periplasm of sensitive cells via a receptor-dependent energy-coupled process. It kills *E. coli* cells by inhibition of murein (peptidoglycan) precursor incorporation into the existing murein in that it cleaves the phosphate ester bond between the precursor and the lipid carrier that translocates the precursor across the cytoplasmic membrane. The resulting C_{55} polyisoprenol no longer enters the reaction cycle, murein synthesis stops and cells lyse. *E. coli* cells that synthesize Cma are protected by an immunity protein, Cmi, which in the periplasm inactivates Cma.
E. coli mutants which are resistant to Cma carry mutations in genes, *fhuA*, *tonB*, *exbB*, *exbD,* which are involved in Cma import from the outside into the periplasm. We recently found that an additional type of Cma resistant mutant carries a mutation in *fkpA* that encodes a periplasmic prolyl *cis-trans* isomerase (PPIase) / chaperone. Spontaneous *fkpA* deletion and point mutants in the PPIase domain are completely resistant to high titers (10^5) of Cma. The crystal structure of Cma reveals a compact form that must unfold during translocation across the outer membrane. It is assumed that this involves a *trans*-to-*cis* prolyl isomerisation of Cma that is converted back to *trans* upon refolding in the periplasm. Cma refolding is catalysed by FkpA. Regardless whether Cma is imported or secreted with a fused signal sequence into the periplasm, it requires FkpA to be active. To identify the residue that might be *cis-trans* isomerized, the 15 proline residues were individually replaced by alanine. The mutant Cma's were fully active except three which displayed 1% activity. Two of them are not imported. The one that remains inactive in the periplasm has a crystal structure identical to wild-type Cma which makes it unlikely that the mutation changes the phosphatase active center that is located far from the proline residue. It is proposed that the proline residue of the inactive imported mutant is targeted by FkpA.
Sequence and structure of the phosphatase domain of Cma is unique. The active center was therefore mapped by random and site-specific mutagenesis. The mutations center in a surface-exposed region. An aspartate residue was defined as a likely catalytic site since conversion to asparagine or glutamate abolishes Cma activity. The residues implicated in phosphatase catalysis are highly conserved in Cma-like proteins of other species than *E. coli*.

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CBP013
Does RAS-1 regulate adelylate cyclase activity?

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In *Neuropspora crassa*, conidiation is started when an aerated liquid culture is filtered and the resulting mycelial mat is exposed to air. Three morphogenetic transitions take place: hyphae adhesion, aerial hyphae growth and conidia development [1]. Each transition is started by an unstable hyperoxidant state and results in growth arrest, autophagy, antioxidant response and a dioxygen insulation process. These responses stabilize the system and, once stable, growth can start again [2,3].
In a solid medium the band mutant (*bd*) exhibits a conidiation band every 22 h [4] resulting from a Thr79Ile substitution in *ras-1* [5]. The same behavior is observed in a Δ*sod-1* mutant strain. In both strains, N-acetyl-cysteine suppresses the conidiation rhythm and paraquat shortens its period. Compared to Wt, *ras-1*^{bd} has increased ROS formation during conidiation resulting in increased aerial mycelium growth and increased submerged conidiation.
Our hypothesis is that RAS-1 acts as a switch between growth and conidiation in *N. crassa*. Only three proteins have a predicted RAS association domain: NRC-1, *STE50p* orthologue and adenylate cyclase (AC). A Δ*cr-1* mutant strain decreases grow of vegetative and aerial hyphae and increases conidia formation. Upon exposure to air, cAMP levels in a mycelial mat follow a similar pattern to protein oxidation, loss of NAD(P)(H)-reducing power and glutathione oxidation [6]. cAMP levels decrease during the hyperoxidant state, both at the start of hyphal adhesion and of aerial hyphae formation, and recover thereafter. AC and the low affinity phosphodiesterase (NCU00237) activity regulation explained cAMP decrease. However, during conidia formation, cAMP decrease was due to regulation of AC and the high affinity phosphodiesterase (NCU00478).

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CBP014
The complex assembly of the Actinobacterial Rieske protein

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Protein export and assembly is essential for the bacterial cell and is generally realized by two distinct operating translocases, called the Sec and Tat systems. Proteins are transported via the Sec pathway in an unfolded conformation. In contrast, proteins are transported through the Tat (twin arginine translocation) pathway in a folded state and are targeted to the Tat pathway by N-terminal signal peptides harboring consecutive, invariant arginine residues. One of the most important Tat-dependent membrane proteins is the Rieske protein, a fundamental component of the essential energy transduction cytochrome bc_1 complex in the respiratory chain of many bacteria. Usually the Rieske protein is composed of a single transmembrane helix at its N-terminus which is preceded by the Tat motif and followed by an iron-sulphur domain. However, in actinomycetes and other pathogenic relatives such as *mycobacteria* the Rieske protein has three transmembrane domains (TMD) prior to the iron-sulphur cluster. Interestingly and very unusually sequence alignment revealed an internal Tat motif preceding the third TMD, which suggests that the Tat system is required for the transport of the folded iron-sulphur domain across the membrane but probably not for the membrane insertion of the first two transmembrane helices. To investigate the assembly of the TMD of the Rieske protein into the cytoplasmic membrane, a reporter system has been used, whereby the iron-sulphur domain of the Rieske protein of *Streptomyces coelicolor* is replaced with maltose binding protein of *E. coli*. Thus, using different molecular biology and biochemical approaches we demonstrated that the assembly of this chimeric protein is dependent on the Tat pathway. But our data also implies that an additional protein insertion pathway co-operates with the Tat pathway in the assembly of the Rieske TMD.

CBP015
Profiling of SeqA binding to the *Escherichia coli* chromosome using an improved ChIP-Chip method

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The SeqA protein of ***Escherichia coli*** is involved in regulation of replication initiation and is also proposed to act in organization and segregation of daughter chromosomes (Waldminghaus and Skarstad, 2009). SeqA binds specifically to hemimethylated GATC sites that are produced during DNA-replication. The DNA remains hemimethylated until the Dam methyltransferase methylates the DNA fully. SeqA dependent regulation of replication initiation is based on its binding to recently replicated origins of replication. This prevents re-initiation of the new origins and also protects the origins from remethylation by Dam. While the role of SeqA in regulation of replication initiation has been investigated in detail its role in chromosome organization and segregation is poorly understood. We applied a published method of chromatin immunoprecipitation combined with microarrays (ChIP-Chip) to analyze binding of SeqA to the 20.000 GATC sites found on the ***E. coli*** chromosome. Faced with a background signal exceeding the specific signal we reinvestigated the procedure and were able to reduce the background significantly by modifying the protocol (Waldminghaus and Skarstad, 2010). The new protocol allowed us to profile chromosome wide SeqA binding.

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CBP016
Synthetic reconstruction of the chromosome partitioning system from *Corynebacterium glutamicum*

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Corynebacterium glutamicum is a Gram-positive, MreB-lacking and non-sporulating model organism with high industrial and medical relevance.
Compared to other organisms, such as *Escherichia coli* or *Bacillus subtilis*, the cell division machinery in *C. glutamicum* looks much simpler, e.g. proteins for spatial restriction of the divisome such as the Min system and nucleoid occlusion proteins are missing. Further, actin homologues like MreB are not encoded, implicating, that cell wall synthesis for cell elongation is not governed by an actin-like cytoskeleton. Recent data suggest that a corynebacterial homologue of the polar determinant DivIVA is responsible for governing of apical growth. We found evidence, that DivIVA-like proteins in corynebacteria may even be involved in chromosome orientation with the cell via interaction with the ParAB partitioning system, thereby coupling chromosome segregation and cell wall synthesis.
In order to examine the *in vitro* interaction behaviour of partitioning proteins of *C. glutamicum* an expression and purification protocol for DivIVA, ParB and ParA was established. Furthermore, sedimentation experiments for membrane interaction were carried out to establish a synthetic *in vitro* assay for reconstruction of the chromosomal segregation machinery. We could show that DivIVA likely binds to lipids in a dimeric form. Titration of the partitioning proteins ParAB to the assay revealed that ParB binds to DivIVA *in vitro*. Binding of ParA to the complex depends on a pre-existing DivIVA-ParB complex. We will exploid this *in vitro* setup to unravel the molecular mechanism of chromosome tethering.

CBP017
Analysis of functional membrane microdomains in bacteria

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The view on plasma membranes has changed dramatically during the last years. Initially it was proposed that membranes are a homogeneous mixture of lipids with embedded proteins. Though, during the last years it could be shown that plasma membranes contain a high degree of lateral organisation. Specialised regions containing different protein and lipid patches were identified, termed microdomains or lipid rafts. In eukaryotic cells lipid rafts are characterised by a high content of cholesterol, glycosphingolipids and characteristic raft associated proteins such as flotillins and GPI anchored proteins. In contrast lipid rafts in bacterial cell membranes are hardly understood.
Previously, we have described a bacterial flotillin, YuaG that is involved in the signalling pathway which leads to Spo0A phosphorylation. YuaG localises in discrete foci in the membrane and these foci are highly dynamic. Purification of detergent resistant membranes (DRM) revealed that YuaG is firmly associated with negatively charged phospholipids. Here we have used a YuaG-SNAP construct to isolate proteins that are associated with YuaG in bacterial membrane microdomains. One of the identified proteins is YqfA, which also has a flotillin like structure and might be part of a hetero-oligomeric complex of flotillins that are scaffolding the lipid microdomains.

CBP018
SMC shows high condensin-like DNA binding dynamics in *Bacillus subtilis* cells

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Chromosomes must be compacted to fit into the bacterial cell, and the topology of DNA must be regulated to allow efficient transcription and replication to go on. The compaction of the DNA is regulated by a number of proteins including histones, histone-like proteins, topoisomerases and the SMC (structural maintenance of chromosomes) complex.
The *Bacillus subtilis* SMC complex consists of an SMC dimer, an ATPase with ABC-transporter related head domains, and a subcomplex of the two accessory proteins ScpA and ScpB. This complex is essential for chromosome segregation and condensation. Deletion causes severe defects in chromosome organization, whereas overexpression of SMC in *Bacillus subtilis* leads to an excessive overcondensation of the nucleoid.
The SMC complex localizes cell cycle dependent in a bipolar manner to discrete centers on the nucleoid. The mode of formation and function of these Centers is unclear. We observed in Fluorescence Recovery after Photobleaching (FRAP) experiments of SMC-GFP, ScpB-GFP and ScpA-YFP (performed in exponentially growing cells) rapid recovery of the foci within few minutes. This shows that there is ongoing exchange of the SMC complexes between bound and unbound molecules, and in between the centers. Thus the SMC complex binds transiently and highly dynamically to DNA. We provide evidence that these dynamics depend, to a considerable degree, on *de novo* protein synthesis. These findings have important implications on the mode of DNA compaction through the SMC complex.
We further created a dominant negative point mutant in the head domain of SMC, which shows severe effects in chromosome segregation, and disordered formation of the discrete Centers. This mutant will allow us to revealing if dynamic binding is important for the function of the SMC complex.

CBP019
On the role of a new member of the CDK9 kinase family in *Aspergillus nidulans*

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Cyclin dependent kinases (CDKs) are a large group of protein kinases which are regulated by association with cyclins. Members of the Cdk9 family have been described from yeast to human and are known to be part of the basal transcription elongation machinery. Their regulatory subunits are different cyclins (cyclin T1, T2a, T2b and K), which do not oscillate during the cell-cycle.
In *A. nidulans* the cyclin PclA has been characterized as a cyclin involved in development. PclA interacts with the main regulator of the cell cycle, NimX and may help to adjust the cell cycle during asexual sporulation [1]. In a targeted approach it was found that PclA also interacts with another kinase, a Cdk9 family member (PtkA) [2]. Deletion of the *ptkA* gene causes a lethal defect and the mutant arrests in a short germling state. PtkA localizes to nuclei during interphase. PtkA does also interact with a cyclin T (PchA) as it does in other organsims, suggesting a conserved role in transcription regulation.
Performing Y2H screens with PtkA, we identified two more interaction partners, one protein kinase and surprisingly, another Pcl cyclin. These interactions occurred most interestingly only in metulae and phialides and are thus restricted to asexual development. These results point to the

possibility that the transcription elongation machinery is specifically modified during asexual development.

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CBP020

Will not be presented!

CBP021

Subcellular localization of Sortase A in staphylococci

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Cell wall anchored surface proteins play important roles in the pathogenicity of *Staphylococcus aureus*. While the biochemical process of anchoring surface proteins by Sortase A (SrtA) in *S. aureus* has been studied in detail, the spatial and temporal knowledge is largely missing. By anchoring red fluorescent protein Mcherry to the peptidoglycan (Mch-cw) as a model system for localization studies, we found that Mch-cw strongly accumulated at crosswall (septum) when *S. aureus* was treated with cell wall biosynthesis antibiotics, such as moenomycin or penicillin. The accumulation was abolished in *S. aureus ΔsrtA*. Second, in a *S. aureus ΔtagO* mutant that lacks wall teichoic acid, both the presentation of Mch-cw to cell surface and cell division are greatly delayed. A Sortase-GFP fusion showed that Sortase A was predominantly localized at the septum with a few foci localized at the sidewall in *S. aureus* wild type. However, these data were provided by plasmid-based fusion proteins that need to be verified by immunofluorescent microscopy study. Further, we seek to understand the localization of Sortase A in the presence of cell wall biosynthesis antibiotics as well as in *S. aureus ΔtagO.* Our data suggested that anchoring of surface proteins to cell wall is closely connected with cell division and occurs mainly at the crosswall.

CBP022

Bactofilins: a new class of cytoskeletal proteins

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The cytoskeleton plays a key role in the temporal and spatial organization of both prokaryotic and eukaryotic cells. Moreover, the principal set-up of these scaffolding proteins shows striking similarities in both branches, including nucleotide cofactor-dependent and -independent components. Here, we report the identification of a new class of polymer-forming proteins, termed bactofilins, that are widely conserved among bacteria. In *Caulobacter crescentus*, two bactofilin paralogues cooperate to form a sheet-like structure lining the cytoplasmic membrane in proximity of the stalked cell pole. These assemblies mediate polar localization of a peptidoglycan synthase involved in stalk morphogenesis, thus complementing the function of the actin-like cytoskeleton and the cell division machinery in the regulation of cell wall biogenesis. In other bacteria, bactofilins can establish rod-shaped filaments or associate with the cell division apparatus, indicating considerable structural and functional flexibility. Bactofilins polymerize spontaneously in the absence of additional cofactors in vitro, forming stable ribbon- or rod-like filament bundles. Our results suggest that these structures have evolved as an alternative to intermediate filaments, serving as versatile molecular scaffolds in a variety of cellular pathways.

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CBP023

Helicobacter pylori posseses four coiled coil rich proteins (Ccrp) that affect cell shape and form extended filamentous structures

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Pathogenicity of the human pathogen *Helicobacter pylori* relies upon its capacity to adapt to a hostile environment and to escape the host response. Therefore, the shape, motility, and pH homeostasis of these bacteria are specifically adapted to the gastric mucus. Recently, we have shown that the helical shape of *H. pylori* depends on two coiled coil rich proteins (Ccrp), which form extended filamentous structures and are required for the maintenance of cell morphology to different extents. Next to the genes coding for Ccrp59 and Ccrp1143 proteins, we have found that *H. pylori* possesses two additional genes potentially encoding Ccrp proteins. Indeed, Ccrp58 and Ccrp1142 also have an impact on cell morphology indicating a complex system for maintenance of cell shape of this human pathogen. Likewise both new identified proteins build up filamentous structures *in vitro.* Interestingly, although all Ccrp mutants posses a normal flagella formation, the strains displayed a reduced motility. All four Ccrps have different multimerization and filamentation properties suggesting a system of individual filaments. Thus, *H. pylori* cells express four Ccrp-proteins that differentially affect cell morphology and have somewhat different biochemical properties, suggesting that helical cell shape is established through a complex network of individual cytoskeletal components.

CBP024

Localization pattern of a Gram positive conjugation machinery

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Conjugation is an efficient way for the transfer of genetic information between bacteria, even between highly diverged species, and a major cause for the spreading of resistance genes. We have investigated the subcellular localization of several conserved conjugation proteins encoded on plasmid pLS20 found in *Bacillus subtilis*. We show that VirB1, VirB4, VirB11 and VirD4 homologs assemble at a single cell pole, but also at other sites along the cell membrane, in cells during lag phase of growth. SSB-like SsbC protein also localizes to the cell pole, but when overproduced lowers conjugation efficiency, indicating that SsbC is also part of the conjugation machinery, but must be present in moderate amounts. BiFC analyses show that VirB4 and VirD4 interact at the cell pole and, less frequently, at other sites along the membrane, suggesting that this is a preferred site for the assembly of an active conjugation apparatus, but not the sole site. TIRF microscopy shows that pLS20 is largely membrane-associated, and is frequently found at the cell pole, indicating that transfer takes place at the pole. All analysed conjugative proteins localize to the pole or the membrane in stationary phase cells and in cells that have been resuspended in fresh medium, but no longer in cells that enter exponential growth, although at least VirB4 is synthesized at equal level. These data reveal an unusual assembly/disassembly timing for the pLS20 conjugation machinery and suggest that specific localization of conjugation proteins in non-growing cells and delocalization during growth are the reason why pLS20 conjugation only occurs during early exponential (lag) phase.

CBP025

Dynamic range in bacterial chemotaxis

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Most motile bacteria are able to follow chemical gradients in its environment through a mechanism called chemotaxis. Bacterial chemotaxis

relies on sensing temporal changes in concentrations of chemoeffectors while bacterial cell is swimming in the gradient. Dependent on the sensory input, bacteria regulate their swimming duration by changing the rotational direction of the flagellar motor, whereby an increase in positive (attractant) stimulus when swimming up the gradient increases run duration in this direction.
The receptor signal is transduced to the flagellar motor by a receptor-associated cytoplasmic histidine kinase CheA and a response regulator CheY. In addition, the chemotaxis signalling pathway contains an adaptation system which adjusts the activity and sensitivity of the sensory complexes by receptor methylation on four specific glutamate residues. The reactions of receptor methylation and demethylation are mediated by two enzymes, the methyltransferase CheR and the methylesterase CheB, respectively. The adaptation system is necessary to ensure ligand sensing over large dynamic concentration range and therefore to enable cells to follow attractant gradients from very low to very high concentrations.
Here we investigate the mechanisms that ensure broad dynamic range of the chemotaxis system, as well as physiological factors that limit this range. By applying a FRET-based reporter of the intracellular pathway activity, we show how methylation on multiple sites extends dynamic range over many orders of magnitude. We also observe that dynamic range becomes limited by saturation of methylation sites, with different concentration limits observed for different chemoeffectors. Further experiments revealed a correlation between dynamic range of the chemotaxis system and growth inhibition of cells by high concentrations of respective chemoeffectors, suggesting that the dynamic range of the chemotaxis system has been evolutionary tuned to physiologically beneficial ligand concentrations.

CBP026
Isolation of a prokaryotic cell organelle from the uniquely compartmentalized anammox bacteria

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The bacteria capable of anaerobically oxidizing ammonium (anammox) have been discovered only quite recently [1]. Since then their significance for the global nitrogen cycle has become apparent due to their large contribution to the oceanic nitrogen loss [2] and they are already applied for the removal of ammonium from municipal wastewater. Like other members of the phylum Planctomycetes, anammox bacteria exhibit a cell compartmentalization that is otherwise unique for prokaryotes [3]. The cells are subdivided into three compartments. The outermost compartment is the paryphoplasm and has an unknown function, but is presumably not analogous to the periplasmic space in Gram-negative bacteria. It is separated by an intracytoplasmic membrane from the riboplasm, which harbors the RNA as well as DNA of the cell. The innermost compartment is the anammoxosome and is hypothesized to be the side of catabolism and energy generation, analogous to eukaryotic mitochondria [4-5]. Isolation of this prokaryotic cell organelle from the anammox bacterium *Kuenenia stuttgartiensis* was attempted by various physical and chemical disruption techniques and led to separation of two subcellular fractions by Percoll density centrifugation. These were investigated with immunofluorescence microscopy and transmission electron microscopy for their outer appearance, DNA content and hybridization with an antibody targeting the anammoxosome. Future studies will include organelle proteomics and activity assays.

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CBP027
Interaction of Lipid II-binding lantibiotics with the wall teichoic acid precursors Lipid III and Lipid IV

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Lantibiotics are a unique group within the antimicrobial peptides characterized by the presence of thioether amino acids (lanthionine, methyllanthionines). These peptides are produced by and primarily act on Gram-positive bacteria and exert multiple activities at the cytoplasmic membrane of susceptible bacteria [1]. Recently the cell wall precursor lipid II was identified as a specific target for the prototype lantibiotic nisin. Nisin binds to lipid II, thereby inhibiting cell wall biosynthesis [2].
Besides its interaction with the peptidoglycan precursors lipid I and lipid II, we show that nisin also interacts with sugar lipids involved in the synthesis of wall teichoic acid, i.e. lipid III (C55-PP-GlcNAc) and lipid IV (C55-PP-GlcNAc-ManNAc). This specific interaction with wall teichoic acid precursors further resulted in a target-mediated pore formation, as has recently been shown for lipid II [3].
We also show that nisin forms a complex with the various C55P-bound precursors at a stoichiometry of 2:1 (nisin: lipid). Studies with selected lantibiotics of the nisin sub-group, all containing the conserved lipid II - binding motif, e.g. gallidermin also showed an interaction with Lipid III and Lipid IV.

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CBP028
In vitro and *in vivo* site-directed mutational analysis of DnaA in *Bacillus subtilis* - aspects of its functionality in the initiation of replication

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The initiator protein of chromosomal replication, DnaA, and its regulation have intensively been studied in *Escherichia coli*, a model organism of Gram negative bacteria. A variety of functional capacities, such as ATP-binding and hydrolysis, oligomerization and specific DNA binding, have been discovered and led to a model of the underlying mechanism. Because the process of initiation of chromosomal replication seems to work differently in Gram positive bacteria, we investigated these capacities and their implication in initiation in *Bacillus subtilis.* We created several *B. subtilis* DnaA mutants by exchange of highly conserved amino acids that have previously been reported for *E. coli* to be involved in the activities mentioned above. Comparative fluorescence microscopy studies of wildtype and mutant DnaA revealed strong phenotypic effects in the frequency of initiation of replication, on DNA compaction, chromosomal segregation, septum formation and cell length, which are different from those phenotypes observed in *E. coli*. Surface Plasmon Resonance experiments display a specific binding affinity and binding stability to DnaA-box containing DNA for each of the mutant DnaA forms, which correspond to the observed phenotypes *in vivo*. Taken together, our results suggest a novel model for how DnaA initiates chromosomal replication in *Bacillus subtilis*.
Funding: DFG (Deutsche Forschungsgemeinschaft)

CBP029
RodA influences the sites of incorporation of new cell wall material in *Bacillus subtilis* and colocalizes with MreB and Mbl

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RodA is a widely conserved bacterial protein implicated in the maintenance of rod cell shape. We show that a functional GFP-RodA fusion largely colocalizes with the MreB cytoskeleton at the lateral cell membrane in

Bacillus subtilis. BiFC experiments show that RodA interacts with MreB, and with Mbl, the second MreB ortholog. Mbl in turn interacts with the membrane proteins MreC and MreD. Because RodA largely colocalizes with YFP-Mbl, our results indicate that MreB, Mbl, RodA, MreC and MreD form a large morphogenetic complex at and within the membrane. TIRF microscopy revealed highly dynamic localization kinetics of YFP-RodA foci along random paths. Contrarily, YFP-MreB filaments remodelled along regular helical paths, showing that RodA molecules diffuse between helical MreB filaments, but are not statically anchored. Consistent with this, RodA showed diffusion-type kinetics in FRAP experiments. RodA also colocalized with fluorescently labelled vancomycin (Van-Fl) that marks sites of new cell wall synthesis. A partially functional RodA-mCherry fusion mislocalized to large clusters at irregular positions along the lateral cell wall, and concomitantly changed the regular positioning of cell wall synthesis, as well as cell shape, showing that the positioning of RodA influences the localization of new cell wall material and thereby cell morphology.

CBP030

Will not be presented!

CBP031

The scaffold protein Iqg1 plays an essential role during cytokinesis in *Ustilago maydis*

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Cytokinesis is the process by which cells physically separate after duplication and spatial segregation of the genetic material. During cytokinesis in *Ustilago maydis* two distinct septa were formed. Formation of each septa is initiated by the assembly of an CAR. This dynamic structure consists of many different components for example F-actin, the myosin light chain Cdc4 and the FCH-protein Cdc15. From other organisms it is known, that the IQGAP-proteins are also important components of this structure.
To analyze the assembly of the actomysoin ring in *U. maydis*, we have characterized the homologous IQGAP protein Iqg1 (um10730) by genetic, cell biological and biochemical approaches. We will show that Iqg1 is an essential gene in *U. maydis* for haploid growth. In colocalisation studies of Iqg1 with F-actin, Cdc4 and Cdc15 Iqg1 was identified as an actomyosin ring component.
From other studies in our lab it is known, that the GTPase Cdc42 is a key player in assembly of the second actomyosin ring. Deletion mutants of cdc42 display a cytokinesis defect and cannot build the second actomyosin ring. Interestingly, a similar phenotype was observed using mutants with a C-terminal GFP fusion of Iqg1. Using in vitro interaction assays, we could show that the Ras GTPase-activating protein related domain (GRD) of Iqg1 bind to Cdc42. We propose that Iqg1 is an effector of Cdc42 during the assembly of the second actomyosin ring.

CBP032

Interactions between PTS transporters and the chemotaxis system in *Escherichia coli*

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The phosphotransferase system (PTS) and the chemotaxis pathway of *E. coli*, which mediate uptake of and taxis towards carbohydrates respectively, are genetically and biochemically among the best studied bacterial systems. The crosstalk between both pathways is known to be important for taxis to PTS substrates, providing one of few known examples of direct interaction between nutrient transport and signalling. While signal processing by the core of the chemotaxis pathway itself is largely understood, the mechanisms of PTS-mediated taxis, which results from concomitant perception of substrates during their uptake, are largely unclear.
Here, we investigate *in vivo* the interconnection among the PTS transport and taxis on the example of glucose, the preferred carbon source of *E. coli*. Our experiments showed that taxis towards low concentrations of glucose is mediated by membrane receptors, whereas taxis in the high concentration range requires glucose uptake through PTS transporters. Using intracellular pathway activity assay based on fluorescence resonance energy transfer (FRET), we demonstrated several intracellular interactions between PTS and chemotaxis proteins. Moreover, we quantitatively analyzed relative contributions of the receptor-mediated and the PTS taxis towards glucose. We further found that adaptation in the PTS-mediated taxis depends on the receptor methylation enzymes, suggesting that PTS-mediated signals affect receptor activity. We propose a new model of signal exchange between both systems that unifies two types of chemotaxis.

CBP033

Deletion of the *mamXY* operon affects biomineralization and chain alignment in *Magnetospirillum gryphiswaldense*

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Magnetospirillum gryphiswaldense employs magnetotaxis to find favorable environments in freshwater sediments by reducing the complex navigation in three dimensions to only one.
Magnetotaxis is mediated by membrane-enveloped magnetite crystals called magnetosomes, which are arranged into a chain to provide a magnetic dipole moment that passively aligns the cell to the earth's magnetic field. Magnetosomes develop by invagination of the cytoplasmic membrane followed by magnetite biomineralization.
In *M. gryphiswaldense* almost all characterized genes that are involved in regulation of this complex process are clustered within a genomic magnetosome island. Within this island four putative operons have been identified which are either essential for magnetosome formation or involved in control of size and magnetic properties of the magnetite crystals. One of these operons is the *mamXY* operon, consisting of *mamY*, *mamX*, *mamH-like* and intriguingly of *ftsZm*, a gene coding for a homolog of the FtsZ protein. Preliminary data from a *mamXY* operon deletion mutant suggest that this operon has an essential role in biomineralization as well as in magnetosome chain formation. Mutant cells display aberrant membrane vesicles. In addition to wildtype like magnetosomes, the mutant cells also contain heterogeneous polycrystalline magnetite crystals and tiny crystal flakes which potentially do not consist of magnetite but rather of different iron oxides. This is indicating, that not only crystal size and shape are affected but also iron oxide composition itself is altered.
To analyze the role of individual genes within the *mamXY* operon in detail, single gene deletion mutants are currently generated by a cre-*lox* based method. Data will be presented showing the effects of loss of these genes on the formation of magnetite crystals, vesicles and the structure of the magnetosome chain.

CBP034

Simkania negevensis replicates in the host endoplasmic reticulum

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Simkania negevensis is a Chlamydia-like emerging pathogen implicated in pulmonary diseases. Knowledge regarding its intracellular accommodation remains sparse. Questions: How is the replicative vacuole of the obligate intracellular bacterium S. negevensis organized and where is it located within the host cell. Results: We show that expansion of the S. negevensis vacuole within epithelial cells is accompanied by massive spatial reorganization of host mitochondria and endoplasmic reticulum (ER). Spatial reorganization was mitochondria- and ER- specific as the Golgi apparatus appeared intact and was positioned between the nucleus and the S. negevensis vacuole. Ultrastructural analysis and 3D reconstruction revealed that S. negevensis forms one large vacuole located within the ER lumen. Location of the vacuole within the ER led to the formation of a so far not described pathogen-containing triple membrane surrounded structure. Conclusion: Like Chlamydia, Simkania is entering and building a pathogen containing vacuole within the host cell. Interestingly, the vacuoles show an unusual location within the cell. While the Simkania vacuole is located within the ER and thus pre-Golgi, the Chlamydia vacuole is a post golgi structure. Comparison of the Chlamydia and Simkania vacuole might shed light on intracellular trafficking and vacuole accommodation within the Chlamydiae.

CBP035
Export the unexpected. A novel periplasmic targeting signal

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Proteins destined for the periplasm are targeted to the Sec and TAT export machineries via hydrophobic N-terminal signal peptides, which are usually cleaved after export. These signal peptides are readily recognisable, a fact that is exploited by algorithms for the prediction of the periplasmic proteome. Although the Fe/Mn superoxide dismutase (SodA) of *Rhizobium leguminosarum* is exported to the periplasm, it does not carry a recognised signal peptide. Instead, the N-terminus of SodA is highly hydrophilic and bears no resemblance to classical signal peptides, and it remains uncleaved after export in both *R. leguminosarum* and *Escherichia coli*. The export of SodA is unaffected in *tatC* and *secB* mutants, but is diminished in a temperature-sensitive SecA mutant. We therefore propose that SodA export, although Sec-dependent, utilises a previously unknown targeting mechanism that is distinct from classical periplasmic targeting. Sequence scanning analysis revealed that a 10-amino acid sequence within SodA was sufficient to target a reporter protein to the periplasm, and mutational analysis of this sequence determined the conserved residues involved in efficient periplasmic targeting.
Our results demonstrate a novel SecB- and (classical) signal peptide-independent pathway for targeting proteins to the periplasm. The targeting mechanism may be widespread, as export of SodA to the periplasm was also observed in other proteobacteria. The novel consensus motif is not recognised by the current algorithms for predicting signal peptides, and proteins carrying it are missing from the predicted periplasmic proteomes. As proteins such as SodA play active roles in processes such as pathogenesis, these findings have wider implications for the study of periplasmic targeting and its role in virulence and bacterial physiology in general.

CBP036
Metabolic changes in the murine macrophage-like tumor cell line J774A.1 after stimulation with Lipopolysaccharide from *E. coli*

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The murine macrophage-like tumor cell line J774A.1 is used in numerous studies like in vitro infections or macrophage-activation experiments. Since the metabolism of the macrophage plays a central role in these cellular processes we investigated the central metabolism by a comprehensive metabolomic approach. Uptake and secretion of intermediates where monitored by extracellular metabolomics using ^{1}H-NMR. Investigations on the intracellular metabolome level were performed by GC-MS and LC-MS. Since it is known that the metabolism of macrophages is affected by stimuli like pathogen-associated molecular patterns (PAMPS), we compared the metabolome data of non-stimulated cells with cells stimulated with lipopolysaccharide (LPS) from *E. coli*. In this study, we show that LPS affects central metabolic pathways like glycolysis, glutaminolysis and the TCA-cycle.

CBP037
Structural Investigation and Mechanism of bifunctional Fructose-1,6-bisphosphate aldolase/phosphatase from *Thermoproteus neutrophilus*

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The fructose 1,6-bisphosphate (FBP) aldolase/phosphatase is a bifunctional enzyme with both aldolase and phosphatase activities. It is found in most archaeal groups and deeply branching bacterial lineages harbor thermophilic organisms [1]. As an essential gluconeogenic enzyme, it catalyses irreversible aldol condensation of heat-labile dihydroxyaceton phosphate (DHAP) and glyceraldehyde phosphate (GAP) to FBP, and also catalyses the hydrolysis of FBP to stabile Fructose 6-phosphate (F6P) and inorganic phosphate (Pi).
In order to understand the mechanism of this bifunctional reaction, we investigated the structure of FBP aldolase/phosphatase in *Thermoproteus neutrophilus* by X-ray crystallography, activity tests, mass spectrometry and other biochemical methods. We solved the structures of this FBP aldolase/phosphatase (apo) and its complexes with DHAP, FBP and F6P at up to 1.3 Å resolution, the FBP Aldolase/Phosphatase of *Sulfolobus tokodaii* structure was used as the initial search model [2]. These high resolution structures depict large conformational changes in distinct loops surrounding the active center. Supported by mutational studies and mass spectrometry, these conformational changes suggest a distinct mechanism in aldolase and phosphatase reactions. These flexible loops act as a switch between aldolase and phosphatase activities.

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CBP038
Role of chemo- and aerotaxis in magnetotactic behaviour of *Magnetospirillum gryphiswaldense*

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Magnetotactic bacteria (MTB) contain a chain of magnetic particles that imparts a net magnetic moment to the cells. Passive alignment with the Earth's magnetic field is believed to increase the efficiency of chemotactic behaviour by reducing the complexity of a three-dimensional search problem in chemically stratified habitats. However, the precise characteristics of this behaviour as well as its interaction with chemotactic mechanisms have remained unknown.
The swimming direction in various mostly uncultivated MTB has been reported to be set by an internal magnetic field polarity that causes cells to move in one direction with respect to the ambient magnetic field until reaching conditions that trigger motion reversal. Although *M. gryphiswaldense* wild-type cells cultivated under standard conditions show no such bias, we found that magnetic swimming polarity can be selected by serial cultivation in strong magnetic fields, yielding distinct populations of N- or S-seeking bacteria. In addition, preliminary experiments indicated that aerotaxis is the main chemotactic behaviour in *M. gryphiswaldense*.
In order to determine molecular determinants of chemo- and aerotaxis, we performed a genome-wide homology search in *M. gryphiswaldense*. Four putative operons containing canonical chemotaxis genes *cheAWYBR* as well as Methyl-accepting Chemotaxis Proteins (MCPs) and further uncharacterised genes were identified. Furthermore, we found that *M. gryphiswaldense* possesses an unusually high number of chemotaxis signal transducers (≥ 50, compared to 5 in *E. coli*), a large fraction of which was found expressed by proteomic analysis. Amomg them, we identified three putative aerotaxis transducers sharing homology with the *E. coli* protein Aer.
The role that putative chemotaxis operons and aerotaxis transducers play in magnetotaxis and magnetic swimming polarity is currently being investigated by constructing single and multiple deletion mutants of all identified chemotaxis operons and selected signal tranducers.

CBP039
Crystal structure of the colicin M immunity protein

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Colicins are bacterial protein toxins produced by half of *E. coli* natural isolates that kill sensitive *E. coli* cells. Colicin M (Cma) inhibits incorporation of murein precursors into murein. Cma producer cells are protected by co-synthesis of an immunity protein, Cmi, that is located at the Cma target site in the periplasm and anchored to the cytoplasmic membrane by an N-terminal hydrophobic sequence [1]. We resumed our previous studies on Cma and Cmi after we had discovered that Cma activity requires the periplasmic FkpA prolyl *cis-trans* isomerase /chaperone [2].
Since the hydrophobic sequence is not essential for Cmi activity [1], crystallization was performed with a soluble Cmi that lacked the N-terminus. Cmi crystals were obtained under several conditions but only one single crystal diffracted to a resolution of 1.95 Å. By using the recently

published software package ARCIMBOLDO [3], we succeeded to solve the structure by this *de novo* approach (Dayté Rodriguez, Isabel Usón-Finkenzeller, Instituto di Biología Molecular de Barcelona, Barcelona, Spain). In the crystal Cmi forms a dimer that is interlinked by a disulfide bridge. It is a highly charged protein with a surplus of negative charges presumably responsible for interaction with Cma which contains a cluster of positive charges.

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CBP040
Dissecting the role of the seven chitin synthases of *Neurospora crassa* in apical growth and septum formation

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Fungal chitin synthases (CHS) are grouped into seven classes, four of them, III, V, VI and VII being exclusive of filamentous fungi. CHS classes V and VII have a myosin-like motor domain (MMD) at their amino terminus. Previous studies in *Neurospora crassa* showed that CHS-1, CHS-3, and CHS-6 tagged with GFP or mCherry accumulated at the core of the Spk, and also at nascent septa. We endogenously tagged with *gfp* the remaining chitin synthases genes, namely *chs*-2 (NCU05239.3), *chs*-4 (NCU09324.3), *chs*-5 (NCU04352.3) and *chs*-7 (NCU04350.3) to study their distribution in living hyphae of *N. crassa*. CHS-2, CHS-4, and CHS-7, appeared solely involved in septum formation. As the septum ring developed, CHS-2-GFP moved centripetally until it localized exclusively around the septal pore. CHS-5 was localized both at nascent septa and in the core of the Spk. We observed a partial colocalization of CHS-1-mCherry and CHS-5-GFP in the Spk. Total internal reflection fluorescence microscope (TIRFM) analysis revealed putative chitosomes containing CHS-5-GFP moving along wavy tracks. Collectively our results suggest that there are different populations of chitosomes, each containing a class of CHS. Mutants with single gene deletions of *chs*-1, *chs*-3, *chs*-5, *chs*-6, or chs-7 grew slightly slower than the parental strain (FGSC#9718); only Δchs-6 displayed a marked reduction in growth. Both Δ*chs*-5 and Δ*chs*-7 strains produced less aerial hyphae and conidia. Currently, we are analyzing CHS activity and chitin content in all the Knock Out mutant strains to determine the relative importance of each CHS in cell wall biosynthesis.

CBP041
Neurosporacrassa class III chitin synthase 1 (CHS-1): subcellular distribution, vesicular trafficking and cytoskeleton associations

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Apical growth in filamentous fungi is attained through different coordinated cellular mechanisms that include cell polarity establishment and maintenance. For cell wall expansion to occur at apical regions of growing hyphae, the orchestrated delivery of enzymes involved in carbohydrate synthesis is extremely important. In this study,by using different live imaging techniques, we found that chitin synthase 1 (CHS-1), one of the seven putative chitin synthase of *Neurosporacrassa*, localizes at the Spitzenkörper (Spk) core, the apical cell surface and transiently to constricting rings during septum development. Hyphae of heterokaryon strains expressing CHS-1-GFP and CHS-3-GFP or CHS-6-GFP, exhibited partial colocalization of the three different chitin synthases, suggesting that each CHS is contained in distinct chitosomal compartments. Total Internal Reflection Microscopy (TIRFM) allowed us to observe the anterograde and retrograde traffic of rapidly moving CHS-1-GFP vesicles, some of them converging at the Spk. The differential localization of CHS-1-GFP and GS-1-mChFP at the Spk, is the first evidence in living hyphaethat both proteins are contained in different populations of vesicles, as predicted from earlier transmission electron micrographs. Using cytoskeleton inhibitors, we concluded that microtubules are not essential for CHS-1 delivery to the Spk or nascent septa, whereas actin is necessary for the correct accumulation of CHS-1 to the Spk.

CBP042
The morphogene AmiC2 is pivotal for multicellular development in the cyanobacterium *Nostoc punctiforme*

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Filamentous cyanobacteria of the order Nostocales are primordial multicellular organisms, a property widely considered unique to eukaryotes. Their filaments are composed of hundreds of mutually dependent vegetative cells and regularly spaced N_2-fixing heterocysts, exchanging metabolites and signaling molecules. Furthermore, they may differentiate specialized spore-like cells and motile filaments. However, the structural basis for cellular communication within the filament remained elusive. Here we present that mutation of a single gene, encoding cell-wall amidase AmiC2, completely changes the morphology and abrogates cell differentiation and intercellular communication. Ultrastructural analysis revealed for the first time a contiguous peptidoglycan sacculus with individual cells connected by a single-layered septal cross-wall. The mutant forms irregular clusters of twisted cells connected by aberrant septa. Rapid intercellular molecule exchange takes place in wild-type filaments, but is completely abolished in the mutant, and this blockage obstructs any cell-differentiation, indicating a fundamental importance of intercellular communication for cell-differentiation in Nostoc. AmiC2-GFP localizes in the cell wall with a focus in the cross walls of dividing cells, implying that AmiC2 processes the newly synthesized septum into a functional cell-cell communication structure during cell division. AmiC2 thus can be considered as a novel morphogene required for cell-cell communication, cellular development and multicellularity.

EMV001
Anaerobic formate- and CO_2-assimilating prokaryotic taxa in a methane-emitting fen soil

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Natural wetlands such as fens and bogs contribute up to approximately 40% of the global biogenic emission of methane. Biopolymers in wetland soils are anaerobically degraded via intermediary events that terminate in the emission of methane (i.e., 'intermediary ecosystem metabolism'). Formate and CO_2 (together with H_2) are precursors of methanogenesis and have been observed to stimulate methanogenesis in anoxic microcosms of soil from the regional fen Schlöppnerbrunnen. However, active formate- and CO_2-utilizing methanogens of the fen remain unresolved. Active methanogens in anoxic fen soil microcosms were evaluated by stable isotobe probing of *mcrA*/*mrtA* (encode for the alpha-subunit of methyl-CoM reductases I and II) and archaeal 16S rRNA genes. Bacterial taxa were also evaluated. Anoxic fen soil microcosms were incubated in the dark and periodically pulsed with low concentrations of either [^{13}C]-formate or $^{13}CO_2$. The production of methane was stimulated by formate and CO_2; in contrast, only formate stimulated acetogenesis. 411 *mcrA*/*mrtA* sequences and 306 archaeal 16S rRNA gene sequences were analyzed. 12 family-level 16S rRNA archaeal genotypes were detected, 7 of which had no isolated cultured representatives. *Methanocellaceae* and *Methanobacteriaceae* were mainly labeled by [^{13}C]-formate, whereas *Methanosarcinaceae* were mainly labeled by $^{13}CO_2$, suggesting that formate-linked methanogenesis was mostly catalyzed by fen soil-derived *Methanocellaceae* and *Methanobacteriaceae*, whereas CO_2-linked methanogenesis was mostly catalyzed by fen soil-derived *Methanosarcinaceae*. In total, 58 bacterial 16S rRNA family-level genotypes and 15 species-level *fhs* (encodes for formyltetrahydrofolate synthetase) genotypes were detected, of which 29 of the bacterial 16S rRNA genotypes and all 15 *fhs* genotypes were defined as novel. Two of the *fhs* genotypes were affiliated with the acetogenic genera *Sporomusa* and *Moorella*. The collective results reinforce the likelihood that

Methanocellaceae-, *Methanobacteriaceae*-, and *Methanosarcinaceae*-related taxa are integrated to the 'intermediary ecosystem metabolism' and the emission of methane in the fen Schlöppnerbrunnen.

EMV002
LOHAFEX - Investigation of the bacterial community in an iron fertilization experiment

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According to the iron hypothesis of J. Martin, vast parts of the ocean are nutrient rich but iron limited. Therefore, fertilization of these areas with iron sulfate, in order to create algae blooms, was considered as a method of CO_2 sequestration. The main aim of the study was the investigation of side-effects of such events to the ecosystem. Here the effects on the bacterioplankton community structure are reported. Changes in the bacterioplankton community during the iron fertilization experiment LOHAFEX were investigated using Catalyzed Amplified Reporter Deposition Fluorescence *In Situ* Hybridization (CARD-FISH) and semi-automatic cell counting. In response to the iron fertilization an algal bloom dominated by *Phaeocystis* sp. was induced and closely monitored. In order to cover the three main groups of marine bacteria *Alphaproteobacteria*, *Gammaproteobacteria* and *Bacteroidetes*, 9 oligonucleotide probes were used in nested approaches. Additionally a probe for *Crenarchaea* was used in order to cover the main marine archaeal clade. In addition to a monitoring of surface water at 8 time points over the 38 days of the experiment, depth profiles for 4 chosen stations were done covering the first 500 m of depth. After the iron fertilization only a minor increase of total cell abundance was found in the surface layer, while thymidine and leucine uptake rates increased inside the fertilized patch. CARD FISH counts showed no differences in bacterial counts during the experiment, but a minor decrease towards the end. Within the *Bacteroidetes* a decrease of abundance was found in the surface during the first 5 days of the experiment. Depth profiles showed an increase of this group at 300 m depth before and at the end of the experiment. However, the abundance of *Gammaproteobacteria* did not change significantly inside the patch, whereas changes were found in the alphaproteobacterial clade. SAR11 increased during the first days of the experiment at day 5 and decreased only after day 21. A massive grazing pressure on larger cells than SAR11 is hypothesized to cause the remarkably stable community, giving the small SAR11 the niche to increase in numbers.

EMV003
Active hydrocarbon-degrading sulfate-reducing bacteria at marine gas and oil seeps

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Marine sediments play a significant role in the global cycling of carbon and other elements. Since O_2 is rapidly depleted in the upper sediment layers, sulphate reduction (SR) is the main dissimilatory process, accounting for up to 50% of total organic matter remineralization. At gas seeps, SR is tightly coupled to the anaerobic oxidation of methane while in sediments with natural oil seepage methane-dependant SR drops to less than 10% of total SR rates, indicating the degradation of other hydrocarbons in addition to methane. Up to now several sulfate-reducing bacteria (SRB) have been described to be capable of hydrocarbon degradation but their activity and abundance in situ are still largely unknown.
In this study we used CARD-FISH and cDNA-based clone libraries (16S rRNA and *aprA*) to investigate the global distribution and abundance of specific SRB in diverse marine oil and gas seeps. Stable-isotope probing (SIP) was used to identify active key-players in seep sediments. In all habitats, members of the *Desulfosarcina/Desulfococcus* (DSS) branch of *Deltaproteobacteria* were most dominant with up to 15% of total single cells. A major part (6%) of this group could be assigned to the seep-endemic subgroup SEEP-SRB1. Another dominant group detected was the SEEP-SRB2 group, distantly related to *Syntrophobacterales* and *Desulfobacterium anilini* with up to 12%.
SIP experiments using butane and dodecane were performed with sediments from gas and oil seeps. Butane was rapidly degraded in incubations with Amon mud volcano sediments (gas seeps) as determined by sulfide and butane concentrations, while in incubations with Guaymas Basin sediments (oil seeps) degradation was slow and started after a lag phase. Butane dissimilation and assimilation were confirmed by δ^{13}C-DIC and -TOC analysis, respectively. T-RFLP analysis revealed a clear labeling of different butane primary-consumers already within the first sampling points, while potential secondary consumers were found to be labeled in the latest sampling points. Active butane-degrading SRB were identified by rRNA gene sequencing. In contrast to butane incubations, dodecane experiments showed a slower microbial response. The identification of key hydrocarbon-degrading SRB together with cultivation attempts will allow a better understanding of the involved microorganisms and the carbon cycling at marine gas and oil seeps.

EMV004
Enrichment of *Dehalococcoides*-related *Chloroflexi* from marine subsurface sediments

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It is estimated that the marine subsurface contains more than 90% of the microbial biomass on Earth [1, 2] and is probably the least known ecosystem on Earth. "*Dehalococcoides*-related *Chloroflexi*" microorganisms (DRC) are a typical deep subsurface group of uncultured microorganisms from the phylum *Chloroflexi* which appear ubiquitous thriving in subsurface sediments [3-5]. In this study, subsurface sediments from off the coast of Chile were used as inoculum for microcosms targeting enrichment of DRC. Their closest cultured relatives are members of *Dehalococcoides* spp., which use halogenated organic compounds as electron acceptors [6-8]. Halogenated compounds are known to be naturally produced by an array of biological and chemical processes in the environment. Oceans are the largest source of biologically produced halogenated organic compounds on Earth [9]. Halogenated organic compounds such as chlorinated benzenes may thus be a potential electron acceptor for subsurface DRC. Herein we show dechlorination of 1,2,3-trichlorobenzene (80 µM; 123-TCB) to 1,3 dichlorobenzene (13-DCB) by sediment microbiota in enrichment cultures from sediments after an incubation time of 6 months. A subsequently transferred enrichment culture showed complete 123-TCB dechlorination after 2 months of incubation. Relative to total bacterial numbers, DRC numbers in the sediment measured by real-time PCR were low in the starting sediment material. Real-time PCR targeting DRC showed an increase of DRC 16S rRNA gene copy numbers from 7×10^2 to 2×10^4 after complete 123-TCB dechlorination to 13-DCB in the enrichment culture within 2 months. This confirms the enrichment in DRC organisms even though the starting numbers in the sediment were low. A clone library was produced from 16S rDNA amplicons of DRC-specific primers, and subsequentially sequenced. All sequences showed similarity with uncultured *Chloroflexi* sequences retrieved from different subsurface locations.

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EMV005
Anaerobic oxidation of methane in Lake Constance sediments

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Freshwater lakes contribute with 2-10% to the total emissions of the potent greenhouse gas methane. In Lake Constance aerobic oxidation of methane has been described extensively, but anaerobic oxidation of methane (AOM) remained cryptic. AOM with sulfate as electron acceptor has been reported for various environments including freshwater habitats. Recently also nitrate and nitrite were shown to act as electron acceptors for methane oxidation in eutrophic freshwater systems, and bacteria belonging to the NC10 phylum are capable to carry out this process.
We performed tracer experiments to follow $^{14}CO_2$ formation from $^{14}CH_4$ anoxically in sediment incubations in the presence of different electron acceptors, namely nitrate, nitrite, and sulfate. The diversity of NC10 phylum bacteria was assessed via clone libraries, and RFLP patterns were used to compare the community composition between different sediments.
No evidence for sulfate-dependent methane oxidation was found, but addition of nitrate significantly increased $^{14}CO_2$ formation in incubations of profundal sediment. In addition, *pmoA* and 16S rRNA genes and of the NC10 phylum were detected in Lake Constance sediments and revealed that the community structure differed between profundal and littoral sediments.
These results clearly indicate that Lake Constance sediments have the potential for anaerobic methane oxidation coupled to denitrification. This process seems to be more important in profundal sediments than in the littoral zone, and the differences in the community structure of the NC10 bacteria may reflect this disparity. Anaerobic oxidation of methane *in-situ* is possibly often masked by aerobic methane oxidation in oligotrophic habitats due to the close spatial proximity of the reactant transition zones but may still play a significant role in mitigating methane emissions.

EMV006
Half a millimeter makes a difference: a microscale study on distribution and specific activity of methanotrophs at an oxic-anoxic interface

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Rice paddies are one of the main global sources of methane, a major green house gas. Considering the importance of rice as a staple crop for a growing human population, source strength may even increase further. With the methane emission from rice paddies being drastically reduced by the activity of methanotrophic bacteria, understanding these microorganisms is essential. While diversity and activity of these bacteria on rice roots is intensively studied, the soil surface layer with its overlapping methane-oxygen counter-gradients is neglected so far.
To build a physical model of the surface of a flooded soil, we used microcosms supplementing a thin membrane supported layer of water-saturated paddy soil with methane from below and with air from above. For sampling, the soil was shock-frozen with liquid nitrogen and sliced horizontally to 0.1 mm thick layers. Community structure was analyzed by T-RFLP, a diagnostic microarray, and by competitive RT-PCR targeting the *pmoA* gene, a functional and phylogenetic marker for methanotrophs. *pmoA* transcripts served as a proxy for species-specific activity.
The active community consisted of type I methanotrophs: *Methylobacter*, *Methylococcus* and *Methylomonas*, and representatives of some rice-specific environmental clusters. This subcommunity was responsible for methane oxidation, while type II methanotrophs were abundant, but not detectable at the mRNA level.
It has already been known that the surface layer of flooded soils acts as a biofilter preventing up to 90% of the methane formed in the anoxic bulk soil to escape into the atmosphere. Here we show at the submillimeter scale, how the very oxic-anoxic interface selects for certain type I methanotrophs that are the main players, while type II were omnipresent but rarely active.

EMV007
Archaea dominate the ammonia-oxidizing microbial community in an acidic fen

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Nitrification in fens and bogs is often hampered by low pH, high content of humic acids, and lack of oxygen in the water-logged peat soils. So far, only little is known about microbial communities involved in nitrification in these environments. The goals of this study were (i) to assess the potential for nitrification in an acidic fen and (ii) to investigate the community composition, abundance, and transcriptional activity of the microbial groups involved in ammonia oxidation, the first and rate-limiting step of nitrification, in the peat soil. Samples were obtained from the acidic fen Schlöppnerbrunnen (Fichtelgebirge/Bavaria). Pore water chemical profiles and measurements of potential nitrification activity provided evidence that the fen soil harbors active nitrifiers. Communities of ammonia-oxidizing archaea (AOA) and bacteria (AOB) were analyzed targeting the *amoA* gene as molecular marker, which encodes ammonia monooxygenase, the key enzyme of ammonia oxidation. AOA constituted about 1 % of the total microbial community in the upper ten cm of the peat profile and outnumbered AOB by up to three orders of magnitude. Quantification of *amoA* gene transcripts suggested a higher transcriptional activity of AOA under field conditions as well as in laboratory incubations of peat samples. The diversity of AOA and AOB was low with only a few different phylotypes. Ongoing experiments aim to estimate the contribution of AOA and AOB to overall nitrification activity in the fen soil.

EMV008
How does land use influence bacterivorous protists in soils?

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The goal of our project is to correlate molecular diversity patterns of active and abundant single-cell eukaryotic predators of soil bacteria, the protists, with a land use gradient of agriculturally used grasslands. Bacterivorous soil flagellates represent an integral component of the terrestrial microbial loop. For instance, nutrients immobilized in the microbial biomass can be transferred to higher trophic levels such as plants and thus enhance the nutrient cycle significantly. A well studied example is the increase of nitrogen uptake in plants due to protist activity. In the framework of the DFG-funded „Biodiversity Exploratories" we hypothesize that the diversity of the protistan „seed bank" (total diversity including inactive dormant cells) and that of the established active population („realized" diversity) will differ in response to biotic and abiotic factors. Therefore we choose a cultivation-independent molecular biological fingerprinting tool, i.e. the T-RFLP method that allows us to gain a rapid and reliable overview of the active (i.e. on the RNA-level) and the overall (i.e. on the DNA-level) protist community composition. We studied different phylogenetic levels and taxa (all eukaryotes, the Chrysophyceae and the Kinetoplastea) at four time points in 2009 and correlated the obtained patterns with environmental factors like soil properties, plant diversity and land use regimes. By comparing the patterns of the realized and total community we could show a strong relevance of dormancy for soil protists. Furthermore, using quantitative PCR the underlying abundances of protistan species were estimated. Land use intensity seems to influences not only the protistan abundance but also the proportion of dormant cells in soil. We could partly uncover the response of the protists in grasslands to land use regimes and the relevance of dormancy for the diversity and activity of protists.

EMV009
Dynamics and drivers of ammonia oxidizing microbes in soil

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In the last 20 years the use of molecular methods has revolutionized microbial ecology. Today we know that only a small part of the soil microflora can be cultivated using classical isolation procedures and functional diversity of soils is the best playground on earth, when enzymes with new properties are in focus. Mainly the role of archaea which has been

largely ignored 20 years ago for stability and resilience of soil ecosystems is nowadays better understood and many studies have shown that archaea have the capacity to contribute to all major nutrient cycles. However there is still a controversial discussion in literature about activity of archaea in soils and their contribution to functional traits like nitrification compared to their bacterial counterparts.
In the presentation data will be presented that confirms the importance of archaea for soil quality of agricultural ecosystems and key drivers will be defined that steer abundance, activity and diversity of functional groups involved in carbon and nitrogen cycle. In additions concepts will be presented how this new data could be used to understand more about general questions related to functional redundancy or ecosystem resilience, theories that were built up for macroecology.

EMV010
Microbial model systems and ecological theory: How does increasing environmental stress affect microbial interactions and ecosystem services?

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Despite the recently increasing interest in ecological theory, the application in microbial ecology is currently still rather limited. One explanation might be the sceptical attitude of many ecologist and microbiologist to integrate general ecological concepts mainly originating from experiments with higher organisms into microbial systems.
Here we argue that microbial model systems are in particular promising due to their simplicity and their high degree of control and replication to answer questions regarding the relationship of biodiversity and ecosystem functions. We established microbial microcosms to investigate the influence of changing environmental conditions on microbial performance along a diversity gradient. Current theory suggests that complementarity is a major mechanism explaining a positive relationship between biodiversity and ecosystem functioning. We show that exposure to increasing levels of abiotic stress or additional trophic levels (e.g. predators) results in altered inter-specific interactions. While under benign environmental conditions competition is controlling the communities, mutualism dominates under stressed conditions. Moreover, higher microbial diversity seems to be in particular important to provide sufficient possibilities for positive interactions between the members of a community - a relevant insurance for maintaining the functioning of a microbial system under stress.

EMV011
Disturbance ecology controls natural attenuation in contaminated aquifers

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Disturbance ecology aims to understand the consequences of perturbation on biota within ecosystems. For aquifers, which are mainly governed by microbes, we are only beginning to grasp their functioning as ecosystems. Especially, aquifers are classically perceived as extremely stable environments, where dynamics occur only over long time scales due to their very limiting conditions (low temperature, nutrients, oxygen). Also after anthropogenic pollution, this may require decades for being naturally attenuated. Yet, the ecological principles governing, and potentially limiting natural attenuation in aquifers are still poorly understood. Here we unravel how anaerobic hydrocarbon degraders established in a thin lower fringe beneath a groundwater contaminant plume were unexpectedly disturbed by relatively minor hydraulic dynamics. Such dynamics have been hypothesized to either increase (by increasing the mixing) or decrease (by imposing unfavourable conditions on locally established degraders) net contaminant removal. Fine scale monitoring of hydrogeochemistry as well as massively paralleled pyrosequencing of bacterial rRNA gene fragments obtained over three years of repetitive sampling from different depths of the aquifer was performed. We established bidirectional sequencing of bacterial rRNA gene amplicons (~520 bp) which even allowed for assembly of amplicon contigs, T-RF prediction and phylogenetic reconstruction. More than 135,000 pyrotags helped us to unravel how degrader populations were affected by hydraulic dynamics. Prior to the disturbance, a highly selected, low-evenness degrader community of sulfate-reducing toluene degraders dominated by *Desulfobulbaceae* established at the lower plume fringe was detected. After relevant groundwater table dynamics, we observed a dramatic collapse of this standing degrader population connected to a transient loss of biodegradation activity. Subsequently, a distinct but functionally redundant population of degraders within the Gram-positive *Peptococcaceae*, over a longer time scale, restored functionality and thus insured natural attenuation against ecosystem disturbance. These findings highlight that aquifers are not steady-state habitats, and call for a new understanding of the ecological controls of hydraulic disturbance on microbes in groundwater ecosystems.

EMV012
The Baltic Sea microbiome:bacterial transitions along a 2000 km salinity gradient

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Aquatic ecosystems are controlled by abiotic environmental factors, including salinity. As early as 1934, Remane described a brackish water transition zone between salt water and freshwater, inhabited by only a few benthic invertebrates living at the edge of their salinity tolerance limits. Yet despite the abundance and importance of microorganisms in marine aquatic ecosystems, it is still unclear how they are distributed along the salinity gradient. We assessed bacterial community succession along the salinity gradient of the Baltic Sea, one of world's largest brackish water environments, using 454 pyrosequencing of partial (400 bp) 16S rRNA genes of 213 samples collected along vertical profiles at 60 sampling stations in summer. Along the salinity gradient a change in the bacterial composition was manifested at broad phylogenetic levels as well as at fine-scale phylogenetic levels, with closely related populations occupying different salinity and depth ranges. A major shift in the bacterial communities in the surface water was observed at salinity 8-10 and at salinity 3-4. Between these abiotic barriers, the bacterial community was composed of a diverse combination of freshwater and marine groups, along with populations unique to the brackish environment. Since water residence times in the Baltic Sea exceeds five years, this brackish bacterial community cannot be the result of conservative mixing of freshwater and saltwater, but reveals the first detailed description of an autochthonous brackish microbiome. In contrast to benthic faunal diversity, a lower bacterial diversity was not observed at intermediate (brackish) salinity levels, which suggests that the rapid adaptation of bacteria has enabled a diversity of lineages to fill what for higher organisms remains a challenging and relatively unoccupied ecological niche.

EMV013
Complex interactions between marine phages and their *Flavobacterium* hosts

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Phages are thought to regulate bacterial community composition through host-specific infection and lysis. However, our work with a marine *Flavobacterium* phage-host system consisting of 40 phages and 21 bacterial strains suggests that specificity and efficiency of infection and lysis is highly variable among phages. Pronounced variations in genome size (8 to >242 kb) and host range (infecting 1 to 20 bacterial strains) was found among the phages. Most of the phages had double-stranded DNA genomes; however, DNase I and S1 digestion of 8 phage genomes suggested that these were single-stranded DNA phages, consistent with their faint staining by SYBR Gold in gels and for microscopy. Further, we were unable to enumerate them by flow cytometry when stained with SYBR Gold or SYBR Green I. Hence, a diverse assemblage of phages was infectious to a suite of *Flavobacterium* hosts that were geno- and phenotypically very similar. Further, our data indicated that susceptibility to infection was strain-specific and that

resistance exists as a continuum between highly sensitive and resistant. Acquisition of resistance and subsequent proliferation of resistant strains was demonstrated on model particles as well as in solution. Loss of susceptibility to phage infection was associated with a reduction in the strains' ability to metabolize various carbon sources. Our work with this model system indicates that phage-host dynamics are extremely complex. If the observed patterns are valid for indigenous marine phage-host systems, they imply that i) continuous ranges of infectivity and susceptibility to infection exist in phage-host system, ii) in turn, that the concept of virus-host system has limited use, and iii) that functional diversification of bacterial hosts occurs at the clonal level. Thus far, the ecological consequences of this intriguing complexity are poorly understood.

EMV029
Aggregate-colonizing microbial communities - a comparison of marine vs. freshwater systems

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We observed the formation of particulate organic matter (POM) aggregates after a dinoflagellate bloom in coastal waters at Helgoland Roads in autumn 2009. These structures are known to function, both, as a sink for colloidal particles that adhere to the aggregates during the process of sinking as well as a source for dissolved substances due to rapid decomposition. The latter is greatly due to the dense colonization by microorganisms. Such an enhanced microbial activity affects the biochemical cycles of carbon, nitrogen, phosphorus, iron and other potential nutrients in the respective aquatic environment. We analyzed samples applying CAtalyzed Reporter Deposition-Fluorescence In Situ Hybridization (CARD-FISH) and epifluorescence microscopy, using different oligonucleotide probes on size fractionated plankton, >10 µm, 10-3 µm, and 3-0.2 µm. The total number of cells in the 3-0.2 µm fraction decreased after the algal bloom before stabilizing after 2 months. Despite this trend the total number remained high and the general ratio between Archaea and Bacteria was maintained. A more in-depth observation showed that different phyla followed different trends as a reaction to the environmental conditions. For example at the group level, in the 3-0.2 µm fraction, Alphaproteobacteria appeared to be the most dominant ranging between 30-50% of the total community. The relative abundances of the Bacteroidetes in this fraction decreased after the algal bloom (from 37% to 12%) whereas Gammaproteobacteria increased (from 9% to 14%). In case of particle associated bacteria (>10 µm) the relative abundance of Gammaproteobacteria (reaching 40%) was significantly higher than that in the free-water phase (reaching 25%). Their number on the aggregates decreased after the bloom whereas the number of Roseobacter increased. SAR11 as well as Crenarchaeota and Actinobacteria clearly preferred the non-attached phase. These data are to be compared with similar analysis on freshwater samples. Lake samples were chosen based on a Denaturing Gradient Gel Electrophoresis (DGGE) analysis which showed seasonal fluctuations in both the epilimnion and the hypolimnion of Lake Stechlin similar to that observed in the marine system. Preliminary results show that in the fraction smaller than 3 µm Gammaproteobacteria consist a minor part of the community (~1%) while the Betaproteobacteria are much more significant than in the marine system, reaching 11% in the epilimnion and 16% in the hypolimnion of Lake Stechlin.

EMV015
The bacterial community in the digestive tract of the small aquatic crustacean *Daphnia magna*

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In aquatic environments, bacteria play a key role in the carbon cycle but their importance in zooplankton guts remains mostly unknown, although their presence was regularly documented. Recently, denitrification by ingested bacteria in anoxic guts of benthic aquatic invertebrates was demonstrated indicating their possible symbiotic participation in digestion. However, the guts of most important zooplankton organisms, e.g. small *Daphnia spp.* which are a significant trophic link in freshwater systems, are probably only partly anoxic if at all. This leads to the question how the microorganisms interact with their host, i.e., whether they symbiotically participate in digestion, whether they prevent success of pathogens or whether they compete for food. The aim of this study was to characterise the intestinal microbial community and to estimate if *Daphnia* have a specialised stable gut microbiota or if the community just reflects surrounding bacteria. Therefore, the intestinal microbial community of *D. magna* clones was analysed via 16S rDNA clone libraries. To investigate the stability of their microbiota, *Daphnia* were incubated under different conditions (food sources, exposure to defined bacteria) while changes in the intestinal community composition were followed by T-RFLP. The *D. magna* microbiota was dominated by clones affiliated to the β-proteobacteria *Limnohabitans sp.*, which were described to respond rapidly to environmental changes. Overall, the intestinal microbial community did not contain known fermentative or obligately anaerobic gut bacteria. *Limnohabitans* spp. were also always prominent in the T-RFLP profiles despite changing food sources and independent of applied bacteria, thus indicating that they are specialised stable community members. Other intestinal microorganisms were stimulated by differing food sources but never dominated the community. Just when *Daphnia spp.* were starved to death their microbial community changed distinctly.

EMV016
Microbial engineers control sediment dynamics in aquatic habitats

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Background. Sediments and their microbial communities (biofilms) feature to a great extend the essential functionality of marine and freshwater habitats and provide important ecosystem services such as nutrient (re)-cycling or self-purification. This study addresses the ecosystem function biostabilisation where the microorganisms modify the response of the aquatic sediments to erosive forces (flow velocity, turbulence) by the secretion of extracellular polymeric substances (EPS).
Methods. The colonization of natural assemblages of estuarine bacteria and diatoms, as well as freshwater biofilms, was studied over several weeks using non-cohesive glass beads (< 63µm) as an artificial substratum. The adhesion capacity and the substratum stability of the growing biofilms has been determined by Magnetic Particle Induction (MagPI) and Cohesive Strength Meter (CSM), respectively. In parallel, bacterial cell numbers, microalgal biomass, the composition of the bacterial and microalgal assemblages as well as EPS quantity and quality (carbohydrates, proteins) have been monitored.
Results. Microbial colonization resulted in significant enhancement of adhesion and stability of the substratum as compared to the controls (up to a factor of 12) irrespective of the environment (freshwater, marine). The stabilization potential of the bacteria exceeded that of the axenic diatom assemblages; however, the overall stabilization was highest in mixed assemblages. The assemblage composition, their physiology and the secretion of EPS quantity and quality were important for sediment stabilization, but strongly influenced by changing abiotic conditions.
Conclusions. While biostabilisation has been mainly linked to microalgae (ecosystem engineers), our results point out the importance of bacterial assemblages for microbial sediment stabilization. Thereby, changes in abiotic conditions can significantly affect the ecosystem service biostabilisation by microbes. The data further suggest that the EPS matrix determines sediment adhesion and stability; however the binding strength was less related to quantity than to quality with possible synergistic effects between proteins and carbohydrates. This information contributes to our conceptual understanding of microbial sediment engineering that represents an important ecosystem service.

EMV017
Calcite biomineralization in a karstic cave - bacteria hidden in the dark

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Karstic caves represent one of the most important subterranean carbon storages on Earth and provide excellent „windows" to the subsurface. Our multidisciplinary study took advantage of the recent discovery of the Herrenberg Cave to investigate its mineralogy and the diversity and potential role of bacteria in carbonate mineral formation. Stalactites consisted of calcite, while dolomite and calcite (besides quartz, muscovite and clay minerals) were found in sediments, suggesting that only calcite was precipitating from seepage water as stalactites. Confocal laser scanning microscopy detected bacterial cells on the surface and in the interior of stalactites. Molecular analyses revealed the dominance of Proteobacteria inhabiting stalactites and fluvial sediments in addition to the phyla Bacteroidetes, Acidobacteria, Nitrospira, Chloroflexi, Planctomycetes, Verrucomicrobia, Actinobacteria and Firmicutes. Up to 16 % of the sequences were related to yet unclassified Bacteria. A large fraction of these bacteria were metabolically active. *Arthrobacter sulfonivorans* and *Rhodococcus globerulus* strains isolated from the cave formed mixtures of calcite, vaterite and monohydrocalcite. *R. globerulus* precipitated idiomorphous, rhombohedral carbonate crystals, while with *A. sulfonivorans* xenomorphous globular crystals were observed. The different crystal morphologies refer to species dependent calcite formation and underline the importance for biomineralization in karstic habitats.

EMV018
Abundance of microbes involved in nitrogen transformation in the rhizosphere of *Leucanthemopsis alpina* (L.) Heywood grown in soils from different sites of the Damma glacier forefield

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Glacier forefields are an ideal playground to investigate the role of development stages of soils on the formation of plant-microbe interactions, as within the last decades many alpine glaciers retreated, whereby releasing and exposing parent material for soil development. Especially the status of macronutrients like nitrogen differs between soils of different development stages in these environments and may influence plant growth significantly.
Thus in this study, we reconstructed major parts of the nitrogen cycle in the rhizosphere soil/root system of *Leucanthemopsis alpina* (L.) Heywood as well as the corresponding bulk soil by quantifying functional genes of nitrogen fixation (*nifH*), nitrogen mineralization (*chiA, aprA*), nitrification (*amoA AOB, amoA AOA*) and denitrification (*nirS, nirK*, and *nosZ*) in a 10-year and a 120-year ice-free soil of the Damma glacier forefield. We linked the results to the ammonium and nitrate concentrations of the soils as well as to the nitrogen and carbon status of the plants. The experiment was performed in a greenhouse simulating the climatic conditions of the glacier forefield. Samples were taken after 7 and 13 weeks of plant growth. Highest *nifH* gene abundance in connection with lowest nitrogen content of *L. alpina* was observed in the 10-year soil after 7 weeks of plant growth, demonstrating the important role of associative nitrogen fixation for plant development in this soil. In contrast, in the 120-year soil copy numbers of genes involved in denitrification, mainly *nosZ*, were increased after 13 weeks of plant growth, indicating an overall increased microbial activity status as well as higher concentrations of nitrate in this soil.

EMV019
Ultrastructural, genomic and ecological analysis of „*Candidatus* Magnetobacrterium bavaricum" reveals a mechanism homologous to proteobacterial magnetosome formation

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Magnetotactic bacteria (MTB) are phylogenetically diverse. They use intracellular membrane-enclosed magnetite crystals called magnetosomes for navigation in their aquatic habitats, which are of broad interdisciplinary interest. Due to the lack of cultivated representatives from other phyla, the genetic background of magnetosome formation was exclusively analyzed in a few closely related members of the *Proteobacteria* thus far, in which all essential functions required for magnetosome formation are encoded within a large genomic magnetosome island. However, the evolutionary origin and phylogenetic distribution of this magnetosome island has been unknown, and it has been questioned whether homologous genes are present in MTB from other phyla.
Here, we present the analysis of the uncultivated „*Candidatus* Magnetobacterium bavaricum" (Mbav) from the *Nitrospira*-phylum by combining ecological and geochemical techniques with metagenomics, single cell sorting and a variety of advanced electron microscopic methods. Micromanipulation and whole genome amplification of individual sorted cells revealed Mbav-specific sequences that were used for screening of metagenomic libraries. This led to the identification of a genomic cluster containing several magnetosome genes with homology to those in *Proteobacteria.* Different electron microscopic imaging techniques, such as focused ion beam milling or ultrathin sectioning of high-pressure frozen and freeze-substituted cells revealed a complex cell envelope and an intricate magnetosome architecture. In particular, the presence of magnetosome membranes as well as cytoskeletal magnetosome filaments suggests a similar mechanism of magnetosome formation in Mbav as in *Proteobacteria*. Altogether, our findings suggest a monophyletic origin of magnetotaxis, and relevant genes were likely transferred horizontally between *Proteobacteria* and representatives of the *Nitrospira*-phylum.

EMV020
Comparison of Genotypic, Proteotypic and Phenotypic Methods for the Identification of Bacteria

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Accurate classification of unknown bacterial isolates is an essential first step in understanding the impact these organisms have on an environmental monitoring program. There are many methods, technologies, and strategies utilized to determine the identity of unknown microorganisms, however, the selection of these methods is often impacted by more than the performance of the technology. Cost, time and the amount of expertise required to perform an assay are major points to consider during the selection process. Current available methods of identification range from genotypic to phenotypic, with 16S sequencing being universally acknowledged as the standard for routine bacterial identifications. Still, there is even variability within this process as not all 16S sequencing methods are comparable. When identifications are based on phenotypic characteristics, the methods are more subjective and results can be impacted by many variables. The first practical proteotypic identification systems utilize matrix-assisted laser desorption/ionization - time of flight (MALDI-TOF) spectroscopy for microbial identification. This technology is based on whole cell protein profiles that are subject to less expression variability than phenotypic systems. This study directly compared performance between several of these technologies, including metabolic profiling (bioMerieux VITEK® 2 Compact), MALDI-TOF (Bruker BioTyper™), automated DNA sequence analysis (ABI MicroSEQ® 2.1), and DNA sequencing with a reference-quality, customized data analysis process and curated libraries (Accugenix). These microbial identification methods were used to analyze 60 unknown environmental bacterial isolates. Accuracy, as well as assay cost, time, and

ease of use for each method are discussed. While 16S rRNA gene sequencing remains the standard for microbial identification of environmental isolates, proteotypic MALDI-TOF technology outperformed more phenotypic methods without compromising assay cost and turnaround time.

EMV021
Insights into the community structure and activity of the iron oxidizing bacteria in the Äspö -Hard Rock Laboratory

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Processes of iron mineralization leading to phenomena such as Banded Iron Formations (BIF) are of great significance to the understanding of the early geochemistry of Earth. Whether the formation of iron minerals was to a large extent biologically induced is yet unknown. The Äspö Hard Rock Laboratory (Sweden) offers a unique opportunity to gain a better understanding of biological iron mineralization. This system consists of a 3.6 km tunnel that runs under the Baltic Sea to a depth of 460 m below sea level. Through a series of fractions various aquifers of Fe(II)-rich brackish to saline waters penetrate the system and harbour a variety of mineralizing microbial mats.
To better understand microbially-induced mineral formation, 3 sets of 4 flow bio-reactors were set up with different combinations of light and aeration conditions and were connected to 3 aquifers of differing chemical composition and age. The waters of the reactors were controlled periodically over a period of 4 years for physico-chemical fluctuations.
We have used a combination of 454 tag sequencing and CAtalyzer Reporter Deposition Fluorescent In Situ Hybridization to analyze the total bacterial community in these reactors. A large number of genera related to known iron oxidizing bacteria was found, including autotrophic (e.g.,s *Mariprofundus* sp. and *Gallionella* sp.) as well as iron precipitating organisms (e.g., *Crenothrix* sp. and *Hyphomicrobium*). We found the marine iron oxidizing bacteria *Mariprofundus* sp. to be dominant in many of the sampled sites, in contrast to the previously reported dominance of *Gallionella* sp. and *Leptothrix* sp. in this ecosystem. To assess the biological vs. abiotic iron oxidation, we incubated microbial mats from the flow reactors and from various locations in the tunnel with $^{57}Fe^{++}$ and $H^{13}CO_3^-$. The samples were used for bulk as well as single-cell nanoSIMS analysis. The latter showed that diverse filamentous and unicellular bacteria were enriched in either or both ^{57}Fe and ^{13}C. The rate of iron oxidation derived from single cell analysis was ~10 times higher than that measured by bulk analysis and normalized to total cell count. This is in agreement with the relative fraction of sequences of iron oxidizing bacteria per sample.

EMV022
Adaptation of the iron-reducing bacterial community to iron oxide availability in anoxic soil

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Question: In many soils, crystalline iron(III) oxides are more abundant than the much more bioavailable amorphous iron(III) oxides but the microorganisms involved in reducing crystalline iron oxide minerals have not been recognized in the environment.
Methods: By RNA-based isotope probing with ^{13}C-acetate, we identified iron-reducing bacteria in anoxic rice field upon amendment of different crystalline iron(III) oxides.
Results: Depending on the iron oxide present, specific taxa were predominantly labeled. *Geobacter* (~up to 69% of clones) and *Anaeromyxobacter* (~14%) populations were found strongly labeled in the presence of ferrihydrite (amorphous), lepidocrocite, and goethite, but Chloroflexi (~19%) and beta-proteobacterial Rhodocyclales (~50%) were predominantly labeled in hematite and control incubations, respectively. Within the family Geobacteraceae, distinct clone sequence clusters emerged in the presence of lepidocrocite and hematite, only distantly related to cultivated Geobacter spp. (93-96 % sequence identity).
Conclusions: The presence of different iron(III) oxide mineral phases apparently selected for distinct microbial populations in anoxic rice field soil, which suggests that individual populations might be specifically adapted to interact with different iron oxide mineral surfaces.

EMV023
Insights in microbial communities - Functional marker genes in the anaerobic degradation of aromatic compound

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In anaerobic bacteria, most aromatic growth substrates (e.g. benzene, toluene, phenol, cresols, xylenes, ethylbenzenes, benzoate analogues, etc.) are channelled to the central intermediate benzoyl-CoA, which is dearomatized by reduction to a cyclic cyclohexadienoyl-CoA. In facultative anaerobes this step is catalyzed by an ATP-dependent class I benzoyl-CoA reductase [1], whereas in obligate anaerobes it is catalyzed by a ATP-independent class II BCR [2]. The subsequent steps of the benzoyl-CoA degradation pathway are highly similar in both facultative and strict anaerobes, yielding three molecules of acetyl-CoA and one CO_2.
The detection of functional marker genes involved in the degradation of aromatic compounds in anaerobic bacteria is important for bacterial community analysis at contaminated environmental sites. So far only two PCR based assays have been developed for the analysis of contaminated anoxic environmental samples that targeted either the *bssA* gene encoding the A-subunit of the benzylsuccinate synthase, or *bcr* genes encoding subunits of class I BCR. *In situ*-microcosms from two different benzene contaminated aquifers were analyzed using molecular tools for detecting the 16S rRNA gene in combination with four functional genes (*bssA*, *bcr*, *bamA*, encoding the ring opening hydrolase, and *bamB*, encoding the active site subunit of class II BCRs). In both microcosms different species related to the genera *Azoarcus* (high benzene concentration) and *Geobacter* (low benzene concentration) dominated, respectively. The results revealed a good consistency of the assays applied, but also indicated that the combined application of assays targeting several functional genes and the 16S rRNA gene is required for a reliable community analysis at environmental sites.

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EMV024
Response of Cellulose- and Cellobiose-degrading Soil Bacteria to Different Redox Potentials in an Aerated Soil

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Cellulose is degraded by aerobic and anaerobic soil microbes. Redox conditions in oxic soil may change rapidly when dry soil becomes wet after a rain fall. Although many novel bacterial taxa involved in cellulose degradation have been identified by gene marker-based methods, little is known about how they respond to fluctuations in oxygen and redox potential. Slurries of agricultural soil were supplied with either carboxymethyl-cellulose (CMC) or cellobiose, and were subjected to oxic conditions, followed by an anoxic period, and then a final oxic period. Ribosomal RNA content of five family-level bacterial taxa and microbial processes were measured. Redox potential was stable (>500mV) during aeration and decreased under anoxic conditions to 300 and -330mV in CMC- and cellobiose-supplemented slurries, respectively. CMC and cellobiose was degraded with similar rates at any redox potential. *Micrococcaceae* (*Actinobacteria*) responded most pronounced to cellobiose supplementation under oxic conditions as compared with the other detected taxa. Saccharolytic *Clostridiaceae* (Cluster I; *Firmicutes*) were induced under anoxic conditions.
Kineosporiaceae/Nocardioidaceae, and 'Deha' (novel family-level taxon of *Dehalococcoides*) did not respond to experimental conditions. Cellulolytic species of *Planctomycetaceae* (*Planctomycetes*) have not been described. Nonetheless, the ribosomal content of *Planctomycetaceae* significantly increased in CMC-treatments during re-aeration, reinforcing previous stable isotope probing studies that suggested this family might contain hitherto

unidentified cellulolytic species. The different responses of cellulose- and cellobiose-degrading bacteria to differing redox potentials may be important to the capacity of the soil microbial community to degrade plant-derived saccharides during fluctuations in oxygen and redox potential.

EMV025
Genome analysis and transcriptome profiles of *Methylocystis* sp. strain SC2 grown at different methane concentrations

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Methane-oxidizing bacteria, or methanotrophs, are crucial players in the global cycle of the greenhouse gas methane. They are strict aerobes that use methane as their only source of carbon and energy. The two key factors that determine methanotrophic activity in *situ* are the CH_4:O_2 mixing ratio and nitrogen sources. In the methane-oxidation pathway, the first step is mediated by *pmoCAB*-encoded particulate methane monooxygenase (pMMO), which converts methane to methanol. It has long been believed that type I and type II methanotrophs possess a single type of pMMO. However, it has recently been shown that the type II methanotroph *Methylocystis* sp. strain SC2 contains two pMMO isozymes (pMMO1, pMMO2) with different methane oxidation kinetics. The conventional pMMO1 is expressed and oxidizes methane only at high concentrations (>600 ppmv), while the novel pMMO2 is constitutively expressed and oxidizes methane at low mixing ratios (<600 ppmv), even at the trace level of atmospheric methane. The pMMO2 should provide type II methanotrophs with a selective advantage, enabling them to survive under methane conditions at which most type I methanotrophs do not thrive, presumably <450-600 ppmv.
We postulate that in strain SC2, the up- and down-regulation of pMMO1 relates to two different metabolic states of methane oxidation, thereby involving major changes in the transcriptome and proteome. The whole genome of strain SC2 was sequenced using a combination of 454 GS-FLX Titanium pyrosequencing (50-fold coverage) and Sanger paired-end sequencing of fosmid clones. The genome size of strain SC2 was found to be 4.2 Mb. To determine how the transcriptome of strain SC2 responds to changes in methane concentration, cells were grown at low (300 ppmv) and high (2000 ppmv) methane mixing ratios. Enriched mRNA was isolated from the cells and sequenced using the Illumina platform. The identification of transcripts whose expression is stimulated or down-regulated under the contrasting growth conditions will be confirmed by quantitative RT-PCR. The long-term goal is to elucidate the signaling pathways controlling the cellular response of strain SC2 to environmental changes in the methane concentration.

EMV026
Suppression mechanisms involved in the antagonistic activity of *Pseudomonas syringae* strain 22d/93 against the soybean pathogen *Pseudomonas syringae* pv. *glycinea*

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The use of naturally occurring antagonists to suppress plant diseases offers an alternative to classical methods of plant protection. The epiphyte *Pseudomonas syringae* strain 22d/93, isolated from a healthy soybean leaf, shows great potential for controlling *P. syringae* pv. *glycinea*, the causal agent of bacterial blight of soybean. Its activity against *P. syringae* pv. *glycinea* is highly reproducible even in field trials, and the antagonistic mechanisms involved are of our special interest.
Three toxins are known to be produced by *P. syringae* 22d/93. Beside syringomycin and syringopeptin, 22d/93 produces a toxin that strongly inhibits the growth of *P. syringae* pv. *glycinea in vitro*. The inhibition can be overcome by supplementing the growth medium with L-arginine, which suggests that the toxin acts as an inhibitor of the arginine biosynthesis. The structure of the natural product was identified as 3-methylarginine. To examine if the toxins produced by 22d/93 are responsible for the antagonistic effects *in planta*, the pathogen was co-inoculated with toxin-negative mutants into leaves of soybean plants.
Moreover, we found that *P. syringae* 22d/93 produced a significantly larger amount of siderophores than the pathogen *P. syringae* pv. *glycinea* produced. While *P. syringae* 22d/93 and *P. syringae* pv. *glycinea* produce the same siderophores, achromobactin and pyoverdin, the regulation of siderophore biosynthesis is very different in both bacteria. The effect of siderophore production by 22d/93 on epiphytic fitness and biocontrol activity against *P. syringae* pv. *glycinea* was determined following spray inoculation of soybean leaves with mutants defective in siderophore biosynthesis.

EMV027
Effects of sulfadiazine entering via manure into soil on the abundance of antibiotic resistance genes and their transferability

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Manure contains considerable amounts of administered antibiotics and represents a hot spot for antibiotic resistance genes as well as broad-host-range plasmids. In consequence, the large amounts of manure applied to arable soils may stimulate the spreading of resistance genes in environmental bacterial communities by horizontal transfer and selection of antibiotic resistance determinants. Manure containing SDZ enhanced the abundance of sulfadiazine resistance genes and their transferability in soils but repeated manure application led to an accumulation of *sul* resistance genes only if the manure contained sulfadiazine as shown in soil microcosm experiments. Two plasmid types were identified that play a major role for the horizontal spreading of resistance genes between manure and soil bacteria. The analysis of sulfadiazine resistance genes in total community DNA in bulk and rhizosphere soil from mesocosm or field samples determined by real-time PCR is still in progress. But the preliminary data suggest that the relative abundance of *sul2* genes was significantly increased in the rhizosphere of grass when the spread manure originated from pigs treated with SDZ. Transferability of *sul* resistance genes was found to be enhanced in the rhizosphere compared to bulk soil, but similar plasmids carrying multiple antibiotic resistance genes were isolated from bulk and rhizosphere soils. Plasmids belonging to the LowGC-type and the IncP-1e group plasmids were frequently captured by exogenous isolations bulk and rhizosphere soils of the mesocosm and field experiments. Thus it can be assumed that these plasmids contribute to the dissemination of SDZ resistance in soils.

EMV028
Viral infections as controlling factor for the deep biosphere?

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The deep-subseafloor biosphere harbours a major part of the total microbial biomass on Earth. However, how life and death in this environment are regulated is not yet understood. While organisms from higher trophic levels appear to be absent, viruses might be a factor for microbial mortality. In this study [1], we found an increasing ratio between viral and total cell counts with depth in deep-subseafloor sediments recovered during Leg 201 of the Ocean Drilling Program (ODP). A phylogenetically diverse culture collection from corresponding sediment layers was tested for the presence of inducible prophages. A treatment by mitomycin C as inducing agent indicated the presence of prophages in 46% of the bacterial isolates. Different morphotypes of myoviruses and siphoviruses were detected by transmission electron microscopy. Pulsed-field gel electrophoresis applied to viral DNA-extracts showed their genetic diversity. Three host strains even harboured more than one prophage. Thus, our results prove the existence of functional viruses in the deep-subseafloor biosphere. Under the specific conditions in deep-subsurface sediments, bacteriophages might take over the role of the main predators, as anoxia and oligotrophy favour the importance of viruses as mortality factors. Furthermore, the fact that the viral shunt

recycles organic compounds might be of special relevance to this severely nutrient-depleted habitat.

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EMP001
Biodegredation of 2 – Methooxyethanol by a new bacterium isolate *Pseudomonas* sp. Strain VB under aerobic conditions

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Microbial biodegradation of 2-methoxyethanol also known as Methyl glycol (MG) under anaerobic conditions has received much attention during the past decade. However, not much is known about the aerobic degradation of 2-methoxyethanol. Samples from various environmental niches were enriched to isolate and determine bacterial isolates capable of utilizing 2-methoxyethanol as a sole source of carbon and energy under aerobic conditions. A 2-methoxyethanol degrading bacterium was isolated from anaerobic sludge of a municipal sewage from a treatment plant in Bayreuth, Germany, by selective enrichment techniques. The isolate was designated strain VB after it was shown by the 16S rRNA phylogenetic sequence analysis as belonging to the genus *Pseudomonas*. Under aerobic conditions Pseudomonas sp. strain VB was capable of mineralizing 2-methoxyethanol and its intermediary metabolites. Stoichiometrically, the strain utilized one mole of oxygen per one mole of 2-methoxyethanol instead of four mole oxygen per one mole of 2-methoxythanol for the total oxidative metabolism.

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EMP002
The Molybdenum Storage Protein - a special kind of metalloprotein

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The diazotrophic soil bacterium *Azotobacter vinelandii* utilizes a FeMo-cofactor containing nitrogenase in larger amounts to accomplish nitrogen fixation. This requires a lot of molybdenum, which is extracted from the environment and stored in a special Molybdenum Storage Protein (MoSto). This extraction strategy is rather efficient and limits the available Mo contents for other soil bacteria.
The MoSto is a remarkable protein due to its capability to store huge amounts of Mo in form of polyoxomolybdate clusters [2]. X-ray studies of the loaded MoSto after purification revealed different types of Mo-oxide based clusters some being covalently bound while others are not. Synthesis of these clusters is an ATP-dependent process whose mechanism is not yet known. *In-vitro* experiments showed that it is possible to fully deplete the MoSto of its metal clusters and later on reload it again. The depletion proved to be a pH-driven triphasic process which can be varied with temperature and time of incubation [1]. Depending on the method of protein purification this can lead to a total reload of 120 Mo-atoms per protein molecule. Further research is necessary to determine the way the clusters are built from single Mo-ions and how their release from MoSto is organized.

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EMP003
Impact of extreme weather events on the microbial function of soil

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Prolonged drought periods as predicted in future climate scenarios will affect ecosystem functions in multiple ways. Water stress not only affects plants but also soil microorganisms. As important soil functions, nutrient turnover processes will be affected during the vegetation period when plants have highest demands. Drought is one of the factors addressed in the EVENT-Experiment established at the Botanical Garden of the University of Bayreuth. In this project, we hypothesise that hydrolytic enzyme activities will be reduced and oxidative processes will be favoured under drought conditions. Therefore, biochemical parameters such as soil enzymatic activities of hydrolytic (phosphatase, chitinase, proteases, cellulases) and oxidative enzymes (phenoloxidases, peroxidases) are measured. In addition the gene and transcript pool of these enzymes will be studied using molecular biological studies on nucleic acids extracted from soil (chitinase, cellulases, xylosidase). The project focuses on experimental and natural grassland communities. First results will be presented on drought effects (1000 year extremes) and an outline of the overall design of the study is presented.

EMP004
Low-temperature denitrification in wastewater by using of encapsulated biomass: The choice of appropriate organism

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Wastewater treatment is one of the fields of industrial application of microbial processes. Recently, the biological treatment is provided by so-called activated sludge, the dynamic polyculture containing huge number of bacterial species.
This technology can be modified, for example by using encapsulated biomass. This kind of immobilisation technique encases the microorganisms into porous polymerous gel, in the case of our study into polyvinylalcohol. The structure of gel enables diffusion of substrate to organisms as well as the microbial growth, but prevents the bacteria from outside of the pellet from intrusion to the inside. As the pellet contains only bacterial species introduced during the fabrication process, careful selection of suitable culture to be immobilized is necessary.
The denitrification process in wastewater as well as in drinking water treatment can be slowed down by low temperature of water. In this study, three types of immobilized denitrification cultures have been compared. The first type contained pure culture of *Paracoccus denitrificans* with optimal temperature of 30 - 37 °C [1], which has already been used for fullscale denitrification [2]. The second type contained pure culture of *Pseudomonas fluorescens* as a representative of psychrophilic bacteria. The last type comprised of highly-adapted mixed culture of psychrophilic denitrifiers cultivated for one year at 5 °C from activated sludge.
The aim of this work was to compare denitrification activity of these types of encapsulated biomass. The experiments were held with synthetic wastewater containing 50 $mg \cdot L^{-1}$ $N\text{-}NO_3^-$ under the temperature 15, 10, 8 and 5 °C. Specific denitrification rates were calculated and the temperature coefficients describing the dependence of denitrification rate on the temperature were determined. The culture composition and dislocation within the pellets was observed. Since the low-temperature denitrification is supposed to be performed in industrial-scale, it is necessary to consider not only the denitrification rates and courses, but also the possibility of easy, steady and sustainable cultivation when choosing appropriate organism.

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EMP005
Identification of indigenous bacteria in an As-high aquifer of Hetao Basin, China

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Endemic arsenicosis poses a huge threat to habitant's health in the areas with high arsenic concentration of Hetao Basin, China, where As concentrations range between 1 and 1000 µg/L. Here, a significant proportion (up to 90%) of the As occurs as As (III). From a geological, geochemical and microbiological viewpoint, there are many studies trying to find the sources of high arsenic concentration in groundwater. Recently, it is generally accepted that microbial activities play a critical role in the releasing of arsenic from the sediments.
Four drillings were carried out in aquifers with different As concentration levels in Hetao Basin. Polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE) were used to identify relevant bacteria species responsible for As release in different sediments of those drillings. The sediments with indigenous bacteria were cultured with DEV-agar plates. For this experiment, 300 µg/L As (V) were added and the growing conditions were comparable to the aquifer conditions on-site.
The PCR-DGGE profiles indicated that the bacteria species, which can grow well with high-As concentration under lab conditions, were very different from the indigenous bacteria that were found in the raw sediments. Comparative sequence analyse for the raw sediment revealed various DNA band patterns, suggesting a population shift in different depths and different sediments. Some bacteria, which may affect the release and mobilization of As in aquifer, like *iron-reducing bacterium*, *Siderooxidans paludicola* and *Novosphingobium hassiacum* were found in the sediments. There are many arsenic resistant bacteria, which have isolated from the incubations. For example *Pseudomonas sp.*, which is gram-negative, rod-shaped, non-motile, non-spore-forming, and noncapsulated, is a very efficient As (V) reducing bacterium. The DGGE profiles also indicated that most of indigenous bacteria species had high G+C, but most of bacteria that were incubated in lab had low G+C.

EMP006
Bacteria associated with coexisting macroalgae: seasonal, interspecies variation and antibiotic effects

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Bacteria associated with 2 macroalgae, a brown and a red macroalga, coexisting in the Kiel Fjord (Baltic Sea, Germany) were investigated seasonally by scanning electron microscopy and cultivation methods. Significant differences between both macroalgal species with regard to their associated bacteria and seasonal variations were observed.
166 bacterial strains were isolated from both macroalgae and classified by phylogenetic analysis of 16S rRNA gene sequences. The strains belonged to 82 phylotypes according to sequence similarities of >99.0%. They affiliated to *Actinobacteria, Alphaproteobacteria*, *Bacilli*, *Betaproteobacteria*, *Flavobacteria*, *Gammaproteobacteria*, and *Sphingobacteria*. Samples from *Fucus vesiculosus* revealed 43, and samples from *Delesseria sanguinea* 57 phylotypes. Certain phylotypes are consistently found as epiphytes, suggesting their specific association to macroalgae.
Culture extracts of all bacteria were tested for antimicrobial activity. More than 60% of the phylotypes inhibited the growth of at least one microorganism of a standard and an ecologically relevant test panel (Gram-positive and Gram-negative bacteria, including macroalgal pathogens and surface associated strains, and one yeast). A higher proportion of the strains showed antimicrobial activity against the ecologically relevant bacteria as compared to the standard set of microorganisms. In contrast, extracts of the macroalgae presented only a weak inhibition of test panel microbes, but a general growth stimulating effect on the macroalgae-associated strains.
Significant activity of the associated bacteria against macroalgal pathogens and competitors and the stimulating effect of the algal host extracts indicate specific functions and adaptations of these bacteria to algal host and *vice versa*.

EMP007
Biogeochemical mobilization of arsenic from aquifer sediments in West Bengal, India

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The aim of this field experiment was to test the potential role of microorganisms in the occurrence of As-rich groundwater, by stimulating the activity of indigenous microbial populations within a shallow aquifer, in West Bengal, India. Local groundwater is of Ca-HCO_3^--type and hydrochemical parameters indicate low redox conditions, in the range of iron reduction.
Sucrose was inserted as readily degradable organic carbon source into five nested monitoring wells by circular pumping, thus generating concentrations from 8.3 to 873 mg/L within the screened depth intervals from 12 - 44 m. For the following 14 days, field parameters were measured and water samples were taken every second day to examine the geomicrobiological effects involved in the mobilization of As. Significant changes in hydrochemical parameters have been observed soon after insertion, indicating that the microbial stimulation was successful. Sucrose concentrations decreased continuously, while degradation products like acetate were formed, disturbing the hydrogeochemical equilibrium in the water-sediment-system. Formation of organic acids (like acetate anions) led to a partial dissolution of carbonates. The absence of dissolved oxygen, nitrate and sulphate combined with a strong increase in the concentration of dissolved Fe^{II} (up to 36 times relative to its initial value) indicates ongoing dissimilatory Fe-reduction, which is believed by many authors to be responsible for As mobilization (e.g. [1]). Dissolved As concentrations showed a considerable temporary increase of up to 49% of the initial value. Nevertheless, this increase appeared to be relatively low as compared to the mobilization of other trace elements, most likely also associated with Fe-oxyhydroxides. Our field experiments strongly support the assumption that the mobilization of As is primarily influenced by the biotransformation of Fe-mineral phases [2; 3]. The dissolution of some mineral phases (e.g. Fe-oxyhydroxides, carbonates, etc.), and the mobilization of associated trace elements, including As, is controlled to a large extent by microbial metabolism, which ultimately depends on the availability organic electron donors in the groundwater environment.

[1] Islam, F. S. et al (2004): Role of metal-reducing bacteria in arsenic release from Bengal delta sediments. Nature, 430, 68-71.
[2] Kocar, B.D. et al (2006): Contrasting Effects of Dissimilatory Iron(III) and Arsenic(V) Reduction on Arsenic Retention and Transport. Environ. Sci. Technol., 40, 6715-6721.
[3] Tufano, K.J and S. Fendorf (2008): Confounding impacts of iron reduction on arsenic retention. Environ. Sci. Technol., 42, 4777-4783.

EMP008
Identification of nitrifying bacteria in activated sludge

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Nitrogen removal is an important process in wastewater treatment system. Nitrifying bacteria have slow growth rates and are sensitive to toxic shocks, pH- and temperature swings. This is the reason why many wastewater treatment plants (WWTPs) fail to establish stable nitrification. The microbial ecology of nitrifying bacteria from Czech WWTPs was investigated using fluorescence *in situ* hybridization (FISH) with 16S rRNA-targeted oligonucleotide probes. This paper is focused on detection of single cells or various clusters of nitrifying bacteria in activated sludge samples taken from sewage WWTPs. Bacteria were identifying and quantifying using epifluorescence microscopy and image analysis.

EMP009
Isotope fractionation of nitrate-dependent microbiological sulfide oxidation

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The degradation of organic contaminants with sulfate as electron acceptor leads to a transfer of redox-equivalents to form reduced sulfur species such as hydrogen sulfide or elemental sulfur. These compounds can be re-oxidized under oxic or anoxic conditions for example with nitrate as electron acceptor and may therefore compete with the oxidation of organic pollutants in contaminated environments. The isotope composition of sulfur compounds is a useful tool to describe and assess these redox processes.
In the present study we analyzed the sulfide oxidation under denitrifying conditions in a column experiment at a BTEX contaminated field site in Zeitz. We used two dimensional stable isotope systems by measuring the ^{34}S and ^{18}O isotopes of the produced sulfate and the ^{15}N and ^{18}O isotopes of the used nitrate. For certain experimental conditions, our results suggest an inverse isotope fractionation for oxygen in the produced sulfate, indicated by very negative ^{18}O values compared to the surrounding water. No evidence for reduction processes of the produced sulfate was found.
To interpret the measured isotope values, enrichment factors for pure strains are needed. Therefore, we performed fractionation experiments with the sulfide-oxidizing model strains *Thiobacillus denitrificans* and *Sulfurimonas denitrificans* to measure the isotope fractionation of ^{34}S and ^{18}O. Both organisms use different enzymatic pathways to oxidize sulfide to sulfate.

EMP010
Effect of oxygen availability on catabolic gene expression of aerobic and anaerobic toluene degrading bacteria

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Bacteria in the environment are constantly exposed to oxygen variations and gradients as they occur, e.g., in aquifers, in microbial mats and the rhizosphere. Microorganisms living in polluted sites have the ability to process these input signals in order to develop adaptive responses to survive fluctuations of external conditions.
The relative expression of catabolic genes under oscillating oxygen conditions was studied in two toluene degrading bacteria capable of aerobic toluene degradation, *Pseudomonas putida* mt-2 and an anaerobic toluene degrader, *Thauera aromatica* K172. The central catabolic genes targeted were *xylM* and *xylE* for *P. putida* and *bssA* and *bcrA* for *T. aromatica*; the quantification was done using real-time PCR. A decrease in the expression level of *xylM* and *xylE* was observed under oxygen limiting conditions when *P. putida* mt-2 was grown on toluene as carbon source. Thus, oxygen is needed as a kind of co-inducer for the expression of the catabolic genes of the TOL plasmid. *P. putida* mt-2 was able to modulate the expression of its catabolic genes according to the oxygen availability in the media. During anoxic periods these bacteria decrease the growth rate and the expression of catabolic genes to a level which allow them to recover the activity when oxygen is present again in the medium. The addition of oxygen to *Thauera aromatica* K172 cultures grown with toluene as the carbon source immediately caused a repression of *bssA* and *bcrA* expression. In mixed binary cultures of *P. putida* and *T. aromatica*, submitted to anoxic/oxic cycles, a regulation of catabolic genes depending on the presence of oxygen was observed. After two oxic cycles *T. aromatica* showed an up-regulation of catabolic genes once oxygen was depleted by *P. putida*.

[1] Martínez-Lavanchy P.M. et al (2010): High stability and fast recovery of the expression of the toluene catabolic TOL genes of *Pseudomonas putida* mt-2 when grown under oxygen limiting and oscillating conditions. Appl. Environ. Microbiol. 76:6715-6723.

EMP011
Influence of root exudates on the structure biofilm formation in the rhizosphere

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The rhizosphere is defined as the soil compartment which is directly influenced by the activity of the plant root system. Plant roots secrete a variety of organic compounds - the root exudates - which cause the high metabolic activity of microorganisms in this special soil compartment. The stimulation of the microbial biomass compared to bulk soil is known as „rhizosphere effect". On the surface of plant roots microorganisms form biofilms which are multispecies communities enclosed by a polymeric matrix and attached to surfaces. Biofilms are the prevalent bacterial mode of life in nature rather than free-living, planctonic cells. These microbial communities give protection from the environment and enable metabolic cooperativeness between different microbial species. Furthermore, biofilms are ecologically important for driving the biogeochemical cycles on earth.
Until now, there is little known about how root exudates influence the development of root-associated biofilms. To assess this question we established an artificial rhizosphere system to manipulate root exudation and analyze the influence of exudate compounds on the microbial community in the rhizosphere.
Our artificial rhizosphere system consisted of a glass slide which was covered by an exudate mix containing carbohydrates, organic acids and amino acids. The glass slides were incubated in soil and the diversity of the attached microbial communities was analyzed with the molecular fingerprinting technique T-RFLP (Terminal Restriction Fragment Length Polymorphism) based on 16S rRNA gene amplicons. To validate the system we compared microbial community structure of our artificial biofilms with the „real" rhizosphere of *Arabidopsis thaliana* Col 0. Subsequently, we tested the influences of following exudate compounds on biofilm formation: Glucose, malic acid and serine. The results show that the artificial rhizosphere system is reproducible as well as comparable to the rhizospherial microflora of *Arabidopsis thaliana* Col 0. The tested root exudates indicate to have an influence on the temporal development of rhizosphere microbial community.

EMP012
Worker's exposure to airborne microorganisms in paper recycling facilities

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In 10 facilities in which waste paper and cardboard are sorted and packed prior a further processing, worker's exposure to airborne microorganisms at two permanent workplaces (delivering area and sorting cabin) was investigated. Culture-dependent the concentrations of airborne moulds and bacteria were determined using the agar based media DG-18 and TSA. The total cell counts were quantified in the inhalable dust fraction after DAPI staining using a fluorescence microscope.
Depending on the examined facility the concentration of cultivable bacteria in delivering area varied between 1.6×10^3 and 2.8×10^5 CFU per m^3 whereas the concentration of airborne moulds extend to 1.8×10^6 CFU per m^3. The concentrations of cultivable airborne bacteria and moulds in investigated sorting cabins ranged between 1.9×10^3 and 8.9×10^4 CFU and 1.8×10^3 and 6.8×10^5 CFU per m^3 air, respectively. The total cell count in the corresponding samples generally exceeded the detected concentration by cultivation approaches.
All quantification approaches clearly showed a workplace related exposure to microorganisms which was at least one magnitude higher as in corresponding outdoor samples. Indeed, in 70% of the examined sorting cabins the technical control value for moulds of $5x10^4$ CFU per m^3 defined for waste management facilities was exceeded.
Based on morphological features the prevalent cultivated moulds were identified as species of the genera *Penicillium*, *Aspergillus* and *Cladosporium*. The predominant bacterial genus was *Staphylococcus,* which was determined by analyses of 16S rRNA gene clone libraries. Additionally

the clone library analyses indicated bacterial sequences which were most closely related to *Aerococcus viridans*, *Pantoea agglomerans* and *Acinetobacter* spp. which are well known as causatives of different respiratory diseases. These results underline the necessity of improved ventilation and last but not least adequate breathing protection at special workplaces.

EMP013

Structure and function of an *m*-xylene degrading, sulfate-reducing enrichment culture revealed by molecular and stable isotope tracer techniques

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A *meta*-xylene degrading, sulfate-reducing mixed culture originally enriched from ground water of a hydrocarbon contaminated field site was investigated in this study. Xylene-isomers belong to the group of BTEX compounds (benzene, toluene, ethylbenzene, xylene) and as toxic and common substances they all represent a big threat to humans and the environment. The aim of the study was to get valuable insights into the anoxic degradation of such compounds following the incorporation of ^{13}C within the proteins of the microbial community (Protein-SIP) [1]. Stable isotopes such as ^{13}C serve as tracers which can be detected in the biomass and the metabolic end products.
For ^{13}C-labeling, we grew the culture using *m*-xylene labeled with ^{13}C at both methyl groups (^{13}C-content of *meta*-xylene: 25 atom%). Control cultures were grown with non-labeled *m*-xylene, acetate and benzoate, respectively. Protein analyses were carried out by 1-DE gels and UPLC Orbitrap-MS/MS.
Labeled and non-labeled *m*-xylene was metabolized in similar rates with sulfate as electron acceptor. Two species dominated the enrichment culture under all cultivation conditions, as revealed by Terminal Restriction Fragment Length Polymorphism (T-RFLP) analyses. One phylotype is affiliated to members of the genus *Desulfobacterium*, the other is related to *Epsilonproteobacteria*. The *Desulfobacterium* phylotype is believed to degrade *m*-xylene. The metabolic function of the *Epsilonproteobacterium* is not yet known.
About 110 proteins were identified and the majority belonged to members of *Deltaproteobacteria*. Proteins of the following metabolic pathways were found: xylene degradation, sulfate reduction and C1 metabolism. The preliminary protein analyses of both ^{12}C- and ^{13}C- xylene samples revealed that the majority of *Deltaproteobacteria* peptides contained approximately 20 atom% ^{13}C, indicating that both methyl-groups were predominantly assimilated by the *Deltaproteobacterium*. The time course of ^{13}C-incorporation will be tracked by a time-series experiments in the near future

[1] Jehmlich, N. et al (2010): Protein stable istope probing (Protein-SIP). Nat. Protoc. 5 (12), 1957-1966.

EMP014

Insights into an anoxic benzene degrading consortium provided by protein based stable isotope probing (Protein-SIP)

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Microbial communities play a key role in the Earths biogeochemical cycles, performing a huge variety of complex converting and degradative processes under oxic and anoxic conditions, e.g. the degradation of benzene. Benzene is a major environmental contaminant of anthropogenic source, belonging to the group of BTEX compounds (benzene, toluene, ethylbenzene, xylene). It is highly stable due to resonance stabilization of the π electron system, turning its degradation into a biochemical challenge especially under anoxic conditions. Benzene is posing a threat to human health and environment due to its toxic and carcinogenic effects. Although it is a widespread pollutant, knowledge about its degradation under anoxic conditions is still sparse. One of the reasons is a lack of suitable methods for analysing complex microbial communities. To open new ways for the analysis of microbial communities, we expanded the classical stable isotope probing (SIP) methods to metaproteomic analysis [1]. This method is based on the analysis of metabolization of substrates labeled with nonradioactive heavy isotopes (e.g. ^{13}C), and the subsequent incorporation of the label into proteins. High resolution mass spectrometry is used to detect the heavy isotope incorporation on peptide level, together with the identification of peptides and subsequently of proteins. This allows a direct linkage between taxonomic and functional information as well as metabolic conditions, hence offering a powerful tool to study trophic structures of microbial communities.
Object of our research is a benzene degrading, sulfate reducing culture from a contaminated aquifer near Zeitz, Saxonia-Anhalt. First clues on taxonomic composition of the culture have been acquired by DNA-SIP experiments [2]. In our recent Protein-SIP study either $^{13}C_6$-labeled benzene or ^{13}C-labeled carbonate is used to trace the carbon flux within the microbial culture, allowing an overview of the usage of these carbon sources. A time resolved picture of the metabolization and utilization of the labeled carbon was achieved by sampling at several times during cultivation. Extensive analysis of the metaproteome also allowed the identification of proteins possibly involved in sulfate reduction and aromatic hydrocarbon degradation. Combining metaproteomic information on phylogeny and metabolic activity will enable us to draw a more detailed picture of the process of anaerobic benzene degradation.

[1] Jehmlich et al. (2010): Protein-stable isotope probing (Protein-SIP). Nature Protocols. 5 (12), 1957-1966.
[2] Herrmann et al. (2009): Functional characterization of an anaerobic benzene-degrading enrichment culture by DNA stable isotope probing. Environ Microbiol. 12(2):401-411.

EMP015

Effects of a genetically modified potato line with altered starch metabolism on carbon fluxes within the plant-soil system and on microbial community structure and function in the rhizosphere

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From the two potato starch components amylose and amylopectin, the second one is of greater interest for industry. To avoid the costly process of separating, genetic engineers developed a potato cultivar, which contains only amylopectin by blocking amylose production through insertion of an artificial gene with antisense orientation to the starch synthase gene. Despite the use of a tuber-specific promoter, it cannot be excluded that the genetic modification might affect the whole plant metabolism, resulting in modified root exudation pattern and thus in altered microbial community structure in rhizosphere. While most rhizosphere microorganisms provide benefits to their host plant, this in turn may reduce plant growth and health.
Hence, to assess potential effects of genetically modified (GM) amylopectin-accumulating potato line #1332 (*Solanum tuberosum* L.) on carbon transformation within the plant-rhizosphere system with special focus on changes in rhizosphere community pattern, greenhouse and field studies were conducted. Besides the parental variety 'Walli', a second non-transgenic potato cultivar was planted, in order to relate possible GM-dependent effects to natural variation among different plant genotypes. Rhizosphere samples were taken at young leaf developmental and at flowering stage of potatoes. For investigation of carbon fluxes within the plant-rhizosphere system and microbial community structure, ^{13}C stable isotope probing in combination with phospholipid fatty acid analysis was chosen. To get a more detailed insight into rhizosphere microbial populations, abundance pattern of the potato pathogen *Phytophthora infestans*, of plant beneficial microbes (*Pseudomonas* spp., *Trichoderma* spp.), and of functional groups involved in soil mineralization processes were examined using quantitative real-time PCR.
Our results revealed that the genetic modification did affect neither carbon fluxes from plant into soil nor microbial community structure and activity in the rhizosphere. Furthermore, no difference in abundance pattern of phylogenetic groups and functional genes under investigation between the GM line and its parental variety was observed. Nevertheless, the non-transgenic potato cultivars varied significantly regarding to all parameters under investigation, and also plant developmental stage affected carbon

fluxes via plant into rhizosphere and, subsequently, microbial community structure and gene abundance.

EMP016
Two extracellular nucleases influence biofilm formation of *Shewanella oneidensis* MR-1

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Many bacteria species, such as the gram-negative, metal-ion reducing bacterium *Shewanella oneidensis* MR-1 are known to form densely packed communities called biofilms. In this structure, cells are enclosed by a self-produced matrix of extracellular polymeric substances (EPS) consisting of proteins, exopolysacharides, lipids, and extracellular DNA (eDNA). Beside its role during biofilm formation eDNA is widespread among aquatic environments and can be exploited by *S. oneidensis* MR-1 as a source of carbon, nitrogen and phosphorus. In this study we investigated the role of two extracellular endonucleases, ExeM and ExeS, for biofilm formation and utilization of eDNA. In contrast to ExeS, which was previously found in supernatants, ExeM contains a predicted membrane anchor and has been identified in outer-membrane fractions of *S. oneidensis* MR-1 cultures. We demonstrated that deletions of both nucleases do not influence the ability of utilizing eDNA. Interestingly, biofilm formation is influenced in mutant strains. Under static conditions, a strain lacking *exeS* forms a more robust biofilm, whereas the mutation does not affect biofilms under hydrodynamic conditions. Deletion of *exeM* results in a strongly decreased biofilm in a static assay. Under hydrodynamic conditions, Δ*exeM* forms more densely packed structures covered by a very thick layer of eDNA compared to *S. oneidensis* MR-1 wild type. In addition, the expression of both nucleases is differentially regulated during biofilm formation and is under control of two master regulatory systems, the Arc and the cAMP/CRP system. These results indicate an important role of the two extracellular nucleases during biofilm formation by degradation of the important structural matrix component eDNA.

EMP017
Role of flagellar glycosylation for *Shewanella oneidensis* MR-1 motility and flagellar assembly

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The γ-proteobacterium *Shewanella oneidensis* MR-1 is motile by a single polar flagellum. Depending on the environmental sodium-ion concentration, the flagellar motor is driven by Na^+- or proton gradients. In the past years it has been elucidated that flagellins, the major structural components of the flagellar filament, of many bacteria are posttranslationally modified by glycan species via *O*-linkage. Glycosylation of flagellin appears to be more common as previously thought; however its underlying mechanism is quite versatile among prokaryotes. It has been shown that glycosylation of flagellin subunits plays an important role in virulence of many bacterial pathogenic species and can also be involved in the flagellar assembly.
A transposon mutagenesis screening for motility identified a FliA (σ^{28})-dependent gene cluster encoding for proteins which may be important for glycosylating proteins. This prompted us to elucidate the role and function of glycosylation of two identified flagellins for the assembly of a functional flagellum and the motility of *S. oneidensis* MR-1. Mutations in each of the five genes within the cluster displayed a defect in flagella mediated motility on swarm plates which was due to aberrant flagellar assembly, which was also confirmed by electron microscopy. Alteration in flagellar assembly and motility was most likely due to posttranslational modification as mutants lacking genes of the putative glycosylation cluster displayed a significant mass shift of the major flagellin subunit. MALDI-TOF and LC coupled tandem MS analyses of purified flagellar filaments clearly identified glycosylation at least at 13 potential sites of either serine or threonine within the variable region of both flagellin subunits of *S. oneidensis* MR-1. The exact sugar composition and the resulting glycan structure remains to be determined.
Concluding from these results we could demonstrate that *S. oneidensis* MR-1 possesses two flagellin subunits which are required to assemble a functional flagellar filament and that glycosylation of these subunits is essential for flagellar assembly and function.

EMP018
Will not be presented!

EMP019
Identification and characterisation of microbial communities converting hydrocarbons to methane

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Against the backround of decreasing conventional resources, especially oil, the search for new energy sources becomes increasingly important. One contribution might be the microbial conversion of oil or coal to methane. In recent years, the exploration of deep microbial life in the earth´s subsurface has become an intriguing and challenging new topic in modern geoscience. Still, only little knowledge has been gained about the metabolic processes and the involved microorganisms in methanogenic hydrocarbon biodegradation.
Consequently, in the DFG-SPP 1319 project we started to investigate the physiological characteristics of microbial consortia with enrichment cultures. These microbials are capable to degrade alkanes and aromatic hydrocarbons under methanogenic conditions. Our study aims at investigating the specific methanogenic community composition participating in the hydrocarbon degradation process. This will lead to a better understanding of the actively degrading microorganisms, their mechanistic aspects, formation of metabolites, kinetics and carbon flows.
Laboratory microcosms from different habitats (e.g. freshwater ditches, marine sediments, contaminated aquifers) showed high methane production rates after the addition of oil or coals, and single hydrocarbons. The T-RFLP fingerprints of microbial enrichments showed a large bacterial diversity while the archaeal one was limited to three or four dominant species. The quantification showed high abundances of *Archaea* and *Bacteria* in all enrichment cultures. Genes indicative of metal reduction, sulphate reduction, and methanogenesis were also detected in high numbers in these incubations. In a second stage we performed stable isotope probing experiments with several ^{13}C-labelled substrates to reveal the carbon flow in freshwater enrichments. These experiments revealed after molecular and biochemical analysis the active community taking part in degrading the hydrocarbons.
In conclusion our study has shown that an active hydrocarbon degrading community can be enriched from different habitats. These methanogenic consortia will be further characterised to evaluate enzymatic pathways and the individual role of the syntrophic partners.

EMP020
Will not be presented!

EMP021
Spatial and temporal dynamics in a tar oil contaminated aquifer and its biochemical consequences to biodegradation

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Introduction of organic contaminants, in a nature state, as aromatic hydrocarbons into a porous aquifer leads to the formation of a plume with a characteristic redox zonation along its horizontal and vertical axes. The identification of steep small-scale physical-chemical and microbial gradients by high-resolution multi-level sampling of groundwater and sediment allowed the identification and localization of biodegradation processes in a tar oil contaminated sandy aquifer in Duesseldorf, Germany. Sulfate

reduction was found to be the dominant redox process coupled to BTEX and PAH oxidation. Five years of repeated sampling revealed pronounced vertical dynamics of physical-chemical and microbial gradients including the spreading of the contaminants with time. These dynamics can be hypothesized to either enhance (via increased mixing) or hamper (by disturbance of established sessile degrader populations) net contaminant removal. There is serious evidence from compound specific stable isotope data and from microbial community analysis that minor hydraulic changes have the potential to impair key degrader populations. Now, the timescale of the temporal and spatial dynamics of biodegradation is focus of our current work. It is still poorly understood how attached microorganisms cope with the unpredictable changes in environmental conditions and how fast they can adapt to the changing redox conditions. Recently, three sampling campaigns proved short-term dynamics for e.g. toluene, sulfate and sulfide along a vertical plume cross section. While microbial patterns, i.e. total cell numbers and active biomass (ATP) lagged behind. In conclusion, severely contaminated sites with highly specialized anaerobic degrader populations are characterized by low resilience. Minor and short-term hydrogeochemical dynamics were found to interfere with effective overall biodegradation, with degraders lacking behind the associated physical-chemical changes.

EMP022
Niche partitioning among nitrite-oxidizing bacteria

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Nitrification is of fundamental significance for the global nitrogen cycle and recent discoveries of novel microorganisms refreshed the traditional textbook knowledge. Chemolithoautotrophic nitrite-oxidizing bacteria (NOB) perform the second step of nitrification and, in contrast to ammonia oxidation, no archaea were identified so far to perform this reaction. NOB are phylogenetically diverse and belong to different subclasses of the *Proteobacteria* or the deeply branching phylum *Nitrospirae.* An increasing diversity of novel strains and even genera became available when the growth parameters were better adapted to natural conditions. With regard to their ultrastructure, NOB can be separated into two groups, characterized by the presence or absence of intracytoplasmic membranes (ICM). NOB without ICM, like *Nitrospira* and the new candidate genus *Nitrotoga*, possess an extended periplasmic space, which serve as cell compartment for the energy gaining reaction. Simultaneously, *Nitrospira, Nitrospina* and *Nitrotoga* are adapted to low substrate concentrations, whereas *Nitrobacter* and *Nitrococcus* containing ICM are very tolerant against nitrite. Besides nitrite, temperature has been identified as another ecophysiological factor determining niche separation. For example, *Nitrotoga* was detected in permafrost-affected soils and prefers temperatures below 20°C. The most versatile genus *Nitrospira* occurs in a wide range of habitats and dominates in geothermal springs with temperatures up to 60°C, where a coexistence of several new species was found. Additionally, *Nitrospira* is the key organism of nitrite oxidation in engineered ecosystems like activated sludge or recirculation aquaculture systems. Here, it has to compete for the substrate with *Nitrotoga* and *Nitrobacter* [1]. Members of *Nitrospina* and *Nitrococcus* are restricted to marine habitats and despite the aerobic nature of nitrification, some NOB are also active under microaerophilic conditions. For example, a novel species of *Nitrospina* originated from the suboxic zone of the Black Sea and the strain was co-isolated with an unknown heterotrophic gammaproteobacterium. Therefore, the whole physiological potential of NOB and their interaction with accompanying bacteria remain to be uncovered.

[1] Alawi et al (2009): Environ. Microbiol. Reports 1, 184.

EMP023
Sequence Comparison and Gene Deletion of Three Redundant Oxygenase Subunits of (Chloro-) Phenol Hydroxylases in *Rhodococcus opacus* 1CP

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Rhodococcus opacus 1CP is a gram-positive bacterium and belongs to the class of *Actinobacteria*. Strain 1CP has the ability to use a wide range of (chlorinated) aromatic compounds as sole energy and carbon sources. Phenol, 2-chlorophenol, 4-chlorophenol, 2,4-dichlorophenol, and 4-methylphenol are degraded *via* their corresponding catechols. These central intermediates are then further catabolized by enzymes of the (modified) *ortho*-cleavage pathway which have been shown to differ significantly from their counterparts in *Proteobacteria*.
Three putative two-component phenol hydroxylases could be identified in *R. opacus* 1CP of which one (*pheA*(1)) was found to be located on the megaplasmid p1CP. All of them seem to play an active role in the degradation of phenol as indicated by their expression pattern. However, protein purification proved to be extremely difficult due to a highly similar chromatographic behavior. Attempts were additionally hampered by a low stability.
Homologs of all of these phenol hydroxylases could also be found in *Rhodococcus jostii* RHA1 by database comparison and it is remarkable that the RHA1-equivalent of *pheA*(1) is localized on pRHL1 and thus shows a plasmid location, too.
To elucidate the catabolic functions of the three two-component phenol hydroxylases in *R. opacus* 1CP during the degradation of chlorinated/methylated phenols the corresponding oxygenase subunits were inactivated by gene knockout. In total seven mutants were generated and characterized by their growth parameters on phenol and methylphenol. Gene deletion was confirmed by DNA sequencing and by analyzing protein expression.

EMP024
Subtyping of F17- related genes in the wastewater from small abattoirs

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The zoonotic pathogens of E.coli can survive over long periods in sewage sludge as well as on pasture land and in association water systems. They could be widely spread in the environment by direct land application of sludge or by regular contamination of surface water, but limited information is available concerning the spreading of these pathogens in sewage of slaughterhouses. The F17 family includes F17a, F17b, F17c, F111 fimbriae produced by bovine E.coli strains , positive Escherichia coli isolates. A total of 88 wastewater samples were collected in wastewater treatment plants at different stages of wastewater processing in small abattoirs, located in different regions in France, screened for the presence of F17 genes (F17 a- A gene, F17 b- A gene, F17c-A/gafA gene and F111-A gene) by multiplex PCR . F17 positive E. coli isolates were 47 samples , detection of the virulence factor F17 (F17 a- A gene, F17 b- A gene, F17c-A/gafA gene and F111-A gene) in the positive E. coli strains showed that the more frequent genes are F17c-A/gafA gene and F111-A gene and the less frequent gene is F17 b- A gene . suggesting that they could be spread into the environment. Our results suggest that the diversity of the E. coli-associated virulence factors in the strains indicates that the environment may play an important role in the emergence of new pathogenic E. coli strains and to increase our knowledge of the important prevention needed in our environment from the pathogenic E. coli and their mutual correlation.

EMP025
Fungi on *Abies grandis* wood

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Abies grandis (Grand Fir) is a neophyte in Germany. Little is known about fungi infesting its wood. In this study we isolated fungi from dead wood from forests for molecular identification. Most species were either ascomycetes or related deuteromycetes but also a few basidiomycetes were detected, some of which are brown rots. Most of the isolates could only be identified to the genus level and some only to a family level, indicating a large range of unknown fungi occuring on dead wood. Generally, for most tree species dead wood has yet little been studied with molecular methods in terms of fungal occupants. Conservative estimates predict that 1.5 million different fungi exist worldwide, most of which are still undiscovered. The study showd that dead wood represents one of the biotops in which many missing fungal species can be detected.

EMP026
The environmental fate of the NSAID fenoprofen - Microbial transformation processes in water sediment systems

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During the last two decades the availability of highly sensitive MS-based techniques of organic trace analysis allowed the detection of cocktails of pharmaceutical residues in large parts of the aquatic environment. As a consequence considerable concern about the (eco)toxicological impact of such compounds has arisen. Compared to the vast number of studies dealing with the detection of drugs in the environment very little is known about the mechanisms of microbial transformations representing an important sink for many of those compounds. Knowledge on metabolic pathways and on involved intermediates is of considerable interest in order to better assess the (eco)toxicological impact of pharmaceutical residues.
The present work deals with investigations towards the microbial transformation of the non-steroidal anti-inflammatory drug (NSAID) fenoprofen by fresh water and sediment of the creek Münzbach (Freiberg, Saxony, Germany). It was shown by RP-HPLC that spiked fenoprofen loads between 160 µM up to 180 µM were eliminated within 10 to 20 days in the presence of sediment and water. Comparison of removal rates of this drug in the presence of water as well as by a water/sediment mixture pointed to a localization of fenoprofen-transforming activity in the aqueous phase.
In such incubations the (transient) occurrence of two different metabolites could be detected. These compounds could be characterized by ^{1}H-NMR to be 4′-hydroxyfenoprofen (3-(4-hydroxyphenoxy)-α-methylbenzeneacetic acid) and 3-hydroxyphenyl-α-methylacetic acid and a complete degradation by hydroxylation and ether cleavage is suggested.

EMP027
Detection of antibiotic resistances in surface water using culture methods and PCR

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Antibiotic resistances represent a serious problem in clinical therapy, and resistant bacteria have been detected frequently in hospitals. However, only limited data are available with respect to microbial antibiotic resistances in the environment.
In order to assess the occurrence and transport of antibiotic resistances in the aquatic environment, water samples were analyzed using culturing methods as well as molecular biological techniques (polymerase chain reaction, PCR). 100 coliform bacteria from River Rhine water were isolated and screened for antibiotic resistance by determining the minimum inhibition concentration (MIC) of trimethoprim/sulfamethoxazol, tetracycline, amoxicillin, gentamicin, meropenem, cefotaxime, ciprofloxacin and chloramphenicol in agar diffusion assays. For the coliform bacteria, resistances against amoxicillin, trimethoprim / sulfamethoxazol and tetracycline were observed. Some bacteria were multiresistant against two, three or four of the tested antibiotics (4%, 5%, and 1%). Using PCR analysis, most of the observed resistances could be attributed to specific resistance genes.
PCR also was applied to analyze resistance genes in water samples without pre-cultivation. The bacteria were concentrated via filtration and total DNA was extracted. The DNA was analyzed for 24 resistance genes according to eight antimicrobial groups. In conclusion, genes encoding for resistance against sulfonamides, trimethoprim, tetracycline, and erythromycin were detected frequently in surface water samples. In particular the resistance genes *sul1*, *sul2*, *dfrA1*, *tet*(C) and *erm*(B) proved to be important.
The authors gratefully acknowledge financial support by the German Waterworks Association (DVGW, project no. W1/01/05).

EMP028
Investigation of the Microbial Gut Flora in Vietnamese Stick Insect *Medauroidea extradentata*

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Insects contribute positively to the earth´s ecosystem, but less is known about their microbial gut population. This also applies to our experimental organism *Medauroidea extradentata,* which eats only leafs and lives under wet tropical conditions. Thus, we focused onto its bacterial gut flora in respect to microbial diversity. The blackberry leafs are highly populated with different bacteria and fungi. However, the gut is scarcely populated as shown by raster - and transmissions electron microscopy as well as microbiological methods. We isolated a facultative anaerobic, Gram-negative enterobacterial species from the gut of the stick insect. This bacterium is classified into the genus *Kluyvera spp.* basicly due to several biochemical reactions and to sequence analysis. Therefore we compared characteristics of our isolate with those of *Kluyvera cryocrescens* type strain (DSM 4588). The biochemical reactions of our isolate within api®20 E and ID 32 E support the affiliation to *Kluyvera spp.* and the 16S rRNA sequences of both *Kluyvera* strains shared 99% identities. In contrast, antibiograms (Mastring M-14) of both strains revealed surprising results. Our isolate showed resistance to eight different antibiotics whereas the type strain was resistant to only three. The multidrug resistance probably accounts for an integron I system, because a PCR amplification of the corresponding integrase gene was positive, when primers detecting the highly conserved enterobacterial integrase genes were used. We further investigated the genoms of both *Kluyvera* strains for vitamin B_{12} transporters. A putative BtuB, the B_{12} uptake protein associated with the outer membrane of Gram-negative bacteria was present in both enterobacterial strains. Their genes were sequenced, cloned into (plasmids) and expressed in *E. coli*.

EMP029
Structure and mechanism of the diiron benzoyl coenzyme A epoxidase BoxB

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A recently elucidated coenzyme A (CoA) dependent aerobic benzoate metabolic pathway [1] uses an unprecedented chemical strategy to cope with the high resonance energy of aromates by forming the non-aromatic 2,3-epoxybenzoyl-CoA [2]. The crucial dearomatizing and epoxidizing reaction is carried out by BoxB and the two required electrons are delivered by BoxA, a NADPH dependent reductase. We determined the X-ray structure of the key enzyme BoxB from *Azoarcus evansii* including the diiron center without and with bound benzoyl-CoA in the diferric and semi-reduced states, respectively [3]. Complementary Mössbauer and EPR spectroscopic studies revealed the later as well as the diferrous state [3]. These studies showed that the semi-reduced state with bound benzoyl-CoA is a prerequisite for O_2 activation. The crystal structures reveal redox dependent

structural changes, most significantly the movement of Glu150 from a diiron bridging in the oxidized, to a not ligating position in the semi reduced substrate bound state. In contrast to other members of the class I diiron enzyme family the position of benzoyl-CoA inside a 20 Å long channel is accurately known indicating that the C2 and C3 atoms of its phenyl ring are closer to one of the irons (Fe1), and that the attacking oxygen of activated O_2 is essentially ligated to Fe1. We postulate a reaction cycle with a radical attack of this oxygen on C2 leading to a delocalization over the CoA thioester as the essential step. The substrate bound structure doubtlessly indicates the stereoselective 2S,3R-epoxide formation by BoxB.

[1] Zaar, A. et al (2001): A novel pathway of aerobic benzoate catabolism in the bacteria *Azoarcus evansii* and *Bacillus stearothermophilus*. J Biol Chem, 276(27): p. 24997-5004.
[2] Rather, L.J et al (2010): Coenzyme A-dependent aerobic metabolism of benzoate via epoxide formation. J Biol Chem. 285(27): p. 20615-24.
[3] Rather, L.J. et al: Structure and mechanism of the diiron benzoyl coenzyme A epoxidase BoxB, to be published.

EMP030
Bacterial succesion and enzyme activity in two glacier forefield chronosequences on Larsemann Hills, East Antarctica

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Beside the Antarctic Peninsula, the Prydz Bay area in East Antarctica is one of the main regions affected by global warming[1][2]. Increasing temperatures lead to the retreat of glaciated areas, whereby new terrain is becoming exposed to soil formation and accessible for microbial colonisation. On the one hand it is important to find out how these habitats develop due to climate change, on the other hand Antarctic glacier forefields provide a unique opportunity as a natural laboratory to study primary succession in connection to microbial communities in extreme environments. A polyphasic approach, containing geochemical and microbiological examinations, will be used to describe the habitat characteristics and the complex system of microbial communities in two glacier forefield chronosequences on Larsemann Hills, East Antarctica. Preliminary results in molecular fingerprinting (DGGE) indicate a higher diversity in the vicinity of the glaciers, which is being confirmed by T-RFLP analysis. Enrichment cultures on two different media were used to determine the number of cultivable heterotrophs and to isolate and characterise selected microorganism. The number of cultivable heterotrophs increases with increasing distance to the glacier. Isolates obtained from the Glacier Transect could be classified as *Actinobacteridae*, *Sphingobacteria, Flavobacteria* and *Alpha-* and *Betaproteobacteria*. Additional classes in the Black Valley Transect are *Cytophagia*, *Gammaproteobacteria* and *Deinococci*. *Actinobacteridae* dominate in both transects. Present results suggest a lower microbial density but higher diversity in the vicinity of the glacier. A colonisation gradient along the chronosequences could be assumed but will have to be proven in further analyses. Furthermore enzyme activity tests for protease, urease, saccharase, glucosidase and phosphatase shall reveal how microbial processes are related to nutrient and energy fluxes in the initial developing habitat.

[1] Temperature increases in the Antarctic due to climate change, 2090 (NCAR-CCM3, SRES A2 experiment). (2008). In *UNEP/GRID-Arendal Maps and Graphics Library*. Retrieved 11:17, December 13, 2010 from http://maps.grida.no/go/graphic/temperature-increases-in-the-antarctic-due-to-climate-change-2090-ncar-ccm3-sres-a2-experiment.
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EMP031
Dynamics of methane cycling microbial communities in degrading permafrost-affected ecosystems on Herschel Island, Canadian Western Arctic

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In the current context of climate change, the main goal of the research presented is to elucidate the fate of organic carbon stored in permafrost by looking at microbial-driven carbon degradation in the active layer of permafrost affected soils from Herschel Island in the Canadian Western Arctic. The abundance, dynamics and function of microbial communities involved in consuming this organic carbon is analysed, especially those involved in methane production and consumption. Microorganisms involved in methane cycling are of particular relevance in frozen environments, as rising permafrost temperatures eventually lead to an increased degradation of previously conserved organic matter. This in turn leads to an increased methanogenic activity, creating a potentially dangerous positive feedback-loop for climate change. The point of focus here is the biodiversity and function of methanogenic and methanotrophic microorganisms thriving in such difficult conditions and their reaction to warming temperatures and a rapidly changing environment.
Undergoing research activities include terminal-restriction fragment length polymorphism (T-RFLP) analysis of methanogenic and methanotrophic communities in an active layer profile from Herschel Island, showing preferential colonisation of the middle and lower anaerobic layers by methanogenic archaea (5cm to 35cm depth) and of the higher, aerobic layers by methanotrophic bacteria (0cm to 20cm depth). Incubation experiments under controlled variables at 10°C with no added substrate revealed a high methane production rate from middle (3.2 nmol g^{-1} h^{-1} at 15cm depth) and lower (3 nmol g^{-1} h^{-1} close to the permafrost table) active layer samples, and a lower but nonetheless consequent methanogenic activity in the upper sediment layers**.** Soil characteristics including soil moisture, C/N ratio, total organic carbon content and grain size were also investigated in order to help elucidate the observed distribution of microorganisms. Active layer samples generally have a high water content (41-88%) and very high organic carbon content (20-42%). The results obtained are being scrutinized to elucidate the adaptability of methane cycling microbial communities and the fate of organic carbon in the active layer.

EMP032
Application of two-dimensional compound-specific isotope analysis for aerobic and anaerobic oxidation of ethylbenzene

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Some microorganisms have the ability to degrade environmentally dangerous hydrocarbons such as BTEX compounds (benzene, toluene, ethylbenzene, xylenes). Compound-specific isotope analysis (CSIA) is a method to detect and quantify *in situ* biodegradation processes. It is based on the observation that enzymes prefer molecules containing lighter stable isotopes (e.g. ^{12}C, ^{1}H) over the ones containing heavier stable isotopes (e.g. ^{13}C, ^{2}H). Measurement of isotope ratios of two elements may provide additionally insight into the reaction mechanisms and thus distinguish between different pathways or predominant redox-conditions. This is expressed by the slope Λ of the linear regression for hydrogen ($Dd^{2}H$) versus carbon ($Dd^{13}C$) discrimination. The factor Λ can be seen as a fingerprint of the initial biochemical bond cleavage reaction within a distinct degradation pathway.
We applied two-dimensional CSIA to investigate aerobic and anaerobic oxidation of ethylbenzene by microorganisms using different degradation pathways. Ethylbenzene dehydrogenase is the initial enzyme in the denitrifying *Aromatoleum aromaticum* strain EbN1. It is able to oxidize the side-chain of non-activated ethylbenzene without molecular oxygen as co-substrate. The naphthalene dioxygenase of *Pseudomonas putida* NCIB 9816-4 has a relaxed substrate specificity and catalyzes the benzylic monooxygenation and dioxygen-dependent alcohol oxidation of ethylbenzene. Although both enzymes lead to the same intermediate (*S*)-1-phenethyl alcohol, our results show different Λ values for both pathways. This indicates that both reaction mechanisms can be principally distinguished by two-dimensional isotope fractionation analysis.
Furthermore we can demonstrate that the cytochrome P-450-like naphthalene dioxygenase shows especially low Λ factors for initial BTEX-attacking reactions compared to other monohydroxylations.

EMP033
Bacterial Identification for Environmental Monitoring Using MALDI-TOF

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Most technologies and databases designed for rapid identification of microorganisms are designed with clinical isolates in mind. When adapted for use in the microorganism monitoring programs of pharmaceutical,

nutraceutical, and sterile manufacturing environments, these technologies under perform due to the diversity of organisms found in these environments. Whole cell proteotypic analysis with matrix-assisted laser desorption/ionization - time of flight (MALDI-TOF) spectroscopy is an emerging rapid and inexpensive method of identifying bacteria. This study tests the accuracy, robustness, and reproducibility of this technology when applied to an extensive panel of known culture collection strains and frequently seen species found in pharmaceutical, nutraceutical and sterile manufacturing environmental monitoring programs. The MALDI-TOF manufacturer's recommended methods for sample processing and data analysis are directly compared to methods optimized in our laboratory for environmental monitoring. Advantages and limitations of using MALDI-TOF technology for this application are discussed. This study showed that fewer than 70% of frequently seen environmental isolates could be accurately identified with the MALDI Biotyper™ system (Bruker Daltonics). The >30% that failed to be identified were largely due to limitations in the clinically focused Biotyper™ database. At Accugenix, the methods were optimized and additions made to the library such that the number of samples that were identified increased. While a custom database and method modifications are required to optimize this technology for environmental monitoring applications, the low cost and rapid turn around time of MALDI-TOF analysis remain strong advantages for this technology.

EMP034
Detection and activity of halorespiring bacteria at chloroethene contaminated sites

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Chloroethenes are frequently detected in contaminated groundwater. Bacteria from several genera - e.g. *Dehalobacter*, *Desulfomonile*, *Desulfitobacterium* and *Desulfuromonas* - are able to reductively dechlorinate / halorespire perchloroethene (PCE) and trichloroethene (TCE) to cis-1,2-dichloroethene (cDCE), whereas only bacteria from the *Dehalococcoides* cluster are known to catalyze the complete reductive dechlorination of PCE to ethene.
We investigated the distribution and growth of chloroethene-degrading microorganisms in groundwater samples and laboratory microcosm experiments. Using nested PCR, we measured the occurence of the dechlorinating species in groundwater samples. The presence of halorespiring bacteria correlated well with hydrochemical site data, pollutant distribution and the degree of dechlorination observed in microcosm studies. Growth of *Dehalococcoides* spp. in microcosms, assessed by quantitative PCR (qPCR), corresponded with the dechlorination of PCE to ethene. An inhibition in growth was observed at lower pH, whereas *Desulfitobacterium* spp. was less affected by pH changes.
In conclusion, this study demonstrates that 16S-PCR detection of halorespiring bacteria represents a quick and easy means to estimate the degree of reductive dechlorination of chloroethenes to occur at a given site.
The authors gratefully acknowledge financial support by the German Ministry of Economics (BMWi, grant no KF2265705AK9).

EMP035
In situ physiology of *G. metallireducens* under low growth rates during cultivation in retentostat

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The physiology and gene regulation of microorganisms in batch laboratory cultures is well established but our knowledge of gene regulation under carbon limited growth conditions such as in chemostats is scares. Basically nothing is known about microbial physiology under *in situ* conditions in soils or sediments. The current study is devoted to examine the *in situ* physiology of the aromatics-degrading anaerobic deltaproteobacterium *Geobacter metallireducens* under close to natural conditions. Our hypothesis is that under natural conditions (i.e. low amounts of carbon sources, low growth rates, and mixed substrates) all carbon sources will be utilized simultaneously relatively to conditions with high substrate concentrations and/or mixed substrates where diauxic growth prevail and one carbon source is preferentially consumed. *Geobacter metallireducens* was cultivated in a retentostat with medium supply of 50ml/h. Either a single carbon source (5mM acetate) or two carbon sources (2.5 mM acetate and 0.7 mM benzoate) were fed and Fe(III)citrate was added as an electron-acceptor in both cases. The lowest doubling time that was achieved during the cultivation was 229 hours, which corresponds to a growth rate of 0.003 1/h. The consumption of carbon sources, reduction of electron acceptor, cell numbers and total protein concentration were examined. We will report on the growth and utilization of carbon sources by *G. metallireducens* during cultivation in retentostat. The analyses of expressed proteins involved in different substrates degradation under limited substrate conditions will be presented. The retentostat cultivation will be used as a model to explain microbial physiology under natural conditions.

EMP036
Degradation of Amadori products in *Bacillus subtilis*: The physiological relevance and transcriptional regulation of the *frlBONMD* operon

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Amadori products (fructosamines) constitute the first stable intermediates of the Maillard reaction and occur ubiquitously in nature. As rotting fruits and vegetables may contain up to 7% of the fresh mass as fructosamines, Amadori products are released into the soil and are presumed to serve as substrates for many soil microorganisms. The Amadori product degradation (deglycation) systems of these organisms differ with respect to mechanisms as well as substrate specificities. Fructosyl amino acid oxidases of fungi and bacteria decompose extracellular Amadori products by means of oxidation, generating the respective amino acid, glucosone and H_2O_2.
The fructosamine 3-kinases of mammals and homologous, related proteins, which are common to all forms of life, degrade intracellular Amadori compounds and thus focus on cell repair functions.
This study addresses to the deglycating system of the soil bacterium *B. subtilis* which resembles that of *E. coli*. A kinase (FrlD) phosphorylates the fructosamine at C6 prior to further cleavage by a deglycase (FrlB). The physiological importance of the encoding genes was examined, revealing the dependence of their expression for growth on fructosamines. Furthermore the complex regulation of the corresponding transcription unit was analyzed. In addition to the known regulation by the global transcriptional regulator CodY, the *frl* operon is subject to repression by the adjacent and inversely encoded FrlR. FrlR causes the strict repression during growth on substrates other than fructosamines. The expression of *frl* genes increased about 33-fold with the onset of growth on Amadori products suggesting that derepression is brought about by substrate induction. Additionally, in the first intergenic region of the operon a FrlR binding site was identified which is centrally located within a 38 bp perfect palindromic sequence. There is genetic evidence that this sequence in combination with FrlR contributes to the remarkable decrease in the transcription downstream of the first gene of the *frl*-operon.

EMP037
Global transcriptome analysis of soil microbial communities

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Global transcriptome analysis, or metatranscriptomics, is defined as the analysis of microbial gene expression patterns at the community level by massively parallel shotgun sequencing as opposed to the total gene content which is characterized by metagenomic approaches. In future, metatranscriptomics will allow us to study the functional response of complex microbial communities to environmental change. This study aimed to establish a robust procedure for generating metatranscriptome data sets from soil microbial communities. Metatranscriptomics involves (i) extraction of total RNA, (ii) depletion of rRNA, (iii) cDNA synthesis, (iv) parallel shotgun sequencing, and (v) bioinformatic analysis. The extraction of high-quality mRNA from soil is more challenging than from most other environments, mainly due to the high humic acid content. Therefore, we first developed an efficient method for extracting high-quality mRNA from soil.

Key steps in the isolation of total RNA are low-pH extraction (pH 5.0) and Q-Sepharose chromatography. Depletion of the rRNA content by subtractive hybridization appears to be more reliable than exonuclease treatment [1]. The enriched mRNA is of high integrity (RIN > 7) and purity. We currently assess different methods to produce cDNA for 454 GS-FLX Titanium pyrosequencing, in particular with regard to the proportion of rRNA to non-rRNA reads and the average read length. An increase in the average read length is crucial for accurate functional annotation and taxonomic binning. The direct conversion of mRNA into cDNA without in vitro amplification appears to be the most promising approach.

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EMP038
Effects of elevated CO_2 on microbial communities in near surface environments

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The reduction of potential greenhouse gas emissions is part of global strategies to alleviate the climate change consequences. According to the Intergovernmental Panel on Climate Change (IPCC) report of 2007, the carbon capture and storage technique (CCS) in deep geological structures (saline aquifers, gas- and oil-fields) can provide a contribution to reduce conflicts between global energy needs and the reduction of greenhouse gas emission. Our study aims at investigating impacts of a potential CO_2 leakage from deep reservoirs for near-surface ecosystems. This work is integrated into the EU funded Project RISCS (Research into Impacts and Safety in CO_2 storage) and focuses on natural CO_2 seeps (adapted sites) and non adapted test fields like the ASGARD area in Nottingham, England. Natural CO_2 seeps located in the Eastern Eifel volcanic field comprising high (90% CO_2), elevated (20%) and background CO_2 concentrations are studied. The effects of increasing CO_2 in the soil gas content on vegetation profiles and soil chemistry showed clear differences between control and elevated CO_2 site. The overall bacterial and archaeal community size decreased by one order of magnitude with increasing CO_2 concentrations, while the abundance of functional genes involved in anaerobic metabolisms (e.g. dissimilatory sulphate reductase) increased. Both, physiological investigations (activity rates) and molecular biological techniques (DGGE, sequencing) confirmed the shift towards an anaerobic and acid tolerant community under high concentrations of CO_2. The non adapted ASGARD area enables investigation of CO_2 effects before, during and after CO_2 exposure, including analysis of adaption mechanisms of pasture and crops. For this purpose a study of soil and gas chemistry, vegetation profiles, microbial communities and activities is conducted. The overall aim, combining results from both systems, is to estimate effects of CO_2 on microbial and plant communities, their recovery time after CO_2 exposure and the detection of sensitive species. The combined results from both sites will help to reveal effects of CO_2 on near-surface ecosystems, to define thresholds for CO_2 levels in the environment and to estimate risks and chances of CO_2 storage.

EMP039
Characterization of the fungal population in biofilms

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It has been recently shown that the majority of microorganisms live in biofilms as an extremely successful way of life. Only a few microorganisms have a planktonic lifestyle. Biofilms are made of a matrix of organic molecules (extracellular polymeric substrates - EPS) in which microorganisms are embedded and which offers new habitats to other organisms, such as other bacteria or fungi [1]. Bacteria and fungi benefit in this symbiotic life form of metabolic exchange, protection and genetic flexibility. By taking samples of biofilms from a municipal sewage plant, we isolated several known species from the genus *Candida* and *Trichosporon*. The most common species was *Galactomyces geotrichum* but also *Saccharomyces cerevisiae* and *Candida tropicalis*. However, sequence analysis of the ITS-regions amplified directly from biofilms revealed the presence of more than 110 so far unknown fungi. Phylogenetic analyses revealed that most of them are closely related to other species from the genus *Candida* and *Trichoderma.* Investigations whether these fungi are biofilm-specific are under way. Further comparable analysis between biofilms in lakes, rivers the sewage plant, revealed that the fungal biodiversity in the latter is larger. We also made fluorescence *in situ* hybridization to analyze the distribution and interaction of fungi and bacteria in biofilms.
To understand the life of fungi inside the biofilm we are currently using *Fusarium oxysporum.* The genome of this fungus is available and he it is readily found in biofilms [2, 3]. We constructed a strain, which expresses dsRed in nuclei. This will enable us to distinguish this strain in biofilms and quantify the proportion of genetically modified strains in competition experiments.

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EMP040
Mechanisms for the detoxification of environmental pollutants by fungi

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Fungi are widespread in the environment, especially in soil where they make up a great part of the microbial biomass. There, fungi are of particular importance as they decompose a wide range both of economically useful products (food, wood, cotton) as well as environmental pollutants (gas oil, petroleum, phenols). We have focused our studies on the fungal biotransformation of persistent and toxic pollutants such as disinfectants and biarylic compounds (biphenyl, diphenyl ether, dibenzofuran) which consist of one or two aromatic ring systems. Disinfectants increasingly reach the environment due to their increasing use in households, as components of cosmetics, and in industry, in addition to their medical applications. Most of the compounds investigated are persistent environmental pollutants and considerable effort has been devoted to study the mechanisms of their biodegradation and detoxification.
In most cases the degradation is initiated by diverse primary oxidation and hydroxylation reactions. One mechanism for detoxification by filamentous fungi involves the formation of conjugates of the hydroxylated intermediates and their excretion. Our results show that there are other important detoxification mechanisms. Thus yeasts are able to cleave the aromatic ring system of these compounds. The products formed are muconic acid derivatives as well as the corresponding lactones. Though the pollutants are toxic to the yeast strains the ring cleavage products are not. Thus, the oxidation of aromatic environmental pollutants up to ring cleavage represents another specific detoxification mechanism.
In addition the oligomerization of oxidized aromatic pollutants by radical forming extracellular fungal enzymes and various dehalogenation mechanisms (e.g. oxidative and reductive dehalogenation, dehalogenation during cycloisomerization of ring cleavage products, and dehalogenation by oligomerization) can all lead to a progressive detoxification of persistent environmental pollutants.
Thus, fungi possess many bio-degradative options in addition to those available to bacteria and hence the combination of all microbial detoxification mechanisms can contribute to clearing toxic environmental pollutants.

EMP041
Environmental dissemination and accumulation of antibiotics, human pathogens and spread of antibiotic resistance genes in wastewater-irrigated soils in the

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Wastewater reuse for irrigation is a widely used practice to alleviate water shortages. Antibiotics, pathogens and resistance determinants that are released in the environment by wastewater irrigation pose a potential risk to

the environment and to human health. As model compounds we selected the antibiotics sulfamethoxazole (SMX) and ciprofloxacin (CIP). We determined the concentrations of SMX and, CIP and their metabolites in a Mexican soil chronosequence (soil irrigated with wastewater for different time periods, from 10 to 100 years). As typical human pathogens persistent in the environment, *Enterococcus faecalis, Enterococcus faecium*, *Enterobacter spp* and *Klebsiella pneumoniae* were chosen. Furthermore, the sulfonamide resistance genes (*sul1*, *sul2*) and the fluoroquinolone resistance genes (*qnrA*, *qnrB* and *qnrS*) were selected for this study.
The antibiotics were quantified by LC-MS/MS after accelerated solvent extraction (ASE) and solid-phase extraction (SPE). The concentration range in the ASE extracts is between 0 and 177 ng/g dry mass (DM) soil for CIP and between 0 and 108 ng/g DM soil for SMX. *Enterococci* were quantified by a real-time PCR assay on basis of the 23S rRNA sequence by TaqMan PCR. Between 10^4 and 10^6 23S rRNA gene copies per g soil could be detected. The *qnr* resistance genes and the *sul1* and *sul2* resistance genes are quantified by SYBR Green real-time PCR. Bacterial CFUs/g soil were in the range of 10^6. Total bacterial cell counts (DAPI-counts) were around 10^9 CFU/g soil. Plasmid transfer rates were determined by use of a mobilizable GFP monitoring tool based on the multiple antibiotic resistance plasmid pIP501. Transfer is verified by fluorescence microscopy, antibiotic resistance acquisition and *gfp*-specific PCR. The GFP transfer rate with the *Bacillus subtilis* was about 10^{-4} per recipient, the GFP transfer rate to the detached soil bacterial community was in the same range.

[1] Arends, K. et al: GFP-labelled monitoring tools to quantify conjugative plasmid transfer between G+ and G- bacteria (in preparation).

EMP042
Biomarkers indicating the variability of methanogenic communities within Late Pleistocene and Holocene Permafrost deposits of Kurungnakh, Siberia

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Permafrost environments of the Northern hemisphere are suspected to be strongly affected by the currently observed and predicted global temperature rise. Given that about one third of global soil stored carbon is preserved in permafrost, a degradation of permafrost due to an increase of atmospheric and soil temperatures might lead to an increase in the bioavailability of recent as well as ancient carbon. Thus, an intensified microbial turnover of these particular carbon pools might cause the release of large amounts of greenhouse gases such as methane. To predict the risk for future climate and estimate the global atmospheric carbon budget, it is important to understand the microbial driven methane dynamics and their response to climate changes in the past.
Therefore, a combination of quantitative as well as qualitative analyses of recent and fossil methanogenic communities was accomplished to reveal variations in permafrost deposits of the Siberian Arctic. A 23 m long permafrost core drilled in 2002 on Kurungnakh Island, Lena River Delta, Siberia comprising deposits of Late Pleistocene and Holocene was examined, using biogeochemical and microbiological methods in context of a paleoclimate reconstruction done by Schirrmeister et al., 2002.
As a general result it is shown that lipid biomarkers and amplifiable DNA were successfully recovered throughout the whole Kurungnakh permafrost sequence with an age of up to 42 ka. Intervals of high total organic carbon and in-situ methane content were also characterized by high amounts of glycerol dialkyl glycerol tetraethers (GDGTs). GDGTs provide paleo-signals of archaeal and bacterial communities as these core lipids are relatively stable outside intact cells in geological time frames. Total GDGTs varied throughout the core but were dominated by bacterial GDGTs. Archaeal GDGTs were detected to a lesser amount but nicely mirrored the genetic fingerprints of methanogenic archaea obtained from denaturating gradient gel electrophoresis (DGGE). Sequence analyses showed a diversity of methanogens affiliated with *Methanobacteriaceae*, *Methanosarcinaceae* and *Methanomicrobiaceae*.
Both biogeochemical and microbiological methods revealed variation within the composition of past methanogenic microbial communities and showed indications of a response to climate changes.

EMP043
Assessment of the diversity of coliform bacteria in river bank filtrate and river water in Indonesia

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In Indonesia, river water is commonly used as source for drinking water production. Due to the absence of efficient waste water treatment, river water often shows strong faecal contaminations. Instead of complex and costly direct treatment of river water, river bank filtration might be an easy and efficient alternative in order to achieve drinking water of a suitable hygienic quality.
In a BMBF-funded project, drinking water treatment under tropical monsoon conditions in central Java was investigated. Within the project, the chemical, physical as well as microbiological properties of water of the Opak River as well as the river bank filtrate at the site Trimulyo were investigated.
The river water exhibited temporarily a high content of suspended solids as well as severe contaminations with faecal bacteria. *E. coli* reaches numbers of more than 10^4 cells per 100 mL, coliform bacteria more than 10^5 cells per 100 mL. In the river bank filtrate, *E. coli* could not be detected anymore; yet coliform bacteria were still present, albeit in numbers below 10^2 cells per 100 mL. As in Indonesia soil and groundwater temperatures reach almost 30°C, environmental coliform bacteria could grow there. Therefore it was investigated if the coliform bacteria found in the river bank filtrate are of environmental origin and grow during the soil passage, or if they come originally from the river water. In order to address this question the cultivable coliform bacteria from the river water and the river bank filtrate were identified using molecular methods. To identify and classify the coliform strains we amplified and sequenced the 16S rRNA as well as functional genes. It could be shown that the spectrum of coliform bacteria in both waters was identical, including e.g. members of the genera *Citrobacter*, *Enterobacter* and *Klebsiella*. Thus, the coliform bacteria found in the river bank filtrate are derived from the river.
In summary, river bank filtration efficiently removes *E. coli* as well as most coliform bacteria (approx. 4 log removal). Yet a disinfection step is required in order to achieve hygienically safe drinking water.

EMP044
Brazilian sponges as source for novel species and bioactive compounds

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We hypothized biofilm communities on sponges to be controlled both by community members and the sponge host. To prove this, microbial communities associated with different sponge species from the Ilha do Arvoredo National Reserve, Brazil, have been investigated for their diversity and bioactive secondary metabolites production.
Different sponge species were sampled at various places in this Reserve. Homogenized sponge sections were cultivated on different agar media. From these plates i) individual colonies were purified and ii) total DNA of all strains on the plates were analysed for biodiversity using the 16S rRNA gene sequences.
Most sponge species possessed very distinct microbial communities and some bacteria species have only be isolated from a single sponge species. The sponge *Axinella corrugata* harboured unique bacteria species. The same sponge species collected at different sites in the Archipelago had closely similar microbial communities which can be very distinct from microbial communities from a different sponge species collected at the same site. This finding points to close mutualistic interactions between the sponge and its microbial communities. In total more than 200 bacterial strains have been isolated and identified. The majority of them belong to the genera *Vibrio*, *Pseudoalteromonas* and *Cobetia*. However, strains from rare genera, e. g. *Maritimibacter*, *Martelella* and *Donghicola*, and species not fitting in any of the known *Bacteroidetes* genera have also been found. The isolates have been tested for their antibiotic activities and their ability to prevent biofilm formation. A number of them showed even activities against multi-resistant

clinical isolates demonstrating that sponge associated bacteria are a rich source for novel bioactive compounds.

EMP045
Changes in diversity and abundance pattern of microbial communities involved in nitrogen fixation, nitrification and denitrification comparing a tidal wetland to paddy soils cultivated for different time periods

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In many areas of China tidal wetlands have been converted into agricultural land for rice cultivation. However, the consequences of land use changes for soil microbial communities are poorly understood. Therefore, we investigated bacterial and archaeal communities involved in inorganic nitrogen turnover (nitrogen fixation, nitrification and denitrification) based on abundance pattern and relative species richness of the corresponding functional genes along a soil chronosequence ranging between 50 and 2000 years of paddy soil management compared to a tidal wetland. Changes in abundance and diversity of the functional groups could be observed reflecting the different chemical and physical properties of the soils, which changed in terms of soil development. The tidal wetland was characterized by a low microbial biomass and relatively high abundances of ammonia oxidizing microbes. Conversion of the tidal wetlands into paddy soils was followed by a significant increase in microbial biomass. 50 years of paddy management showed a higher abundance of nitrogen fixing microbes compared to the tidal wetland, whereas dominant genes of nitrification and denitrification showed no differences. With ongoing rice cultivation copy numbers of archaeal ammonia oxidizers did not change, while that of their bacterial counterparts declined. The gene coding for the nitrite reduction *nirK*, which was dominating over its functional redundant counterpart *nirS* at all sites increased with rice cultivation time in all soils. Relative species richness showed significant differences between all soils with the exception of archaeal ammonia oxidizers in the paddy soils cultivated for 100 respectively 300 years. In general, changes in diversity pattern were more pronounced than in abundance pattern.

EMP046
Bacteriophages as indicators for changes of the active microbial community in BTEX contaminated systems

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A pilot-scale plant was set up at a former refinery site near Leuna (Germany) as a ***Co**mpartment **Tra**nsfer* (CoTra) project to investigate efficient low-cost and near-natural remediation strategies for BTEX contaminated groundwater (up to 15 mg/l). Significant changes in degradation but also in microbial community composition were postulated. Since bacteriophages represent one of the major factors regulating bacterial abundance and diversity, the question arose whether the development of the bacteriophage community potentially mirrors changes in bacterial community composition.
In this study we aimed at (i) an inventory of the phage abundances in groundwater samples and the two different treatment systems of the CoTra pilot plant, i.e. the constructed wetlands (AP2) and the aerobic trenches (AP5), (ii) an analysis of the composition of the phage community itself, and finally (iii) an evaluation of the option to use transducing phages for identifying the active and thus BTEX consuming part of the microbial community.
The amount of phage particles was found to be nearly constant over the year with phage titres between $1x10^8$ and $2x10^9$ phages/ml in the treatments and the contaminated groundwater and 10-fold less titre in uncontaminated groundwater. Based on PFGE separation of concentrated phages 7-9 different main phage genome sizes were differentiated. Mispacked chromosomal 16S rDNA in so-called „transducing phage particles" allowed to identify growing bacteria as phage hosts and to detect them within the whole bacterial community. The data are discussed with particular reference to two contrary hypotheses for the function of phages in ecosystems, termed as either „surviving of the fittest" or „killing the winner" hypothesis.

EMP047
Characterization of a new *Nitrospira* in competition to known nitrite oxidizing taxa of activated sludge samples from waste water treatment plant

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The nitrification process is one of the most important tasks for modern wastewater treatment. Because cultivation of this autotrophic community is difficult and time-consuming, direct methods targeting to both, identity and activity of nitrifying bacteria, became most important for the analysis of this process. This study is based on analyses of the active *Nitrospira* population in activated sludge samples from the municipal waste water treatment plant of Hamburg (Germany). The autotrophic bacterial community was labeled with ^{13}C-carbonate and analyzed by FAME-SIP. With FAME-SIP it was possible to detect the metabolically-active autotrophic bacterial community in environmental samples. We combined these chemotaxonomic analyses with cloning of the 16S rRNA and fluorescence *in situ* hybridization (FISH). A new *Nitrospira* was detected by means of a characteristic fatty acid profile which was different from that one of *Candidatus* Nitrospira defluvii. The labeled compound was the *cis* 7 isomer of hexadecanoic acid. This lipid was not described before for nitrite-oxidizers from activated sludge. These results were supported by a new 16S rRNA gene sequence achieved by a cloning approach. This sequence was allocated to the *Nitrospira* branch but different from known sequences. Further on cells of *Nitrospira* were detected with fluorescence *in situ* hybridization performed with specific probes designed for the new *Nitrospira* variant. The labeling experiments gave important hints for promising enrichment conditions for this organism. The analyses showed highest incorporation of label at temperatures of 17 - 22°C with low nitrite concentrations of 0.3 mM for the new characteristic *Nitrospira*-related compound from activated sludge, the fatty acid 16:1 *cis* 7. Based on FAME-SIP analyses, the competition of *Nitrospira* populations with other autotrophic nitrite oxidizing bacteria such as *Nitrobacter* and *Nitrotoga* were analyzed in activated sludge samples under different incubation conditions.

EMP048
Optimization of Culture Conditions for Pyrogallol Production from Gallic acid by *Enterobacter sp.*

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The process of tannin biodegradation is initiated by tannase which converts tannic acid into gallic acid. The second step of this pathway is catalyzed by gallic acid decarboxylase which converts gallic acid into pyragallol. Pyragallol has widespread industrial applications as it is used as a developer in photography, for staining fur, leather and hair, for manufacturing various dyes, and for determining oxygen in gas analysis. In the present study a microorganism was isolated from soil and identified as *Enterobacter* sp. Culture conditions for the maximum production of pyrogallol from gallic acid were optimized with the isolate. The maximal production of pyrogallol was observed when the bacterium was cultured at 30°C for 20 hrs in a medium containing 0.2 % gallic acid, 0.4 % $(NH_4)_2SO_4$, 30 mM phosphate buffer pH 6.6, 0.05% $MgSO_4$, 0.001% $FeSO_4$. The other parameters optimized are incubation time, incubation temperature, agitation speed, Inoculum age and Inoculum size.

EMP049
Identification and characterization of aerobic chloroethene degrading bacteria

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The extensive use of chloroethenes as solvents and synthetic feed stocks over decades made those compounds to a major source of groundwater and soil contamination. The bioremediation of chlorinated ethenes such as vinyl chloride (VC) in groundwater via oxidation by aerobic microorganisms is a cost-effective alternative to physical and chemical approaches. Metabolic pathways that use the target pollutant as growth substrate are favourable for bioremediation processes, as compared to cometabolic degradation in the presence of auxiliary substrates. Several mixed cultures and pure bacterial strains that can use VC as sole carbon and energy source have been published and examined in regard to application as bioremediation agent.
Recently, metabolic cis-1,2-dichloroethene (cDCE) degradation has been reported for a mixed culture enriched at TZW [1; 2]. Concentration and temperature range as well as starvation capacity and effects of co-contaminating chloroethenes were determined. In our current joint study, also molecular biological approaches are applied. Two aerobic metabolically VC-degrading isolates from two different sites in Germany were identified by sequence analysis at KIT. *Hydrogenophaga taeniospiralis* and *Mycobacterium tusciae* were identified as VC-degrading bacteria. PCR-DGGE and 16S-DNA sequence analysis allowed the identification and characterization of the degrading organisms using the basic local alignment search tool of the NCBI database. The design of PCR primers and fluorescence in-situ hybridisation (FISH) probes for bacteria involved in the process of aerobic degradation is part of this project.
Funding by BMWi and AiF (grant no. 16224 N) is gratefully acknowledged.

[1] Schmidt K.R. et al (2010): Aerobic biodegradation of cis-1,2-dichloroethene as sole carbon source: Stable carbon isotope fractionation and growth characteristics. Chemosphere 78:527-532.
[2] Zhao H.-P. et al (2010): Inhibition of aerobic metabolic cis-1,2-di-chloroethene biodegradation by other chloroethenes. Water Research 44:2276-2282.

EMP050
Occurrence of acidophilic and halotolerant Fe(II)-oxidizing microorganisms in high saline mine tailings from the Atacama desert, Chile

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A marine shore copper mine waste tailings deposit in arid climate at Chañaral, Chile, was studied to understand the influence of high salinity on the microbial community composition and biogeochemical processes in this extreme environment. Samples were taken from the oxidized zones at several sites which had a paste pH in the range of 2-8. The microbial community was quantitatively analyzed using different methods: 1) total cell numbers by SYBR Green II direct counting, 2) quantitative real-time PCR, 3) most probable number cultivation of acidophilic Fe(II)-oxidizers. The results showed that the composition of microbial communities and the cell numbers of different microbial groups are highly variable at different sampling sites. Depth profiles of cell numbers of the mine tailings deposit showed total cell numbers in the range of 10^4 - 10^8 cells g^{-1} tailings. *Bacteria* dominated over *Archaea* in the mine tailings. The acidophilic Fe(II)- and/or sulfur-oxidizing *Acidithiobacillus* spp. dominated over the acidophilic Fe(II)-oxidizing *Leptospirillum* spp. among the Gram-negative *Bacteria*. In parallel to the microbial community analyses, novel acidophilic halotolerant Fe(II)-oxidizing microorganisms were enriched at salt concentrations of up to 1 M probable suitable for metal bioleaching using seawater. Growth of up to 10^7 cells/ml was observed in case of complete oxidation of ferrous iron in the medium. The growth of these microorganisms and its ability to oxidize ferrous iron were depended on pH, temperature, initial concentration of ferrous iron, and the inoculum. High ferric to ferrous ratios exhibited an inhibitory effect on bacterial growth.

EMP051
Metagenome approach of two microbial biofilms in a biogas system

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Biogas production forms a substantial component amongst the renewable energy technologies converting biomass to methane. In order to improve biogas production, a profound knowledge about the involved microorganisms is essential.
We investigated two biofilms of a thermophilic (55°C) two-phase leach-bed system in laboratory scale using a metagenome approach. This biogas system with two internal circulations of leachate consisted of a gastight hydrolysis reactor, an effluent storage reactor and a downstream anaerobic filter reactor. The retention time of the rye silage and the winter barley straw (w/w 10/1) was 21 days. Afterwards, samples of the digestate of the hydrolysis reactor (cellulolytic biofilm) and from a tower packing of the anaerobic filter reactor (methanogenic biofilm) were taken. The extracted DNA was sequenced by means of 454-pyrosequencing technology in a Genome Sequencer FLX Titanium System resulting in altogether 552,268 reads with a total of 218 mb sequence information. The average read length was 395 bases. Efficient characterization based on reads was applied using several software pipelines as RDP classifier or CARMA software for taxonomical analyses and Pfam or COG classification for functional analyses.
Up to 30% of the obtained reads could be assigned to taxonomic ranks indicating that many up to now unknown microorganisms are participating in the formation of methane. However, *Clostridia*, *Thermotogae* and *Bacilli* are the most prevalent classes among the bacteria in the cellulolytic biofilm sample and therefore may play a key role in carbohydrate degradation. Pfam characterization of enzymes also revealed *Clostridia* and *Bacilli* as prevalent for carbohydrate degradation supporting the previous findings. In contrast, pfam analysis of the methanogenic biofilm sample showed a high abundance of methanogenic enzymes for *Methanobacteriales*, whereas taxonomical analyses revealed that *Methanosarcinales* is highly abundant. These results suggest that the biofilm-based methanogenesis is not only driven by the hydrogenothrophic, but also by the acetoclastic pathway.
In conclusion, the two biofilms, sampled from one biogas reactor, revealed strong differences in taxonomical and functional analysis caused presumably by reactor compartmentation.

EMP052
Genetic and biochemical characterization of a hydrothermal vent enrichment with autotrophic hydrogen oxidizers

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An enrichment culture for autotrophic hydrogen oxidizing microbes, inoculated with diffuse fluids from the hydrothermal vent system Sisters Peak (5°S on the Mid-Atlantic Ridge) was investigated with respect to the microbial community composition, as well as the genetic and biochemical features with regard to hydrogen oxidation and CO_2 fixation.
According to phylogenetic analyses (16S rRNA genes) *Alpha-* and *Gammaproteobacteria* were detected, whereby the majority of sequences was related to *Thalassospira*. In contrast, 16S rRNA sequences generated from isolated RNA by RT-PCR were mostly assigned to *Thiomicrospira crunogena.* Interestingly, the relative abundances of *Alpha-* and *Gammaproteobacteria* were 10 % and 11 %, respectively, whereas nearly 80 % of all DAPI-stained cells could not be assigned to any bacterial group, although common probes targeting different *Proteobacteria* were used. None of the identified species are known for their ability to oxidize H_2.
The only NiFe uptake hydrogenase (responsible for energy yielding H_2 oxidation) identified by a PCR based screening of a metagenomic library from the culture using different primer sets was most similar to the respective gene from *T. crunogena*, which up to now has not been cultivated with H_2 as electron donor. But the transcription of this gene could not be confirmed by RT-PCR yet. Recent investigations hint at the presence of a hydrogenase from *Alteromonas macleodii,* which was not amplified during the screening. But this needs further investigations to be confirmed. The uptake hydrogenase activity of membrane associated proteins was

designated to be 4 fold higher than the activity of soluble proteins. These activities indicate the oxidation of H_2 in the enrichment culture, but the species expressing hydrogenases could not yet be identified.
Amplified sequences indicating CO_2 fixation via the Calvin Cycle matched the respective gene of *T. crunogena.*
Additionally, to confirm and amend these first results, future investigations shall include the identification of the hydrogenase expressing species, determination of hydrogen consumption rates and purification of the hydrogenase.

EMP053
Will not be presented!

EMP054
Bacterial degradation of 1H-benzotriazole
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The complexing agent 1H-benzotriazole (BT) is a widely-used corrosion inhibitor in cooling and heating fluids, dishwashing detergents, and aircraft de-icing fluids. Due to its apparent persistence against biodegradation, it is ubiquitously present in the aquatic environment. This is even more alarming since the compound has toxic effects. So far, BT has been considered non-biodegradable by bacteria under oxic and anoxic conditions. In this study, we investigated the presence of an intrinsic biodegradation potential for BT in two environmentally relevant compartments: sewage sludge and deep aquifer sediments. An aerobic sewage-sludge-derived mixed culture coupled BT degradation with bacterial growth. During biodegradation of BT, N-methylaniline and further transformation products with absorption maxima at 367 and 550 nm were formed. This is the first report on bacterial growth with BT at mesophilic temperatures and underlines that sewage sludge is a habitat of microorganisms can potentially degrade BT. The anaerobic intrinsic biodegradation potential for BT was studied in deep aquifer sediments eventually contaminated by heat transfer fluids leaking from borehole heat exchangers. Concentrations of BT and its derivative methyl-benzotriazole stayed constant over a period of more than 200 days indicating that no intrinsic biodegradation potential was detectable under the various redox conditions investigated.

EMP055
Dynamic of microbial communities in anaerobic biogas reactors
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Knowledge about the composition and function of microbial communities responsible for substrate degradation and formation of biogas in anaerobic digestion reactors is still rather incomplete. In this study, the dynamic of the microbial diversity from start-up to the development of a stable biogas formation process into anaerobic digesters was investigated. The anaerobic digestion was performed into an up-flow leach-bed reactor combined with a methane reactor and recirculation of the liquid phase. Reactors were continuously fed with wheat straw and run at thermophilic (56°C) and mesophilic (36°C) conditions. Samples were taken from the liquid phase and digestate. Additionally, at stable process conditions, carrier bodies from the methane reactor were sampled. Terminal-restriction-fragment polymorphism (T-RFLP) of restriction endonuclease digested PCR-amplified 16S rDNA was applied to analyse changes into the community structures. Furthermore, 16S rRNA gene libraries were constructed to get detailed insights into the composition of the microbial communities at the date of start-up phase and stable process of biogas formation. The results of this study provide a basis for the modelling and optimisation of the overall anaerobic digestion process within the APECS project. The aim of APECS (Anaerobic Pathways to renewable Energies and Carbon Sinks) is to provide the scientific and technical basis for an efficient and sustainable production of bio-methane as a high quality fuel and biochar as a long lasting carbon sink and an efficient soil improver.

EMP056
Comparative and functional genomics of *Methylobacterium* and *Hyphomicrobium* strains degrading halogenated methanes
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The halogenated methanes dichloromethane and chloromethane are volatile toxic halogenated solvents produced both naturally and industrially. Dichloromethane- and chloromethane-degrading bacteria have been models of choice to study microbial dehalogenation metabolism at the physiological, biochemical and genetic levels. A comparative analysis of complete genome sequences of halogenated methane degrading *Methylobacterium* and *Hyphomicrobium* strains (Vuilleumier *et al.*, 2009; Muller *et al.*, accepted), obtained at Genoscope and the US American Joint Genomic Institute, was performed to complement the investigation of dichloromethane- and chloromethane-degrading bacteria using functional genomics approaches (Muller *et al.*, submitted; Roselli *et al.*, submitted). Our study highlights both the importance of horizontal gene transfer in the dissemination of halomethane degradation genes in the environment, and the involvement of the Alphaproteobacterial core genome in specific adaptations to dehalogenative metabolism.

[1] Vuilleumier, S. et al (2009): *Methylobacterium* genome sequences: a reference blueprint to investigate microbial metabolism of C1 compounds from natural and industrial sources. PLoS ONE 4, e5584.
[2] Muller, E. et al (accepted). Dichloromethane-degrading bacteria in the genomic age. Res. Microbiol.

EMP057
Metaproteomics to investigate the impact of sampling-site biogeochemistry on structure and functionality of leaf-litter degrading microbial communities
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The composition of organic matter in natural ecosystems is strongly influenced by the microorganisms present. Conversely, bacteria and fungi are limited by the amount and type of organic matter available in a given environment, most of which is ultimately derived from plants. Changes in the stoichiometry and biochemical constituents of plant litter may therefore alter species composition and elicit changes in the activities of microbial communities and their component parts. The identification of the microbial proteins of a given habitat together with the analysis of their phylogenetic origin and their spatial and temporal distribution are expected to provide fundamentally new insights into the role of microbial diversity in biogeochemical processes.
To relate structure and functionality of microbial communities involved in leaf-litter decomposition we determined biogeochemistry, community structure (PFLA-analyses), enzymatic activities, and analysed the protein complement of different litter types, which were collected in winter and spring at various Austrian sampling sites, by a semi-quantitative proteomics approach (1-D-SDS-PAGE combined with LC-MS/MS). In samples with high manganese and phosphor content a significant increase of fungal proteins from February to May was observed, which was in good agreement with the PFLA-analyses showing similar trends towards an increase of the fungal community. In contrast, the PFLA analysis revealed no temporal changes in the community at Achenkirch and even a decrease in the fungal/bacterial ratio at Klausen-Leopoldsdorf, two sampling sites low in P and Mn; similar trends are reflected in our spectral counts. In conclusion, semi-quantitative proteome- and PFLA-analyses suggest that fungal and bacterial abundance positively correlates with the total amount of P and Mn within the different litter types. Spectral counts of extracellular enzymes demonstrated a significant increase of these enzymes in the May, which was also mirrored by measurements of total enzymatic activities. The finding that almost all hydrolytic enzymes identified from litter were of fungal origin suggests a prominent role of fungi during aerobic litter decomposition.

EMP058
Functional diversity of microbial biofilm communities growing on some halogenated compounds

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γ-Hexachlorocyclohexane (γ-HCH) and 4,4`-diphenylether (DFE) are halogenated pollutants that persist in the environment for a long time. Although degradation of such compounds by either bacteria or fungi is very difficult, nevertheless, bacteria and fungi within a community can help each other making the degradation process easier. The purpose of the present work was to determine the extent of microbial potential for the degradation of γ-HCH and DFE in soils. This study addressed the huge diversity of bacteria and fungi from 12 soil samples collected around insecticide and pesticide producing factories in Egypt. From γ-HCH and DFE enrichment cultures, all samples yielded high biodiversity as revealed by the analyses of the16S rDNA genes for bacteria and 18S rDNA genes for Fungi. Soil and sediment samples were used to inoculate γ-HCH and DFE microcrystals on a substratum (Permanox^{TM}) in microcosms to grow complex biofilm communities on γ-HCH and DFE. The biofilms were monitored for about 42 days by community fingerprinting using single strand conformational polymorphism (SSCP) of 16S rRNA and 18S rRNA gene amplicons. All soil samples yielded biofilms on both γ-HCH and DFE. SSCP analyses of the biofilms revealed rather diverse bacterial and fungal communities. The Structural biofilm development was monitored by Confocal Laser Scanning Microscope (CLSM) using SYBRO, to stain proteins, DAPI to stain DNA for all bacteria and Bac Light kit bacterial viability check (live (green)/ DEAD (red)). From the soil samples, multispecies were obtained and most of them could use γ-HCH and DFE as sole source of carbon in a minimal medium. Bacteria and Fungi in microbial communities play different roles and together were able to form biofilms using γ-HCH and DFE as a carbon source (a functional diversity cooperation). The metabolites of the biodegradation of the two compounds were characterized by GC/MS. The molecular characterization of gene family encoding for the, linA, linB and linC, γ-HCH-degrading enzymes in the bacteria is in progress.

EMP059
Association of hygienically relevant bacteria with freshwater plankton

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In the present study the colonization of plankton organisms by different categories of hygienically relevant bacteria was investigated in a mesotrophic lake (Lake Baldeney, Germany). The target organisms were faecal indicator bacteria (coliforms, *Escherichia coli*, intestinal enterococci, *Clostridium perfringens*), obligate pathogens of faecal origin (*Campylobacter* spp.) and potentially pathogenic environmental bacteria (*Legionella* spp., *Pseudomonas aeruginosa*, *Aeromonas* spp.). Monthly sampling (April to September 2010) of water, phytoplankton and zooplankton was performed at three transects across the lake. The abundance of the bacteria was determined by cultural methods and in the case of legionellae and *P. aeruginosa* additionally by culture-independent quantitative polymerase chain reaction (qPCR).
The concentration of all faecal indicator bacteria, *Aeromonas* spp. and *P. aeruginosa* that were attached to plankton was generally higher compared to that in the water phase. *Camplyobacter jejuni* and *Campylobacter coli* were qualitativley detected in water and on phytoplankton, but not on zooplankton. *P. aeruginosa* was only found in low concentrations in water and on plankton, while *Legionella* spp. could not be detected by cultivation. However, using qPCR *P. aeruginosa* and *Legionella* spp., but not the medically important species *Legionella pneumophila*, were found in all samples at levels of about 10^4 - 10^6 genome units/L in free water and at several log units higher concentrations on phyto- and zooplankton. This may indicate that at least part of the legionellae and *P. aeruginosa* populations existed in a viable but nonculturable state.
This study shows that diverse species of hygienically relevant bacteria accumulate on plankton which can thus act as a vector and reservoir for these organisms. This observation may be relevant for the epidemiological risk assessment of the human use of surface water for recreational purposes and as source water for drinking water production.

EMP060
Detection of viruses in the Jordan valley and elimination in laboratory soil columns

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In particular in arid regions the reuse of waste water is an important issue. As part of the SMART Jordan Valley project (www.iwrm-smart.org), new integrated approaches for water management and aquifer recharge are developed. Elimination of pathogens and persistant pollutants represent key factors in integrated water resources management, and identifying suitable treatment processes to eliminate such compounds becomes inevitably necessary.
Waste water, groundwater, and surface water samples have been taken in Jordan and Palestine since spring 2007 and were analysed with respect to viruses (adenoviruses, rotaviruses group A, noroviruses genogroup I, and MS2 bacteriophages). In our study an advanced cation-coated filter method was developed to concentrate pathogens in large volumes of water, followed by PCR. A total of 23 water samples were examined. In 69% of the samples at least one group of viruses was detected. MS2 bacteriophage appeared together with the other viruses. Rotavirus group A was dominating. The results are consistent with previous reports since rotaviruses seem to be ubiquitous.
Preliminary laboratory soil infiltration studies were conducted with two flow-through soil columns under unsaturated, aerobic conditions at two different temperature settings (2°C and 20°C) to study virus elimination. Soil columns were spiked with MS2 bacteriophages in high concentrations and breakthrough of phages was followed for 84 days. Both, with plaque assay and qualitative PCR the model virus was detected at low concentrations in the effluent at 2°C, but not at 20°C. The column study confirmes that Soil-Aquifer-Treatment can significantly contribute to the reduction of microbiological pollutants. Future studies will include the operation of pilot systems for aquifer recharge in the Jordan Valley.
Funding by the German Federal Ministry of Education and Research (project no. 02WM1082) is gratefully acknowledged.

EMP061
Electron acceptor-dependent identification of key anaerobic toluene degraders at a tar-oil contaminated aquifer by Pyro-SIP

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Bioavailability of electron acceptors is probably the most limiting factor in the restoration of anoxic, contaminated environments. Particularly in aquifers, the oxidation of contaminants such as aromatic hydrocarbons often depends on the reduction of ferric iron or sulphate. At a tar-oil contaminated aquifer in Germany, we have previously detected a highly active fringe zone beneath a toluene plume, where a specialized population of degraders co-dominated by *Desulfobulbaceae* and *Geobacteraceae* had established [1]. Although on-site geochemistry links degradation to sulfidogenic processes, dominating degradation genes *in situ* appeared related to *Geobacter* spp [2]. Therefore, a stable isotope probing (SIP) incubation of sediment samples with $^{13}C_7$-toluene and comparative electron acceptor amendment was performed. We introduce pyrosequencing of templates from SIP microcosms as a powerful new strategy in SIP gradient interpretation (Pyro-SIP). Our results reveal the central role of *Desulfobulbaceae* for sulfidogenic toluene degradation *in situ*, and affiliate detected catabolic genes to this lineage. In contrast, *Betaproteobacteria* related to *Georgfuchsia* spp. became labelled under iron-reduction. The almost absolute absence of *Geobacter* spp. in SIP-DNA excludes its relevance as toluene degrader *in situ*. Furthermore, secondary toluene degraders within the *Peptococcaceae* detected under both redox conditions prompt hypotheses about niche-differentiation and functional redundancy within degradative potentials on site.

[1] Winderl et al (2008): Appl Environ Microbiol 74, 792.
[2] Winderl et al (2007): Environ. Microbiol. 9, 1035.

EMP062
Dominant denitrifiers in grassland and forest soils are *Alpha-* and *Gammaproteobacteria* as determined by isolation and next generation sequencing

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Denitrifying prokaryotes are facultative aerobes that catalyse the reduction of nitrate and nitrite to nitrous oxide and molecular nitrogen. Soil denitrification is the main source but also a temporary sink of the greenhouse gas nitrous oxide and depends on the denitrifying community. The diversity of denitrifiers in soils was assessed with isolation and molecular approaches, which detect different prokaryotic groups. Two grassland and two forest soils under contrasting land use were studied. Maximum velocity of denitrification in these soils varied from 0.27 to 1.87 $\mu mol(N_2O)\ h^{-1}\ g_{DW}^{-1}$ and most probable numbers of denitrifiers from $2*10^5$ to $1*10^7\ g_{DW}^{-1}$. Neither maximum velocity of denitrification nor cell numbers of denitrifiers were significantly different in grassland and forest soils. Five different isolation approaches selective for denitrifiers yielded 179 isolates. These isolates were affiliated to 22 different families from *Proteobacteria*, *Bacilli*, *Actinobacteria*, and *Negativicutes*. 7 of the isolates represented putative novel species. Alpha- and gammaproteobacterial isolates were dominant in both grassland and forest soils, while *Actinobacteria* were also found in forest soils. 454 pyrosequencing of nitrite reductase encoding genes (*nirK/S*) yielded 3,000 *nirK* sequences that grouped into 48 species-level OTUs that affiliated with 7 families within *Alphaproteobacteria* and *Gammaproteobacteria* and *Nitrospira*. Grassland soils were less diverse than forest soils. 7,000 *nirS* sequences were grouped into 30 species level OTUs belonging to 7 alpha-, beta and gammaproteobacterial families. Three and 24 OTUs were only found in forest and grassland soils, respectively. *NirS* diversity was higher in grassland soils than in forest soils. Phylogenetic analyses indicated many novel *nirK* and *nirS* OTUs. A higher family-level diversity was obtained with cultivation methods than with cultivation-independent methods, but more novelty was detected with the latter approach. Both methods indicated that denitrifiers in grassland soils are different to those found in forest soils. The collective data indicates that *Alphaproteobacteria* and *Gammaproteobacteria* are dominant denitrifiers in both grassland and forest soils.

EMP063
Carbon stable isotope fractionation of hexachlorocyclohexane isomers during aerobic and anaerobic dechlorination

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In biochemical processes the preferential reactivity of the lighter stable isotope over the heavier stable isotope results in enrichment of the heavier isotopes in the residual substrate and relative enrichment of the lighter isotope in the products.
The stable isotope fractionation of organic contaminants such as the pesticide Lindane (γ-hexachlorocyclohexane (γ-HCH)) may be used to assess their degradation in the environment. The extent of in situ transformation may therefore be inferred by using experimentally determined compound specific isotope fractionation factors during biotransformation by defined microbial cultures. In this study, carbon isotope fractionation factors were determined for the dechlorination of γ-hexachlorocyclohexane (γ-HCH) by the anaerobic strain *Clostridium pasteurianum* DSM 525 and the aerobic strain *Sphingobium indicum* DSM 16412. *C. pasterianum* dechlorinated γ-HCH in two weeks, and the metabolites γ-3,4,5,6-pentachlorocyclohexane (γ-PCCH) and chlorobenzene (CB) were formed. *S. indicum*, known to mineralize α, β and γ-HCH, degraded γ-HCH in four weeks with 1,2,4-TCB as metabolite. For both strains the carbon isotope fractionation of γ-HCH dechlorination was quantified by using the Rayleigh equation. The bulk enrichment factor (εC) of - 4.8 ± 0.6 for *C. pasteurianum* was similar to the one previously reported for sulfate reducing strains. In the case of the aerobic strain a similar trend for the isotopic fractionation was observed.

EMP064
Alphaproteobacteria are prevalent methylotrophs in aerated soils as determined by cultivation and pyrosequencing of structural genes

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Methanol augments the formation of ozone in the troposphere. Aerobic methylotrophic microorganisms reduce the emission of methanol from plant-derived carbon and soil organic carbon. Most methylotrophs utilize methanol. The biogeography and diversity of methanol-utilizing methylotrophs in soils is not well resolved. Methylotrophic communities of mineral soils from two forests and two grasslands (Nationalpark Hainich, Germany) were analyzed by cultivation and gene marker based methods. 77 strains were isolated on methanol-containing mineral medium and belonged to seven classes of *Bacteria*. The most abundant class was *Alphaproteobacteria* (39%). Viable cell numbers of methylotrophs varied but averaged $5 \times 10^7\ g_{DW}^{-1}$. 19,000 sequences of *mxaF* (gene of the alphasubunit of methanol dehydrogenase), *mch* (methenyl-tetramethanopterine dehydrogenase), and *fae* (formaldehyde-activating enzyme) were retrieved by tagged amplicon sequencing. The majority of these genes were affiliated with *Alphaproteobacteria*. Only one-third of the detected genotypes occurred in all four soils, indicating that uniformly common genotypes were the minority genotypes. Molecular finger printing of *fae* revealed seasonal differences. The detectable class-level diversity obtained by cultivation was higher than the class-level diversity detected with gene markers. Nonetheless, both approaches suggested that *Alphaproteobacteria* (e.g. *Hyphomicrobium*) were prevalent methanol-utilizing methylotrophs in aerated soils.

EMP065
Novel hydrogenase gene transcripts indicative of active facultative aerobes and obligate anaerobes in the earthworm gut

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Mucus-derived saccharides provide ideal in situ conditions for ingested fermentative bacteria in the anoxic earthworm gut, and high concentrations of H_2 as well as organic acids are indicative of ongoing fermentation. H_2 production during fermentation is catalyzed by [FeFe]- and [NiFe]-hydrogenases that are present in obligate anaerobes and facultative aerobes, respectively. The main objective of this study was to resolve transcript diversities of [FeFe]- and [NiFe]-hydrogenases of potentially active H_2 producers in anoxic gut content microcosms of *Lumbricus terrestris* supplemented with glucose, a representative sugar found in gut contents. 177 of 178 [FeFe]-hydrogenase gene transcripts affiliated to the *Clostridiales* (65-81% amino acid sequence identity) whereas the remaining transcript had an 84% identity (based on translated amino acid sequence) to a hydrogenase of *Pelobacter carbinolicus* (*Deltaproteobacteria*). The [FeFe]-hydrogenase gene transcripts yielded 13 distinct OTUs (based on an amino acid sequence similarity cut-off of 80%). 21% and 79% of 86 [NiFe]-hydrogenase gene transcripts were affiliated to *Aeromonadaceae* and *Enterobacteraceae*, respectively. *Verrucomicrobia*- and *Firmicutes*-affiliated [NiFe]-hydrogenases gene sequences were also detected, mainly of which were highly novel. The collective findings (a) indicate that the microbial community of the earthworm gut hosts microbes containing hitherto undetected [FeFe]- and [NiFe]-hydrogenases, (b) suggest that obligate anaerobes of the *Clostridiales* and facultative aerobes of the *Enterobacteriaceae* were the main H_2 producers in glucose-supplemented gut enrichments, and (c) reinforce previous RNA-based stable isotope probing studies that identified *Clostridiaceae* and *Enterobacteriaceae* as important glucose-fermenting taxa in earthworm gut content.

EMP066
Nutritional physiology of *Sarcinomyces petricola* A95, a model black fungus to study primary successions in terrestrial ecosystems

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Primary successions in terrestrial ecosystems usually involve microbial growth on bare rock surfaces. In these extremely stressed environments which include hot and cold deserts, complex microbial communities must adapt to high solar irradiation, temperature extremes as well as low water and nutrient availability. Subaerial biofilms (SABs) has been used to describe these microbial communities and are dominated by associations of fungi, algae, cyanobacteria and heterotrophic bacteria [1]. Although microbial members of SABs communities vary, the presence of melanized ascomycetous fungi is common on rock and material surfaces in all climatic zones. These fungi grow in restricted compact colonies and are therefore often referred to as microcolonial fungi (MCF, [2]). MCF persist on the interface between a solid substrate and the atmosphere (e.g. on material surfaces, roof tiles, rocks) and actively alter the substrate by physical and/or chemical mechanisms. Despite being ubiquitous and important to rock weathering, soil formation and material deterioration and preservation, relatively little is known about their physiology.
In our laboratory, we work on the black fungus *Sarcinomyces petricola* A95 as a model organism to study life development and persistence on subaerial rock and material surfaces. We report here preliminary results on the physiological characterization of *S. petricola* A95 for what concerns nutrient requirements (e.g. C- and N-sources) and growth phenotypes in different laboratory media. Moreover, results on the symbiotic growth of *S. petricola* A95 with the photosynthetic, diazotrophic cyanobacterium *Nostoc punctiforme* ATCC29133 are presented.

[1] Gorbushina, A.A. and W.J. Broughton (2009): Microbiology of the Atmosphere-Rock Interface: How Biological Interactions and Physical Stresses Modulate a Sophisticated Microbial Ecosystem, *Ann Rev Microbiol* 63:431-50.
[2] Staley et al (1982): Microcolonial Fungi: Common Inhabitants on Desert Rocks?, Science **215**:1093-5.

EMP067
Molecular biological methods for qualitative analysis of cultured bacteria of workplaces

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Current quantitative detection methods of workplace related bacterial exposure levels are based on cultivation dependent approaches. However, routinely a qualitative statement can not be made because of the time consuming but necessary isolation procedure. Here, genotypic identification of bacteria could provide a possibility for the routine qualitative analysis. For this purpose an effective DNA extraction protocol is needed which is capable for isolation of DNA from nearly all bacterial species. To establish such a DNA extraction method the efficiency of three different DNA isolation kits from different manufactures (Sigma Aldrich, Analytik Jena and MP) was investigated, using fifteen different bacterial type species from the phyla Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria. The GenElute™ Plant DNA extraction Kit, offered by Sigma Aldrich was deployed in two different procedures, once according to manufactures instructions and secondly combined with an additional mechanical cell disruption. To determine the DNA extraction efficiency of all investigated DNA isolation kits a defined cell count (10^8 cells) of each bacterial species were deployed per assay. These cell counts were chosen to avoid an exceeding of columns binding capacity. To identify the DNA extraction method, which is most efficient according the amount of extracted DNA of all employed bacterial strains a ranking procedure was applied. Preliminary results showed that the FastDNA® SPIN Kit for Soil, offered by MP, was the most efficient DNA extraction method based on the chosen ranking criteria. Based on PCR amplification efficiencies using universal bacterial 16S rRNA-gene primers (27f/ 1492r) a second ranking will be done in future investigations. Here the quality of isolated bacterial DNA for amplification should be ascertained.The results will be discussed at the poster.

EMP068
Characterization of Steroid Degrading Bacteria from the Baltic Sea at Kiel Germany

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Steroid contamination of sea water is an ever growing problem and impacts population dynamics of all kinds of sea animals. We have long experience with the soil bacterium *Comamonas testosteroni* which is able to catabolize a variety of steroids and polycyclic aromatic hydrocarbons, and which might be used in the bioremediation of contaminated soil. For our studies we use 3α-hydroxysteroid dehydrogenase/carbonyl reductase (3α-HSD/CR) as a reporter enzyme, since it is the key enzyme in steroid degradation. Moreover, the expression of the corresponding gene, *hsdA*, is induced by environmental steroids. In previous investigations we have identified and described several genes being involved in *hsdA* regulation. In this work we isolated several bacterial strains from the Baltic Sea at Kiel, Germany, which degrade steroids and which are able to use steroids as carbon source. Two of them, strain S19-1and H5, were characterized as being gram negative. 16S-rRNA analysis showed that S19-1 belongs to Buttiauxella noackiae and H5 is similar to Vibrio porteresiae. They could be best grown in SIN medium supplemented with 0.6 - 5.1 % NaCl and at 20°C. Both S19-1 and H5 can use testosterone, estradiol or cholesterol as a carbon source in minimum medium. In *Comamonas testosteroni* about 20 enzymes could be induced by 0.5 mM testosterone. A new plasmid pKEGFP-2, suitable for metagenomic studies, and pGEM-EGFP were prepared for isolation of steroid inducible genes in S19-1 and H5. A 4.610 kb DNA fragment which contains the 3a-HSD/CR gene and its regulation elements from *Comamonas testosteroni* was cloned into plasmid pGEM-EGFP and pKEGFP-2. The result showed that testosterone induction could be detected by a microplate fluorescence reader after the plasmids were transformed into *E coli* HB101 cells. Therefore, the system could be used to isolate steroid degradation and steroid regulatory genes from S19-1 and H5. In addition, the exact characterization and systematic classification of these marine steroid degrading bacterial strains is envisaged. The strains might be used for the bioremediation of steroid contaminations in sea water.

EMP069
Earthworms sustain Alpha- and Betaproteobacterial 4-Chloro-2-Methylphenoxyacetic Acid Herbicide Degraders in Soil

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2-Methyl-4-chlorophenoxyacetic acid (MCPA) is a widely used herbicide and subject to aerobic microbial degradation. Earthworms represent the dominant macrofauna in many soils and enhance both growth and activity of MCPA-degrading bacteria in soil. Thus, active MCPA degraders in soil and drilosphere (i.e., burrow walls, gut content, and cast) of the earthworm *Aporrectodea caliginosa* were assessed by 16S rRNA stable isotope probing in soil columns under experimental conditions designed to minimize laboratory incubation biases. The presence of earthworms decreased the time taken to degrade agriculturally relevant concentrations of [^{13}C]MCPA (20 µg g_{dw}^{-1}). 16S rRNA analysis revealed 73 OTUs indicative of active *Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Cyanobacteria, Firmicutes, Gemmatimonadetes, Planctomycetes, Proteobacteria*, and *Verrucomicrobia* in soil and drilosphere. Seven OTUs indicative of *Alpha-, Beta-, Gammaproteobacteria,* and *Firmicutes* consumed MCPA. *Alphaproteobacteria* (*Sphingomonadaceae* and *Bradyrhizobiaceae*) were dominant consumers of MCPA in soil and drilosphere. In contrast, *Beta-* (*Comamonadaceae*) and *Gammaproteobacteria (Xanthomonadaceae)* were MCPA consumers in burrow walls, indicating that this part of the drilosphere is favorable for beta- and gammaproteobacterial MCPA degraders. Approximately 20 and 350 µg [^{13}C]MCPA g_{dw}^{-1} were consumed within 24 hours and 20 days, respectively, in oxic microcosms with drillosphere material (i.e., bulk soil, burrow walls, and cast). Gut contents did not facilitate the degradation of MCPA. *Sphingomonadaceae* dominated MCPA consumers in bulk soil and burrow wall microcosms, while *Beta-* and *Gammaproteobacteria* (*Burkholderiacea*, *Comamonadaceae, Oxalobacteraceae,* and *Xanthomonadaceae*) dominated MCPA consumers in cast microcosms, indicating that the latter taxa are prone to respond to

MCPA in casts. The collective data indicate that *Alphaproteobacteria* are major MCPA degraders in soil and drilosphere.

EMP070
Microbial community adaptation and plasmid spreading in near-natural remediation systems (CoTra) with BTEX-contaminated groundwater

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Aromatic contaminants like benzene, toluene, ethylbenzene and xylene (BTEX) are persistent under anoxic conditions. The transfer of contaminated groundwater from anaerobic into aerobic environments is thus a promising strategy to enhance bacterial degradation of such compounds. A pilot-scale plant named Compartment Transfer (CoTra) with constructed wetlands (AP2, planted and unplanted) and aerobic trenches (AP5) was set up in 2007 at a former refinery site near Leuna (Germany) to investigate efficient low-cost bioremediation strategies for BTEX contaminated groundwater. The systems were investigated after 1 and 3 years of operation, with the aim of understanding (i) the changes of the catabolic gene pool within the microbial communities and (ii) the role of plasmids as carriers of genes involved in BTEX degradation.
Significant changes in degradation but also in community composition determined by T-RFLP analysis of 16S rRNA genes were found. Screening of whole community DNA revealed that all sites were well equipped with upper and lower pathway genes for the degradation of aromatic compounds. However, degradation potential differed between the two time points, e.g. new degradation genes for dioxygenases (TODC1 & TODE) were found after 3 years while other genes became less abundant. Similar shifts were observed for the plasmid pool: initially plasmids carried BTEX-degradation genes coding for subunits of the monooxygenases TBMD and TMOA and a toluene/biphenyl - dioxygenase (BEDe/BEDm). Later on these genes were less abundant; instead a catechol-1,2-dioxygenase (C12O) gene could be detected on plasmids. All sites (groundwater included) contained plasmids of the IncP1 group, while the AP5 and the sediment community of the planted AP2 were additionally equipped with IncP7-like plasmids.
The results indicate that shifts within the microbial communities and/or uptake of catabolic genes resulted in an increased microbial ecosystem function. Plasmid mediated horizontal gene transfer may thus have played a significant role in these events. We concluded that the microbial communities, whilst relying on plasmid-borne degradation genes in the early establishment phase of the treatment sites, now have adapted to a point where they no longer depend on degradation genes located on plasmids.

EMP071
Parasitic growth of *Pseudomonas aeruginosa* in co-culture with the chitinolytic bacterium *Aeromonas hydrophila*

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Polymer-degrading bacteria face exploitation by opportunistic bacteria that grow with the degradation products without investing energy into production of extracellular hydrolytic enzymes. This scenario was investigated with a co-culture of the chitinolytic bacterium *Aeromonas hydrophila* strain AH-1N and *Pseudomonas aeruginosa* strain PAO1 as opportunist with chitin as sole source of carbon, nitrogen, and energy.
Co-cultures of both strains had a biphasic course. In the first phase, strain PAO1 grew along with strain AH-1N without affecting it. The second phase was initiated by a rapid inactivation of and a massive acetate release by strain AH-1N. Both processes coincided and were dependent on quorum sensing-regulated production of secondary metabolites by strain PAO1. Among these the redox-active phenazine compound pyocyanin caused the release of acetate by strain AH-1N by blocking the citric acid cycle through inhibition of aconitase. Thus, strain AH-1N was forced into an incomplete oxidation of chitin with acetate as end product, which supported substantial growth of strain PAO1 in the second phase of the co-culture [1].
To identify the molecular mechanisms underlying this parasitic growth strategy of strain PAO1 transposon mutagenesis was carried out, and mutants were screened for an altered phenotype in co-culture with strain AH-1N. From six mutants obtained three showed only slight growth and did not inactivate strain AH-1N. These mutants had a defect in biosynthesis of arginine, methionine, and histidine, respectively, indicating that prototrophy is important for growth of strain PAO1 in the co-culture. Three mutants showed strongly delayed inactivation of strain AH-1N and were altered in production of quorum sensing-regulated secondary metabolites. In one mutant the gene encoding Lon-protease was inactivated, and in two mutants genes with unknown functions were inactivated. We are currently investigating the function of these genes.

[1] Jagmann et al (2010): Env Microbiol, 12:1787-802.

EMP072
Structure and function of the symbiosis partners of the lung lichen (*Lobaria pulmonaria* L. Hoffm.) analyzed by metaproteomics

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Environmental proteomics, also referred to as metaproteomics, is an emerging technology to study the structure and function of microbial communities. Here we applied semi-quantitative label-free proteomics using one-dimensional gel electrophoresis combined with liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) and normalized spectral counting together with fluorescence *in situ* hybridization and confocal laser scanning microscopy (FISH-CLSM) to characterize the metaproteome of the lung lichen symbiosis *Lobaria pulmonaria*. In addition to the myco- and photobiont, *L. pulmonaria* harbors proteins from a highly diverse prokaryotic community, which is dominated by *Proteobacteria* and including also *Archaea*. While fungal proteins are most dominant (75.4% of all assigned spectra), about the same amount of spectra were assigned to prokaryotic proteins (10%) and to the green algal photobiont (9%). While the latter proteins were found to be mainly associated with energy and carbohydrate metabolism, a major proportion of fungal and bacterial proteins appeared to be involved in posttranslational modifications and protein turnover and other diverse functions.

EMP073
Pyruvate uptake of the CO_2-fixing, sulphide-oxidizing and nitrate-reducing „*Sulfurimonas*" sp. GD1

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Chemolithoautotrophy, mainly driven by *Proteobacteria*, plays a major role in pelagic redoxclines of the central Baltic Sea. Interestingly, representative chemolithoautotrophic organisms are widely distributed over a relatively broad depth interval where physico-chemical features change from oxic to sulfidic conditions. Because nitrate-reducing or aerobic respiratory processes as major energy source can be excluded in sulfidic depths, alternative carbon and energy pathways in the metabolism of chemolithoautotrophs have to be taken into consideration to explain high cell abundances in within these areas.
Our aim was to investigate a potential mixotrophic lifestyle of the epsilonproteobacterial strain GD1, which was isolated from a pelagic redoxcline of the central Baltic Sea, using pyruvate as a proxy for utilization of organic substrates *in vitro*. Phylogenetically, GD1 is a member of the genus *Sulfurimonas* and supposed to be a key player for autotrophic denitrification in central Baltic Sea redoxclines. The experimental approach included radiocarbon measurements, mass spectrometric analyses of biomarkers and rRNA-based SIP analyses.
In vitro, the uptake of radioactive pyruvate was present, with about 85% of the signal recovered in fractions usually containing lipids and proteins, whereas in nucleic-acid containing fractions the radioactivity could hardly be detected. Mass spectrometric analyses of biomarkers of ^{13}C-labelled GD1 cells revealed an absolute ^{13}C content of up to 30% in individual amino

acids, indicating that pyruvate is a substantial basis for primary metabolites. Our results provided detailed information on the carbon metabolism of the strain GD1, indicating a metabolic versatile lifestyle *in situ*.

EMP074
Detection of tar oil degrading bacteria with PCR and a novel most probable number (MPN) test

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Abandoned manufactured gas plant sites and landfills containing gasworks residues are important emittants of tar oil pollutants. Additionally to BTEX and PAH, the NSO-heterocyclic aromatic compounds are increasingly considered in monitoring programmes. The presence of bacteria with the capability to degrade tar oil pollutants is a pre-requisite for bioremediation. In our study, molecular biological methods (PCR) and culture techniques (MPN) for the detection of pollutant degrading bacteria are examined.
PCR-analysis of oxygenases, key enzymes of the aromatic metabolism of aerobic bacteria, represents a promising approach to detect hydrocarbon degrading bacteria. A qPCR method for the detection of mono- and dioxygenases was established. Specific aromatic dioxygenase (*nahAc, PAH-RHD*) and monooxygenase (*tmoA*) genes were demonstrated to occur in tar oil contaminated groundwater.
Bacterial numbers of BTEX, PAH and NSO-HET degraders in groundwater samples are additionally determined by the MPN (Most Probable Number) microplate technique. For the detection of 2- and 3-ring NSO-HET degraders a novel MPN method was established. Mixtures of 2- and 3-ring heterocyclic compounds were provided over the gas atmosphere. MTT, a tetrazolium salt, is absorbed by living cells only and reduced by the bacteria metabolically active. Formation of blue formazan crystals enabled the enumeration of NSO-HET degrading bacteria.
Funding by the BMWi (project no KF2265706SA9) is gratefully acknowledged.

EMP075
Establishment of a standard operational procedure for characterization of work related microbial exposure level

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Today's large-scale poultry production is often accompanied by high concentrations of airborne microorganisms at working places. To characterise the work related microbial exposure level (WoRMEL) standardisied operational procedures are essential to compare the exposure level on the manifold agricultural working places.
Current quantitative detection methods of airborne bacteria are based on cultivation approaches using non selective culture medium like TSA. However, a qualitative statement can not be made because of the time consuming but necessary isolation procedure. Furthermore, nonviable or dead bacterial cells which can also cause negative health effects remain undetected. Here, molecular approaches can be a useful alternative. Therefore, the aim of this study was the establishment of a cultivation independent assay to characterise the bacterial diversity in bioaerosols obtained from poultry processing plants. A fundament within this assay is an optimal DNA extraction from collected bioaerosols. To establish a validated DNA isolation protocol four commercial DNA extraction kits (GenElute™ Plant Genomic DNA Miniprep Kit, Sigma-Aldrich; innuPREP Bacteria DNA Kit, Analytik Jena Biometra; peqGOLD Bacterial DNA Kit, Peqlab; FastDNA® Spin Kit for Soil, MP Biomedicals) each in combination with mechanical treatment were used in parallel. To compare the results one defined bioaerosol sample from an exhaust air flue of a broiler shed were investigated. The amount of extracted DNA was determined fluorometically. The qualitative comparison was done by RFLP analysis of 16S rRNA gene PCR products using agarose gel electrophoresis and automated chip based electrophoresis (Biorad Experion™). Preliminary results showed that two of the four used DNA extraction kits were unusable because no positive PCR products could be obtained. RFLP analyses of 16S rRNA PCR products obtained from DNA which was extracted by the remaining kits resulted in quite similar restriction pattern indicating an equivalent quality. For more detailed qualitative analyses PCR products will be analyzed by generating 16S rRNA gene clone libraries. Results of phylogenetically assignment and comparison of 16S rRNA gene sequences will be discussed at the poster presentation.

EMP076
Microbial activity in schwertmannite

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Mining activities in Lusatia result in mine water that is loaded with high concentrations of iron and sulfate. A biological technology has high potential for mine water treatment, since the immobilization of iron can occur by microbial iron oxidation. Such an approach is realized in a pilot plant at the open pit Nochten where indigenous bacteria oxidize iron which subsequently precipitates as the iron-oxyhydroxysulfate schwertmannite.
As shown previously [1], bacteria can be found in the water as well as in the schwertmannite that is deposited on carrier material. For stabilizing the process of iron oxidation and increasing the iron oxidation rate, the recirculation of schwertmannite was considered. Therefore, the activity of microorganisms in schwertmannite on carrier material and in aging schwertmannite was investigated.
The investigations of the activity of bacteria in schwertmannite on carrier material showed that the total cell number decreases with increasing depth of the mineral layer. The determination of the percentages of living and dead cells with the LIVE/DEAD® BacLight™ Bacterial Viability Kit (Invitrogen) revealed a decrease of living cells and a corresponding increase of dead cells with increasing depth of the schwertmannite layer. Furthermore the composition of the microbial community was analyzed by fluorescence in situ hybridization (FISH) and T-RFLP. Heinzel et al. [2] reported that '*Ferrovum myxofaciens*' and *Gallionella*-relatives dominated in the water. In the schwertmannite layer these two groups could be also found as the dominant species. However, a significant change in the structure of the microbial community in the different depths of the schwertmannite layer was not observed.
In the aging schwertmannite the activity of the microorganisms, determined with LIVE/DEAD® BacLight™ Bacterial Viability Kit, was almost constant over a several weeks.
The results suggest that a recirculation of schwertmannite could increase the oxidation rate, since relatively high bacterial activity exists in the mineral sludge.

[1] Heinzel, E. et al (2009): Applied and Environmental Microbiology 75(3):858-861.
[2] Heinzel, E. et al (2009): Environmental Science & Technology 43(16): 6138-6144.

EMP077
The Earthworm *Aporrectodea caliginosa* Augments the Microbial Degradation of 2,4-Dichlorophenol in Agricultural Soil

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Earthworms play an important role in processing soil organic matter and contribute to the removal of organic pollutants from soil. 2,4-dichlorophenol (2,4-DCP) represents the initial degradation product of the widely used herbicide 2,4-dichlorophenoxyacetic acid (2,4-D). Aerobic microbial processes are important to mineralization in soil. 'Hotspots' of microbial activity in soils include the drilosphere, i.e., earthworm gut content, cast, and burrows. Earthworms (*Aporrectodea caliginosa*) accelerated the disappearance of 2,4-DCP in soil columns. Most probable numbers (MPNs) of 2,4-DCP degraders (a) in bulk soil of columns with and without earthworms approximated $6{\cdot}10^5$ and $6{\cdot}10^3$ g_{DW}^{-1}, respectively, and (b) in burrow walls approximated $9{\cdot}10^6$ g_{DW}^{-1}. Mineralization of [$U^{14}C$]-2,4-DCP was enhanced in oxic microcosms of soil that was pre-incubated with earthworms. Over 300 2,4-DCP putative degraders were isolated under oxic and anoxic conditions, and belonged to 19 genera. The majority of the isolates belonged to the *Gammaproteobacteria* (i.e., *Pseudomonadaceae*, *Enterobacteraceae*). Approximately 30% of the aerobic isolates were not previously known to degrade 2,4-DCP. Analyses of *tfdB* (encodes a 2,4-DCP-hydroxylase) and *pheA* (encodes a phenol hydroxylase) as structural marker genes for 2,4-DCP degraders indicated the presence of novel and diverse 2,4-DCP degraders in the drilosphere and soil. *tfdB* of burrow walls were most diverse. The collective data indicates that (a) earthworms stimulate the degradation of 2,4-DCP in soil by enhancing the growth of 2,4-

DCP degraders, and (b) soils influenced by earthworms harbor highly diverse and novel 2,4-DCP-utilizing microorganisms.

EMP078
Bacteria emissions from broiler sheds

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Emissions of microorganisms from live stock buildings and their environmental impact are hardly characterized. In particular residents in the area of poultry processing plants are increasingly interested in this characterization because of a potential negative health effect. Therefore we investigated the microbial load and the bacterial diversity in emission samples from broiler sheds by cultivation independent analysis. Distributed over 2.5 fattening periods emissions samples from a broiler shed were collected by impingement into isotonic NaCl solution. Concentrations of microorganisms in emission samples clearly increased during the fattening period from 3.7 x 10^7 cells per m^3 at the beginning to 9.4 x 10^8 cells per m^3 at the end (after ~ 40d). Depending on the ventilation rate an enormous number of > 10^{10} microbial cells was emitted from one broiler shed per second. Qualitative analyses of bacterial diversity in emission samples for the first time via a generation of 16S rRNA-gene clone libraries revealed that the most abundant sequences (60%) of all 257 investigated clones could be assigned to the genus *Staphylococcus.* Among them sequences which are most closely related to *S. cohnii* subsp. *cohnii, S. cohnii* subsp. *urealyticum, S. nepalensis, S. lentus* and *S. arlettae*. With respect to risk assessment, the largest percentage of the identified 16S rRNA gene sequences can be assigned to bacteria which are classified to biological agents of the risk group 1 (German technical rule TRBA 466). However, bacterial species of the risk group 2, like *Staphylococcus saprophyticus, Aerococcus viridans, Enterococcus hirae, E. faecium* and *Escherichia* spp. were detected, too. All together 28 different and well described bacterial species within 11 different genera were detected. But the remaining 21% of the analysed sequences were next related to yet uncultured bacteria. Against the background of increasing numbers of poultry fattening plants, both from ecological and medical point of view the environmental impact of these emissions should be considered in further investigations.

EMP079
Distribution of deep-biosphere bacteria in sediments and the water column of the Black Sea

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The marine subseafloor biosphere is one of the largest biotopes on Earth. The microbial community is composed of a broad range of so far uncultured phyla with unknown metabolic properties. However, several community members have pelagic or even terrestrial relatives (Batzke et al. 2004). The scientific question of this study is to understand the origin of deep-subseafloor bacteria. Therefore, two different hypotheses were tested: (i) Bacteria that are abundant in the water column enter their habitat by sedimentation and survive long-term burial. (ii) The extreme environmental conditions select for specific phylotypes that thrive in the deep-subseafloor biosphere.
In this study, samples were collected from the Black Sea (Meteor cruise M72/5) which was chosen as a model habitat as it is characterized by a stratified water column. To span the whole range of redox regimes, samples were taken from oxic surface waters, the oxic-anoxic transition zone, anoxic bottom waters and sediments down to a depth of 8 meters below seafloor (mbsf). The goal was to focus on four model organisms and not to analyse the whole microbial community composition. Those were members of the *Roseobacter* clade, the *Chloroflexi, Photobacterium* sp. and *Rhizobium radiobacter.* While *Roseobacter* affiliated bacteria and *Photobacterium* sp. are abundant in the water column but are also found in surface sediments (Sass et al. 2010, Süß et al. 2008), the *Chloroflexi* and *R. radiobacter* represent highly abundant members of the deep-subseafloor biosphere (Süß et al. 2006, Wilms et al. 2006). Analyses were performed by using quantitative PCR (qPCR) on original samples and enrichment cultures in combination with molecularly steered cultivation.
Based on the results of the qPCR, we were able to quantify the natural abundance of the four model organisms in the different habitats and could show a successful enrichment in our culture media. The molecular screening of serial dilution cultures by specific PCR was used to identify and isolate unique phylotypes. Isolates belonging to the four model organisms were subjected to further physiological characterisation.

EMP080
Diversity and abundance of *Roseobacter*-affiliated bacteria on various algal surfaces and in sediments

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Roseobacter-affiliated bacteria belong to the most abundant marine microorganisms. They inhabit a wide range of ecological niches, especially in coastal environments and the polar oceans. Members have been found to be free-living, particle-associated, in relationships with marine phytoplankton, invertebrates and vertebrates. This widespread distribution is reflected in a broad physiological spectrum. Most of the *Roseobacter*-affiliated bacteria are known to be aerobic heterotrophs, some are facultative anaerobes and others are even capable of performing aerobic anoxgenic photosynthesis (AAP). The ecology, physiology and molecular biology of the *Roseobacter* clade is now investigated in a transregional collaborative research center (SFB/TRR51).
Sediments have been identified as the third-most important habitat for the *Roseobacter*-clade [1]. It has been shown that this group contributes with an average of 3% to the microbial communities thriving in marine surface sediments, but they were also detected in deep anoxic layers. However, the abundance, distribution and metabolic potential of sediment-dwelling *Roseobacter* have not been studied systematically so far.
To elucidate their role in marine sediments in comparison to other surfaces, we have analyzed algal and sediment samples from Helgoland on different phylogenetic levels. DGGE was performed using primers specific for *Bacteria*, *Rhodobacteraceae* and *Phaeobacter*. The fraction of AAP bacteria was traced by specifically targeting the *puf*ML gene, which encodes for two highly conserved structural proteins of the light harvesting complex. All phylogenetic and physiological groups were analyzed by quantitative PCR. To confirm the results, numbers of bacteria and *Rhodobacteraceae* were determined by CARD-FISH. Additionally, a cultivation approach was conducted to obtain isolates for further physiological studies.
An unexpected high number of *Rhodobacteraceae* was found within the sediment samples. Diversity analysis via group specific PCR revealed a higher diversity on algae surfaces than in the sediments with representatives spreading over the whole *Rhodobacteraceae* lineage.

[1] Buchan, A. et al (2005): Overview of the marine Roseobacter lineage. Appl Environ Microbiol 71: 5665-5677.

EMP081
Reconstituting lantipeptide biosynthesis in *E. coli*

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Lantipeptides are ribosomally synthesized and posttranslationally modified peptides containing lanthionine or methyl-lanthionine rings. They are mainly produced by Gram-positive bacteria and exhibit significant biological activities targeting components of the bacterial plasma membrane, e.g. lipid II. Since their bactericidal effect often stems from more than one mechanism, the emergence of resistance to lantipeptides is rarely observed. Therefore, lantipeptides represent promising leads for drug development.
Recent genome mining studies unveiled that the genes responsible for lantipeptide biosynthesis are widespread in the eubacterial kingdom [1; 2]. The Gram-negative filamentous bacterium, *Herpetosiphon aurantiacus* ATCC 23779, harbours two putative lantipeptide gene clusters in its genome

[1]. Interestingly, the locus located in 34.6-34.8 centisome region of the chromosome encodes six different prepeptides, but only one candidate lanthionine synthase for their chemical transformation. To determine whether all prepeptides are processed by a single promiscuous enzyme, a series of co-expression experiments was conducted in *E. coli* and monitored by MALDI-TOF MS. The results indicated that the annotated lanthionine synthase readily dehydrates all six prepeptides notwithstanding their structural differences in the C-terminal region. Moreover, it was revealed that three lanthionine rings are formed in the peptides upon action of the enzyme.

[1] Begley, M. Et al (2009): Appl. Environ. Microbiol., 75, 5451- 5460.
[2] Li, B. Li et al (2010): Proc. Natl. Acad. Sci. USA, 107, 10430-10435.

EMP082
Occurrence of *Roseobacter* subclusters in the German Bight of the North Sea

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The *Roseobacter* clade of *Alphaproteobacteria* is an important component of the marine bacterioplankton. Studies all over the world have demonstrated that members of this clade can constitute large proportions of total *Bacteria* which can vary greatly, seasonally and as a function of environmental factors. Most of the *Roseobacter* clusters identified in pelagic environments consist predominantly of uncultured phylotypes and only scarce information exists on the simultaneous occurrence of distinct subclusters.
In order to elucidate the occurrence of the major pelagic subclusters of the *Roseobacter* clade, we investigated these subclusters during a phytoplankton spring bloom in May in the German Bight of the North Sea. Due to the fact that members of the *Roseobacter* clade are often found in association with algae we sampled stations in- and outside the phytoplankton bloom and analysed the particle-associated (PA, >5 µm) and the free-living (FL, 0,2-5 µm) fraction for the presence of the following subclusters: RCA, NAC11-6, NAC11-7, CHAB-I-5 and SH6-1. DNA extracted from the PA and FL bacterial fractions was analysed by PCR with cluster-specific primers. Further, we applied DGGE of 16S rRNA gene fragments amplified with primers specific for the *Roseobacter* clade. In addition inorganic nutrients (phosphate, nitrate and nitrite), dissolved amino acids, plankton-related parameters (chlorophyll, POC, suspended particulate matter) and bacterial cell counts were assessed.
All five clusters of interest were detected in the investigated area but predominantly in the FL bacterial fraction. However, only the RCA and SH6-1 clusters were detected consistently in the entire area. The other clusters were not detected at all stations and exhibited less uniform patterns, e.g. the NAC11-6 cluster was not detected at the stations with the highest concentrations of chlorophyll *a* (13-15 µg Chl l^{-1}). The *Roseobacter*-specific DGGE showed rather diverse banding patterns and a higher number of bands in the PA fraction than in the FL bacterial fraction, especially at stations with high chlorophyll concentrations. The *Roseobacter* community of the PA and FL bacterial fractions showed pronounced differences as revealed by a cluster analysis.

EMP083
Role of light in the survival of the aerobic anoxygenic phototroph *Dinoroseobacter shibae* during starvation

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Dinoroseobacter shibae was isolated from a culture of marine dinoflagellates. The strain belongs to the *Roseobacter* clade and is an aerobic anoxygenic phototroph (AAP; Biebl *et al,* 2005). AAPs are capable of using light as a source of energy under oxic conditions without the generation of oxygen. They possess light-harvesting systems, reaction Center, bacteriochlorophyll a (Bchl a) and carotenoids with spheroidenone as a major component. Light was shown to induce ATP formation and proton translocation by the cells [Holert *et al,* 2010]. However, the cells do not grow by light energy alone. Instead there is only a certain level of light-dependent increase in the amount of biomass, protein and pigment concentrations [Biebl *et al,* 2006].
Our question is under which conditions does light have the maximum competitive advantages for the bacteria. Accordingly, we tested the postulates (i) that the role of light energy for the cellular metabolism is proportional to the degree of starvation and (ii) that the metabolism is specifically adapted to the day-and-night rhythm. Batch cultures of *D. shibae* were maintained for several months under starvation in a) the dark, b) under continuous illumination and c) under dark-light cycles. To record the physiological fitness respiration, chemiosmotic proton translocation and the adenylate energy charge were determined.

[1] Biebl, H. et al (2005): *Dinoroseobacter shibae* gen. nov., sp. nov., a new aerobic phototrophic bacterium isolated from dinoflagellates. Int J Syst Evol Microbiol 55: 1089-1096.
[2] Holert, J. (2010): Influence of light and anoxia on chemiosmotic energy conservation in *Dinoroseobacter shibae.* Environmental Microbiology Reports, no. doi:10.1111/j.1758-2229.2010.00199.x
[3] Biebl, H., and I. Wagner-Döbler (2006): Growth and bacteriochlorophyll *a* formation in taxonomically diverse aerobic anoxygenic phototrophic bacteria in chemostat culture: influence of light regimen and starvation. *Process Biochem* 41: 2153-2159.

EMP084
Genome mining in the plant pathogen *Ralstonia solanacearum*

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Genomic analyses have unveiled the tremendous potential of microorganisms for natural product biosynthesis and have initiated a paradigm shift in isolation programs from bioassay-guided fractionation to genome mining. By means of computational sequence comparison tools and biosynthetic precedence, the structures of many previously unobserved metabolites can be predicted from genomic data, which in turn allows the development of suitable fermentation and genetic methods to activate or enhance their production.
This work is focused on the secondary metabolism of *Ralstonia solanacearum*, a Gram-negative soil bacterium that causes bacterial wilt in solanaceous plants like tomato, potato and tobacco [1]. Analysis of the genome sequence of this phytopathogen revealed the presence of a biosynthetic gene cluster related to the yersiniabactin locus from the plague bacterium *Yersinia pestis*. Variation of culture conditions eventually led to the activation of the biosynthetic genes in *Ralstonia solanacearum* and enabled an isolation of the encoded metabolite. The subsequent structure elucidation unveiled a molecular architecture which, albeit related to yersiniabactin, was not expected from computational analysis.

[1] Gabriel et al (2006): MPMI, 9, 1, 69-79

EMP085
Analysis of nitrogen transforming microbial communities in shallow and deep karstic aquifers

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Microbial nitrogen transformation processes in aquifers play an important role for the suitability of groundwater as a drinking water resource. However, only little is known about the microbial communities mediating those processes in aquifer systems. In this study, we are analyzing samples taken from karstic limestone aquifers at different depths ranging from 12 to 88 meters. Sampling sites are arranged along a gradient from forest to agriculturally used land in the national park Hainich (Thuringia/Germany). Here, high levels of oxygen availability in the groundwater with an oxygen saturation of up to 50 % point to an important role of aerobic nitrogen transforming processes. Therefore, our goal is to investigate seasonal and spatial patterns in the community composition, abundance, and transcriptional activity of microorganisms mediating the first and rate-limiting step of nitrification, the oxidation of ammonia, using the *amoA*-gene as a molecular marker. Preliminary results obtained with a combined DGGE/cloning approach suggest differences in the community composition of ammonia oxidizing bacteria and ammonia oxidizing archaea between different depths as well as between different sampling times. Moreover, at some sites, elevated concentrations of nitrate in the groundwater coincide with increased bacterial *amoA* gene copy numbers as determined by

quantitative PCR. In situ nitrification activities as well as the relative importance of planktonic microbial communities versus communities that are associated with the aquifer matrix for nitrogen transformation processes remain to be investigated.

EMP086
Comparative occurrence and detectability of fumarate-adding degradation genes in hydrocarbon-contaminated anaerobic aquatic ecosystems

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The degradation of hydrocarbons via the addition of fumarate to methyl groups is an important catabolic pathway in anaerobic ecosystems contaminated with hydrocarbons of natural or anthropogenic origin. Because of the unique reaction mechanism, genes for fumarate adding enzymes (FAE) are an ideal genetic marker for the targeted detection of bacteria involved in anaerobic hydrocarbon degradation. The knowledge about the occurrence and diversity of anaerobic hydrocarbon degrader populations gives important insights into the structure and function of degrader communities as well as natural attenuation.
FAE sequences show a ramified phylogeny which is determined by both degrader affiliation as well as substrate spectrum. It has been shown that distinct gene lineages can be recovered by different specific primer sets, i.e. as for enzymes involved the degradation of monoaromatic, polyaromatic and aliphatic hydrocarbons. However, a thorough assessment of degrader detectability in contaminated samples by different assays and also rapid screening tools are still at lack. Here, by a comparative screening for FAEs in hydrocarbon-impacted samples of marine, subsurface and limnic origin we provide new insights into the global distribution of key anaerobic hydrocarbon degradation gene pools. By a *bssA*-targeted T-RFLP assay, additional insight into the diversity and relative abundance of key FAE gene lineages within degrader populations are given. Comparing the results of FAE gene detection with ribosomal markers, it even becomes possible to associate previously unidentified FAE lineages to defined taxonomic phyla, as we demonstrate for ^{13}C-enriched DNA of uncultured toluene degraders within the *Desulfobulbaceae*, which were detected in a stable isotope probing experiment with contaminated aquifer sediments.

EMP087
Metagenomics and metatranscriptomics of the *Roseobacter* clade

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The *Roseobacter* lineage is a widely distributed, abundant and biogeochemically relevant group of marine bacteria. Members of the clade have been identified in a large variety of habitats (costal and polar regions, open oceans, ice, saline lakes and hypersaline lakes). The use of a multitude of organic compounds, the production of secondary metabolites, and other metabolic pathways contributes to the success of the group in marine environments.
This study was focused on assessing and exploiting the diversity and metabolic potential of uncultivated members of the *Roseobacter* clade and other marine microorganisms. In this survey, samples derived from the German Sea were analysed by metatranscriptomic and metagenomic approaches.
To gain insights in the metabolic potential, RNA was extracted from filtered sea water samples. For metatranscriptomic analyses, the RNA was purified, ribosomal RNA was removed and enriched mRNA converted to cDNA. The resulting cDNA was sequenced by 454 pyrosequencing and further analysed. To analyse bacterial diversity, a two step 16S rRNA RT-PCR and a 16S rDNA PCR were performed. The generated amplicons were sequenced by 454 pyrosequencing and further analysed. The *Roseobacter* lineage was abundant in all samples. Other bacterial phylotypes were also identified, e.g. the SAR11 clade, another group of abundant marine bacteria.
Along with the other studies, high-molecular weight DNA, extracted from the samples, was used for the construction of metagenomic large-insert libraries. The generated libraries were afterwards prospected for proteolytic activity by a function-driven approach. So far, two novel genes conferring proteolytic activity were identified.

EMP088
From grapes to wine: Monitoring the development of yeast populations by FT-IR

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Nowadays the wine production is mainly based on the use of commercial starter cultures of *Saccharomyces cerevisiae* yeast strains. Nevertheless spontaneous fermentations carried out by non-Saccharomyces yeasts are a common mode in wine production. This can lead to wines with more distinct aromas on the one hand, but wines with off-flavours, partly unsuitable for sale, on the other side.
Wild yeasts derived from the vineyard and the cellar equipment are known to be very important for the wine quality of spontaneously fermented musts.
Therefore, in this project promoted by the AIF yeasts on Riesling grapes from six vineyards regarding different habitats and soil types in Germany were analyzed. The yeast populations were monitored at three different stages pre harvest and at six phases during the spontaneous fermentation. At each sampling point 100 randomized isolated yeasts were identified. The identification was accomplished by FT-IR technology, which allows handling such a quantity of isolates, to gain knowledge of the yeast diversity during wine making.
The results demonstrated that twelve main yeast species like *Hanseniaspora uvarum* (up to 90 %), *Candida oleophila* and *C. boidinii* (up to 54 %) and different *Metschnikowia* and *Pichia* species (~ 10 %) run through the first third of the fermentation process before it is dominated by *Saccharomyces cerevisiae*. The proportion of other species such as *Torulaspora delbrueckii, Debaryomyces hansenii* and *D. polymorphus* or *Issatchenkia orientalis* is small. Interestingly it seems that yeasts responsible for fermentation originating from the grape surface and coming in the cellar are in the minority. Depending on the phytosanitary status of the grapes, genera like *Aureobasidium, Rhodotorula, Cryptococcus* or *Sporidiobolus* are the main yeasts (up to 100 %) on the surface of the grape berries. These are irrelevant for the fermentation process. Therefore yeasts coming from the cellar equipment have a strong influence on the whole population during the fermentation process.
The FT-IR technology leads to a better insight on yeast populations in contrast to molecular based methods, but is still limited corresponding to the total cell count. Using specific media, further studies showed, that fermentation relevant organisms are present on the grapes. Usually these yeasts are under the detection limit, if 100 randomly selected yeasts per sample were isolated.

EMP089
Alteration of compost and topsoil microbial communities affected by acid mine drainage

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Mining processes are widespread over the whole world and independent of the mined material, formation of acid mine drainage (AMD) is a basic problem off all mines. Through oxidation and natural leaching processes, immobile heavy metals become mobile in the water fraction and also bioavailable for all organisms. Most of these mobile heavy metals are harmful for organism, like bacteria, plants and animals.
To handle the heavy metal pollution and to remediate contaminated areas, the addition of soil, especially compost, is a first step in phytoremediation. With this soil addition a great number of bacteria are also added to a new environment with harmful conditions. The non-adapted microbial communities have to cope with these contaminants and to develope tolerance or resistance.
At our study and sampling site near Ronneburg (Thuringia, Germany) uranium mining was performed there for over 40 years. Heavy metals were

mobilized via leaching processes driven by AMD and microbial leaching with *Acidithiobacillus ferrooxidans*. During the leaching process, the leachate percolated through the isolation layer and contaminated the underground with mobilized heavy metals and salts.
After removal of the heap material, the salt and heavy metal rich sediment led to a pH in the range of 3 to 4,5, while the content of organic matter is very limited. As a result, an obvious decrease in numbers of cfu per gram soil of 100 to 1000fold was observed in comparison to an uncontaminated soil. The test field Gessenwiese was installed in 2001. Three large plots were prepared with different treatments: 5cm topsoil, 5cm compost or no amendment as control are investigated.
To understand the interdependencies between affecting conditions and to investigate the influence of heavy metals, the population dynamics and growth characteristics of single isolates were studied including both cultivation-dependent and DNA-based fingerprinting methods. Plating, strain isolation, direct cell counts and respiration measurements were used to establish surface and vertical profiles at the heavy metal contaminated field site to follow microbial diversity over time. 16S rDNA libraries were prepared at different time points to observe changes in community composition.

EMP090
Spatiotemporal patterns of microbial communities in a hydrologically dynamic alpine porous aquifer (Mittenwald, Germany)

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Following the working hypothesis that microbial communities in groundwater are driven by the prevailing hydrological dynamics, a porous 'pristine' groundwater ecosystem in the alpine Isar River valley near Mittenwald (Germany) was investigated with respect to spatiotemporal patterns of suspended and attached microbial communities. Characterized by a very high hydraulic conductivity and groundwater flow velocities of several meters per day, the aquifer underlies strong seasonal hydrological dynamics mainly governed by precipitation events and snow melting. In a two-year study, water collected from the Isar River and groundwater was sampled from 4 selected monitoring wells varying in its distance to the River. The bacterial abundance in groundwater ranged from 1.1 - 8.0 $\times 10^4$ cells mL^{-1} and only 0.1% to 5.6% of the total cell numbers were found as viable counts (CFUs) on R2A agar plates. Water from the Isar River generally revealed higher total cell counts. The proportion of active microbial biomass in river and groundwater, determined via ATP analysis, was highest in spring and autumn. Bacterial carbon production determined by the incorporation of [^{3}H]-leucine into cell biomass was found extremely low in the pristine groundwater, $\leq$ 0.3 µg C L^{-1} h^{-1}, revealed that bacterial activity in groundwater becomes more pronounced in relation to river water in summer. This carbon production is related to concentrations of AOC (assimilable organic carbon) of only 5 to 20 µg L^{-1}, accounting for 0.1 to 1.3% of the DOC. The highest concentration of AOC went along with the highest proportion of active biomass. Bacterial community fingerprinting via T-RFLP analysis showed that community structure in terms of dominating species/groups in groundwater significantly changed with season. The bacterial diversity was highest in spring and lowest in summer. In contrary, bacterial communities on natural sediments from the Isar River exhibited stable community patterns over the time period of several months when exposed to groundwater in monitoring wells. This leads us to the preliminary conclusion that groundwater bacterial communities in such highly dynamic aquifers are strongly determined by the origin of water recharged to the aquifer. The enormous amounts of water from snow melting in spring, which passes the investigated aquifer in summer, result in a severe decline in 'visible' bacterial diversity. Bacterial communities are then dominated by only a few species/groups (T-RFs). In autumn the system returns to more microbiologically stable conditions.

EMP091
The epoxide antibiotic produced by *Pantoea agglomerans* and its function in the biocontrol of bacterial plant pathogens

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The strain *Pantoea agglomerans* 48b/90 (Pa48b) attracted our attention because of its ability to inhibit the growth of important bacterial plant pathogens (i.e. *Erwinia amylovora*, *Pseudomonas syringae* pathovars, *Agrobacterium tumefaciens*) and the human pathogen *Candida albicans in vitro*. Bioassay-guided fractionation using anion exchange chromatography and HPLC under HILIC conditions yielded the bioactive, highly polar antibiotic. This compound was identified as 2-amino-3-(oxirane-2,3-dicarboxamido)-propanoyl-valine (APV) and was first described for this species (1). Similar structures were described for two actinomycete isolates, *Streptomyces collinus* (A19009) and *Micromonospora* sp. (Sch 37137), and for *Serratia plymuthica* CB25-I. Structurally related molecules interact with the fungal (and bacterial) glucosamine-6-phosphate synthase (GlmS) and block the formation of *N*-acetylglucosamine, a key compound of the bacterial peptidoglycan and fungal chitin (2). Supplementation of our minimal medium with *N*-acetylglucosamine indeed resulted in APV becoming inactive against *E. amylovora* and *Candida albicans* suggesting that APV acts as GlmS inhibitor. Interestingly, the formation of APV *in vitro* is growth associated and strongly temperature dependent: its optimal production rate is between 8 °C and 12 °C. Therefore, we conducted plant experiments at moderate and low temperatures. In addition, the coinoculation of an APV-negative mutant with the bacterial plant pathogens should highlight the role of APV within the biocontrol of the bacterial blight pathogen *Pseudomonas syringae* pv. *glycinea* and the fireblight pathogen *Erwinia amylovora*. Surprisingly, independent from the temperature conditions the difference in suppression of disease symptoms between wildtyp Pa48b and mutant was minimal.

[1] Sammer et al (2009): Appl. Environ. Microbiol. 75, 7710-7717.
[2] Milewski (2002): Biochimica et Biophysica Acta 1597, 173- 192.

EMP092
Towards an improved understanding of trophic connectivities in belowground microbial food webs

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The flow of carbon and energy through natural systems is largely controlled by organisms engaged in complex trophic interactions. Although such food webs have been intensively studied for higher organisms, involved microbes are mostly treated as a black box. In the frame of the DFG FOR-918 („Carbon flow in belowground food webs assessed by isotope tracers"), we aim to open this black box and uncover the interactions between bacteria and other trophic levels of a soil food web depending on plant carbon inputs and channelling carbon into deeper unsaturated and saturated zones. To trace this organismic food web from its origins, a model community of microbial plant exudate consumers was enriched from an agricultural soil at our field sampling site (a maize field in Göttingen) with an artificial mixture of ^{13}C-labelled root exudates as substrate. Subsequently, labelled indigenous bacterial biomass was added to mesocosms with the same soil and secondary microbial consumers were traced by rRNA-SIP.
Already after one day of the inoculation, T-RFLP fingerprints of 'heavy' rRNA revealed secondary microbial consumers of the added biomass to be active. Over time, ^{13}C-labelled microbial subpopulations varied and indicated a complex and dynamic food web to be active within the bacteria. For identification of labelled OTUs, selected rRNA fractions are analyzed by massively paralleled 454-pyrotag sequencing. We use bidirectional sequencing of bacterial rRNA amplicons, which allows for assembly, T-RF prediction and phylogenetic placement of dominating amplicon contigs. Furthermore, pyrotag data from the SIP experiment are compared to respective field community data to link the identified trophic connectivities to C-turnover in the field. As next step (in progress), links to other members of the belowground food web (*Fungi, Protozoa*) will be elaborated together with our partners within FOR-918.

EMP093
Fast Response of High Density Planctonic Leaching Microorganisms to Growth Inhibiting Tensids

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An inhibition of microbial leaching is necessary to protect the environment from acid mine drainage (AMD). This was successfully performed with sodium dodecyl sulphate in Romania [1]. In Eastern Germany leaching microorganisms were found in an active lignite mining area [2]. This study shows the effect of surfactants to planctonic microorganisms found in AMD areas in comparison to Escherichia coli. Cultivation experiments for three typical leaching microorganisms revealed no growth after treatment with concentrations above 0.25 g/L SDS. To get insight in a possible inhibition mechanism high cell densities (108-109 cells per mL) were adjusted in photometer cuvettes and optical density was followed over 1000 seconds. For the sulphur-oxidizing Acidithiobacillus thiooxidans DSM 622 a decrease in OD600 between 70 and 80 percent was measured after treatment with 0.5 to 10 g/L SDS, respectively. The iron- and sulphur-oxidizing bacterium At. ferrooxidans ATCC 23270 showed a decrease between 50 and 60 percent for the same concentrations. A comparable sensitivity was observed for Acidiphilium cryptum DSM 2389 a heterotrophic leaching bacterium.Treatment of At. thiooxidans and Ac. cryptum with twelve other surfactants (anionic, cationic, non-ionic or amphoteric) showed a response in one case only. For comparison with a much better known Gram-negative bacterium E. coli was treated with surfactant concentrations from 0.001 to 10 g/L. In contrast to leaching microorganisms growth of E. coli was declined but clearly detectable at all concentrations. In the photometer SDS caused a weaker decrease in OD600 between 15 and 25 percent within the same time and concentration regime. Treatment of all investigated microorganisms with up to 35 percent ethanol showed no change in OD600 although a complete inactivation of all cells was achieved. The fast decrease in OD600 plus a detectable release of proteins and nucleic acids allow the conclusion that a lysis mechanism was triggered in the leaching microorganisms by the surfactant.

[1] Schippers et al (2001): Waste Management, 21:139-146.
[2] Siebert et al (2009): Advanced Materials Research, 97: 71-73.

EMP094
Anaerobic co-digestion of organic wastes in different temperature regime

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This study introduces new knowledge over the process of anaerobic digestion of mixed vegetable organic wastes in continuously stirred tank bioreactors realized in laboratory conditions.
Methods: A laboratory experiments with two anaerobic bioreactors with working volumes of 3 L and 14 L has been performed. Chemical oxygen demand, the volatile fatty acids (VFA) concentration and the ratio VFA/bicarbonate alkalinity, concentrations of total and soluble organics in the substrates and in the bioreactors were determined according to appropriate methods. Different temperature regimes have been realized in two bioreactors - mesopfilic (temperature 34 ± 0.5 ºC) and thermophilic (temperature 54 ± 0.5 ºC).
Results: The mashed wastes form different vegitables - potatos, tomatos, cucumbers and apples have been used as incoming substrates. The experiments with mixtures of them with different ratios under the same dilution rates and dry matter concentration in the incoming substrate have been made. They show in some cases the increase of the daily biogas production.
The comparative studies of productivity of both bioreactors have been made under the same dilution rates and dry matter concentration in the incoming substrate in different temperature regimes. There are no significant differences in yields of biogas from two bioreactors.
Conclusions*:* Multidisciplinary studies of the anaerobic digestion of different wastes in different operation modes have been performed. New knowledge about the process of anaerobic digestion of mixed vegetable organic wastes has been obtained. The main conclusion is that for different substrates and operation modes thorough studies are necessary.

Acknowledgments: This work is supported by the Ministry of Education and Science, NSF, Bulgaria, Contract DO 02-190/08. The support of E. Chorukova by BG051PO001-3.3.04/32 HR Project should also be acknowledged.

EMP095
The role of antibiotic production in the biocontrol of bacterial plant pathogens

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Pantoea agglomerans is naturally occurring epiphytic bacterium. Several strains were described as candidates for the biocontrol of plant pathogens. Some of them are known for the production of antibiotic metabolites which belong to diverse chemical classes and affect different molecular targets. The strain C9-1, isolated from apple blossom, is already registered as BlightBan C9-1 (Nufarms Americas) in the US and produces two antibiotics herbicolin O (also pantocin A) and herbicolin I. The structure, biosynthesis and role in the biocontrol of the fire blight pathogen remained unknown for long time.
We could show that our strain *P. agglomerans* 48b/90 produces an highly polar peptide antibiotic: 2-amino-3-(oxirane-2,3-dicarboxamido)-propanoyl-valine (APV). This compound inhibits the growth of important bacterial plant pathogens (i.e. *Erwinia amylovora*, *Pseudomonas syringae* pathovars, *Agrobacterium tumefaciens*) and the human pathogen *Candida albicans*. Comparing the properties of APV with the information available for the antimicrobial herbicolin I from C9-1, we could show that the two compounds are identical. Meanwhile also the biosynthesis cluster of APV (also Dapdiamide) is well-characterized, whereas the role of APV in the suppression of plant pathogens was unknown.
We conducted our plant experiments at moderate (20-25°C) and low (12-20°C) temperatures because the optimal temperature for APV production *in vitro* is between 8 and 12°C. We could show that Pa48b and its APV-negative mutant establish stable populations in a wide temperature range on apple blossoms and on soybean leaves. The coinoculation of the APV-negative mutant with the pathogens was supposed to highlight the role of APV within the biocontrol of the bacterial blight pathogen *Pseudomonas syringae* pv. *glycinea* (Psg) and the fireblight pathogen *Erwinia amylovora*. Surprisingly, the difference in suppression of the symptoms between wildtyp and mutant was minimal independent from the temperature. In coinoculation with Pa48b or its APV-negative mutant the population size of Psg was 10-fold lower at low temperatures and at least 2 orders of magnitude lower at moderate temperatures. Furthermore, the population size of *Erwinia amylovora* was decreased by 2 to 4 orders of magnitude in the coinoculation with Pa48b and its APV-negative mutant, respectively.

EMP096
Transcriptional response of the photoheterotrophic marine bacterium *Dinoroseobacter shibae* to changing light regimes

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Question: Bacterial aerobic anoxygenic photosynthesis (AAP) is an important mechanism of energy gain in aquatic habitats, accounting for up to 5% of the surface ocean's photosynthetic electron transport. The dominant AAP bacteria in marine communities belong to the Roseobacter clade. For this reason we used Dinoroseobacter shibae as a model organism to study the transcriptional response of AAP bacteria to changing light regimes.
Methods: We used continuous cultivation of D. shibae in a chemostat in combination with time series microarray analysis in order to identify gene regulatory patterns after a change in illumination.

Results: The change from heterotrophic growth in the dark to photoheterotrophic growth in the light was accompanied by a strong but transient activation of a broad stress response to cope with the formation of harmful singlet oxygen during photophosphorylation, an immediate downregulation of photosynthesis-related genes, fine-tuning of the expression of electron transport chain components and upregulation of the transcriptional and translational apparatus. Furthermore, our data indicate that D. shibae might use the 3-hydroxypropionate cycle for CO2 fixation.Analysis of the transcriptome dynamics after the switch from light to dark demonstrates that only few genes are directly regulated in response to light and other signals such as singlet oxygen concentration, electron flow, redox status and energy charge of the cell must be involved in the regulation of the processes accompanying AAP. This study provides the first analysis of AAP on the level of transcriptome dynamics. Our data allow the formulation of testable hypotheses about the mechanisms involved in the regulation of this important biological process.

EMP097
Fine scale depth monitoring of aerobic vs. anaerobic toluene degradation potentials over a redox gradient in a contaminated aquifer

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Microorganisms are important driving forces for degradation of hydrocarbon pollutants in groundwater environments. However, BTEX contaminants can be utilized as carbon and energy source under different hydrogeological and redox settings. It was previously shown that the distribution of anaerobic toluene degraders as traced via functional marker genes and that of typical iron- and sulphate-reducing groups is highly correlated to zones of increased anaerobic degradation at the lower fringe of an actual contaminant plume [1].
Here, we proceeded in order to elucidate the respective contribution of aerobic and anaerobic processes to net contaminant removal at the upper plume fringe, situated at an aerobic/anaerobic redox gradient. Genetic monitoring strategies based on the fingerprinting and sequencing of ribosomal gene amplicons were applied. Well-defined small-scale distribution patterns of typical aerobic and anaerobic degrader lineages were revealed. Via catabolic gene-targeted qPCR, we provide first interesting insights into pronounced quantitative configurations of aerobic and anaerobic toluene degraders over the redox gradient above the BTEX plume. An unexpected stratification of toluene oxygenase (*tmoA*) genes was observed with respect to oxygen availability. Surprisingly, this marker was found at maximal depth-resolved abundance in plume zones considered as absolutely reduced, such as the plume core, and not at the plume fringe. This may point towards unusual ecological controls of these putative aerobic contaminant degraders. In ongoing m-RNA analyses, we want to prove weather this localisation of presumed aerobic degraders could substantiate an importance of aerobic degradation without oxygen, as has been proposed also for other processes of hydrocarbon turnover in anaerobic environments [2]. This knowledge may provide a powerful tool to monitor the real state and capacity of natural attenuation and bioremediation in contaminated field sites.

[1] Winderl et al (2008): Appl Environ Microbiol 74, 792.
[2] Ettwig et al (2010): Nature 464, 543.

EMP098
Raman spectroscopy for the detection of molecular changes induced in bacteria by various heavy metals

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Investigations focused on the diversity of microbial community in different heavy metals contaminated sites indicate that *Acidiphilium* species are a common presence in this type of environment [1]. To gain a better understanding of the role played by these organisms in the remediation process and their resistance mechanisms towards different toxic metals, a study focused on the changes induced by various heavy metals in microorganisms is required.
For the investigation of the biochemical changes induced by various heavy metals in microorganisms, Raman spectroscopy was used. Compared with other spectroscopic approaches, the advantages of this micro-Raman spectroscopic technique represent its non-invasive character, minimal sample preparation, and the fact that only one bacterial cell is required to record the fingerprint Raman spectrum which provides information about the chemical composition of the investigated microorganism. Various studies demonstrate that Raman spectroscopy in combination with different chemometrical methods, e.g. HCA, LDA, SVM or ANN, can be successfully applied in bacterial identification [2; 3]. However, an important factor which could hinder the bacterial identification by means of Raman spectroscopy represents the accumulation of storage materials within the cells. A number of microorganisms are known to produce various polymers as carbon and energy storage molecules. By far, the most common compound produced by bacteria is polyhydroxybutyrate (PHB). Since this polymer can be accumulated in high amounts within the bacterial cells, it is expected that the Raman signals from the above mentioned substance partially or totally overlap the bands from others cell components.
A. cryptum JF-5, a dissimilatory Fe- and Cr reducing bacterium which produce large amount of PHB, was investigated with respect to the influence of chromium, copper, cadmium and nickel on the biochemical composition of the cells. The changes induced by the heavy metals in the chemical compositions of the microorganisms can be correlated to the type and amount of toxic substances present in the environment. The obtained results suggest that various resistance strategies are used by the investigated bacterium to adapt to the environmental conditions.
Acknowledgement: We gratefully acknowledge financial support from the Deutsche Forschungsgemeinschaft (Graduiertenkolleg „Alteration and element mobility at the microbe-mineral interface") as well as the TMC (Microplex).

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[3] Harz, M. (2009): Cytometry, 75A, 104.

EMP099
Sequence analysis of *pxmABC*, a *pmoCAB* homologue with yet unknown function, from rice rhizosphere soil

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Methanotrophs are a group of bacteria that utilize methane for energy and carbon source. pMMO, one of the two enzymes that mediate the first step in methane metabolism, was previously known to be encoded by *pmoCAB* as is its homologue, ammonia monooxygenase (encoded by *amoCAB*). Recently, several genome sequencing projects have identified novel *pmo*-like genes arranged in ABC orientation in strains of *Methylomonas*, *Methylobacter*, and *Methylomicrobium*. Named *pxm*, these genes have also been identified from freshwater sediment and *Methylomonas methanica* S1. Interestingly, sequences of these genes diverge significantly from any previously-known Pmo/Amo and their functional role remains yet elusive.
To examine their evolutionary and ecological context, we have identified and analyzed *pxmA* genes in rice rhizosphere soil. Using newly developed primer sets, a clone library was generated by nested PCR that specifically amplified partial *pxmA* sequences of ~450 bp. Our *pxmA* sequence data were combined with those deposited in the GenBank/EMBL database for further analyses.
All *pxmA* sequences were highly conserved with no indels despite the fact that different sets of primers and PCR techniques were applied for the acquisition of the sequences. Approximately 70 *pxmA* sequences were placed into five different clusters in a phylogenetic tree including one entirely novel cluster with ~60% of clones grouped within, indicating that either previous primer sets were incomprehensive or that composition of *pxmA* genes in soil and aquatic environment may differ. dN/dS value for clusters ranged between 0.0192 to 0.136, strongly indicating that these *pxm* genes have significant functional role. Their relatively uniform codon usage patterns may indicate that their hosts are phylogenetically very closely related and thus be confined to type Ia methanotrophs. From the sequence information, we speculate that these genes may provide insight into the link between methane and nitrogen metabolism and thus, we expect to extend this work to confirm this hypothesis by investigating their functional role from both biological and ecological standpoints.

EMP100
Microbiological water quality including pathogenic bacteria and parasites along the river Rhine

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The European water framework directive 2000/60/EG aims to protect water and to promote water use based on a long-term protection of water resources by using ecological and chemical parameters.
The commission Biologists within the International Association of the Waterworks in the river Rhine catchment area (IAWR) concluded that these parameters are not sufficient to describe the quality of a water body. Especially microbiological aspects are totally missing, but are most important for hygienic aspects of recreational activities and safe drinking water production. Therefore it was decided to do a survey to investigate the river water quality by health related microbiological parameters.
The microbiological parameters included on one hand fecal indicators (*E. coli*, coliform bacteria, enterococci, *Clostridium perfringens* and somatic coliphages) and on the other hand pathogenic microorganisms, namely thermotolerant *Campylobacter* and the parasites *Cryptosporidium* and *Giardia*. Samples were taken at two dates in summer 2009 at three different sampling points from Rhine-km 0 up to Rhine-km 865.
The results of this monitoring program will be presented and discussed. The numbers of the bacterial fecal indicators as well as those of the somatic coliphages increased in direction of the flow of the river Rhine. The pathogenic parasites *Cryptosporidium* and *Giardia* were detectable in the range of 1 to 20 cysts in samples of 100 L. The pathogenic *Campylobacter* were not detected. The most probable reason was the high water temperature (>25°C) during the sampling dates. Since the survival rate of Campylobacter bacteria is higher at lower temperature, further sampling at lower water temperatures would be needed.
In summary, it seems important to add microbiological parameters into a framework directive for the protection of water.

EMP101
Activation of nitrous oxide-producing nitrate reducers during gut passage through earthworms of different feeding guilds

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Nitrous oxide (N_2O) is emitted by the earthworm, and the anoxic conditions of the gut might stimulate ingested nitrate-reducing soil bacteria linked to this emission. Thus, denitrifiers and dissimilatory nitrate reducers in the earthworm gut of three different feeding guilds (epigeic [*Lumbricus rubellus*], anecic [*Lumbricus terrestris*], and endogeic [*Aporrectodea caliginosa*]) were investigated. *NarG* (encodes for a subunit of nitrate reductase) and *nosZ* (encodes for a subunit of N_2O reductase) were detected in the earthworm gut and surrounding soils at both gene and transcript levels. *NarG* transcripts related to nitrate dissimilators were detected in gut contents. *NirS* (encodes for a nitrite reductase) was examined at gene level in gut and soil of *L. terrestris*. Gut-derived sequences of all three genes displayed high similarity to soil bacteria and soil-derived sequences. Gut-derived *narG* sequences and terminal restriction fragments (TRFs) were mainly affiliated with gram positive organisms at both gene and transcript levels. Most transcripts were closely related to *Mycobacterium*-affiliated sequences. Sequences related to Gram negative bacteria were dominant in mineral soil. *nosZ* sequences and TRFs at both gene and gene expression levels belonged mostly to *Alphaproteobacteria*, with high similarity to *Bradyrhizobium japonicum* and uncultured soil bacteria. Differences between gut-derived and mineral soil-derived sequences were large at the transcript level but minimal at the gene level. Feeding guilds affected the detected *nosZ* community at the gene level and to an even greater extent at the transcript level. Feeding guilds also affected the detected *narG* sequences at the gene level but had minimal impact on *narG* expression. Most *nirS* sequences were affiliated with those related to *Bradyrhizobium*, *Rhodanobacter*, and uncultured soil bacteria. These collective observations indicate that (i) denitrifiers and dissimilatory nitrate reducers in the earthworm gut are soil derived, (ii) these functional groups are selectively activated and likely compete for inorganic nitrogen during gut passage, and (iii) feeding guilds affect this selective activation.

EMP102
Co-regulation of multidrug resistance and pathogenicity in the plant pathogen *Erwinia amylovora*

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Erwinia amylovora is the causative agent of the plant disease fire blight, with economic importance on apple and pear. During pathogenesis, the bacterium is exposed to a variety of plant antimicrobials. The bacterial multidrug efflux system AcrAB-TolC, which mediates resistance toward structurally unrelated compounds, was shown to confer resistance to these phytoalexins. While investigating AcrAB-TolC in *E. amylovora*, a linkage between multidrug efflux and pathogenicity has been discovered: a *tolC*-deficient mutant was impaired in multiplication in plant tissue and did not cause characteristic symptoms of fire blight on apple. Moreover, SDS-PAGE analysis of extracellular proteins showed that a hrp-associated virulence protein is no longer secreted by the *tolC* mutant.
A link between antibiotic resistance and pathogenicity was also found in other enterobacteria, e.g. *Salmonella enterica*. An intact AcrAB-TolC system is required for the colonization, and persistence, of these bacteria in the host. Mutants lacking *acrB*, or *tolC* showed decreased expression of major operons and proteins involved in the pathogenic process. Members of the AraC/XylS family of regulators, e.g. *marA*, *soxS*, *rob* and *ramA*, have been shown to activate expression of RND efflux pumps in *Enterobacteriaceae*. Furthermore, overexpression of *ramA* in *Salmonella enterica* lead to decreased expression of virulence genes suggesting that the regulation of multidrug efflux systems and expression of virulence genes show considerable overlap.
This project aims to identify similar regulators in *E. amylovora*. A BLAST search was used to identify homologous sequences in the available genome sequences. Overexpression of the homologous genes will be used to investigate the influence of the regulators on multidrug resistance by using MIC assays and on virulence by determination of *in planta* growth.

EMP103
Long-term investigations of natural remediation processes: adaptation of microbial wetland communities towards high loads of petrochemical aromatic compounds

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Within a long term project on compartment transfer (CoTra Project) at a former refinery site near Leuna (Germany) a semi-natural wetland was set up to investigate natural microbial degradation processes of petrochemical aromatic compounds (7 mg/l MTBE, 23 mg/l BTEX) under controlled, semi-natural conditions. The wetland (5m x 2.3m x 1.1m) is split into a planted (*Carex* spp.) and an unplanted section. Contaminated groundwater from the area is introduced at 11 l/h (~24d residence time). Saturation of >75% of the aromatic compound inflow concentration was reached already after one week. After 19 weeks the system became fully saturated and anoxic in the deeper layers, indicating the onset of microbial degradation processes. Sediment-bound bacterial biomass was generally higher in planted section. Flow cytometry analysis revealed high annual bacterial community dynamics. Beside bacteria, also fungi and phages obviously seem to play an important role in the system. No differences of pollutant removal rates between planted and unplanted section were detectable during the first season, but started to differ significantly after the following second season (~20% for MTBE and BTEX for unplanted; >60% for planted). However, pollutant breakdown efficiency of both strips strongly changed with season and temperature. In general, degradation processes were strongly enhanced by deep-going plant roots and soil-water transition layers, probably due to oxygen transport into deeper section and creation of

favorable environment for degrading microbial community. Detailed investigation of structural and functional microbial compounds revealed establishment of different communities for both strips. Planted wetlands and river banks, thus, inherit a high potential for microbial degradation processes and can act as natural buffer of weak and medium strong contamination.

EMP104
Cryoturbation affects denitrifier communities in N_2O-emitting arctic permafrost peat soil

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Wetlands are sources of the greenhouse gas N_2O. Cryoturbated (i.e., bare-surface soil mixed by frost action) rather than unturbated permafrost peat soil in the Russian discontinuous permafrost zone emits N_2O. The high nitrate and water contents of cryoturbated soil could favor the production of N_2O via denitrification. Thus, denitrifiers in cryoturbated and unturbated soils were assessed. Unsupplemented cryoturbated and unturbated soils produced 600 and <10 nmol N_2O g_{DW}^{-1} d^{-1}, respectively, in anoxic microcosms. Linear consumption of N_2O occurred subsequently in microcosms with cryoturbated soil. Nitrate-supplemented anoxic cryoturbated and unturbated soil produced N_2O at maximum velocities of 20 and <2 nmol N_2O h^{-1} g_{DW}^{-1}, respectively, when the reduction of N_2O was blocked by acetylene. Nitrite-supplemented anoxic cryoturbated and unturbated soil produced N_2O at maximum velocities of 28 and 18 nmol N_2O h^{-1} g_{DW}^{-1}, respectively, in the presence of acetylene. N_2O was below 40% of total N gases produced by cryoturbated soil when concentrations of nitrate and nitrite were <20 µM, suggesting that N_2 was a significant product of denitrification under such conditions. The ratio of N_2O to total produced N gases gradually increased to 100% with increasing nitrate or nitrite concentrations. N_2O approximated 100% of total produced N gases at all nitrate/nitrite concentrations in unturbated soil, indicating that N_2O consumption was not significant. These results indicated that (a) cryoturbated soil had a higher denitrification potential than did unturbated soil, and (b) denitrification was source of N_2O. Pyrosequencing and phylogenetic analyses of *narG, nirK/nirS*, and *nosZ* (encoding nitrate-, nitrite-, and nitrous oxide reductases, respectively) identified many novel denitrifiers in permafrost soil. Major sequence clusters occurred in both soils. Denitrifier diversity was lower in cryoturbated than in unturbated soil, indicating that the conditions in the cryoturbated soil selected for a specialized denitrifier community. The collective data indicates that (a) novel and highly diverse denitrifier communities are associated with N_2O fluxes in permafrost soil and (b) cryoturbation selects for a highly adapted denitrifier community capable of rapid denitrification.

EMP105
Biodegradation of diketopiperazines

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Diketopiperazines (DKPs) are important natural products produced by a variety of microorganisms and animals as quorum sensing signal molecules or antimicrobial agents [1; 2]. Despite their abundance in nature, little is known about the degradation of these smallest possible cyclic peptides.
Three different approaches are under way to investigate the mechanisms of possible DKP degradation pathways:
Paenibacillus chibensis (DSM 329) and *Streptomyces flavovirens* (DSM 40062) are described for the hydrolysis of cyclo(Asp-Phe) [3]. The activity against other DKPs of these two strains is characterized and the purification of the responsible enzymes was started.
Since DKPs are reported to be produced by numerous sponge-associated bacteria [4], enrichment cultures were obtained by incubating different sponge samples in minimal media containing certain DKPs as sole nitrogen source. Several strains isolated from these enrichment cultures are now under investigation for suitable enzymes with the ability to hydrolyze DKPs.
Certain hydantoinases are tested for DKP hydrolysis activity. Recently we have shown that these cyclic amidases are able to cleave different substituted dihydropyrimidines [5]. Due to the structural similarity between hydantoins, dihydropyrimidine derivatives and DKPs, a selective hydrolysis of DKPs by hydantoinases seems possible.

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EMP106
Microbial activities in different forest- and grassland soils

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Here we describe a complex study of soil microbial communities derived from German forest- and grassland soil samples, including phylogenetic and functional analysis. To get insights into environmental factors that may regulate microbial activities we analyzed the metatranscriptomes of 32 different soil samples (16 grassland- & 16 forest soil samples). First the mRNA was separated from the total community RNA. The mRNA of each sample was converted into cDNA without any amplification step and directly used for 454-sequencing (FLX, Roche). In total, 5'729'182 sequence reads were generated. For the evaluation of the data sets we used the MG-RAST- (Metagenome Rapid Annotation using Subsystem Technology[1]), UFO- (Ultra-fast Functional profiling[2]) and the Treephyler tools (fast taxonomic profiling of metagenomes[3]). The functional assignments and the phylogenetic composition of the different soil samples were compared. Analysis revealed significant differences in the microbial activities of the different soil sample areas. Furthermore this study shows that analysis of soil activities have big potential to recover new gene families.

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[3] Schreiber, F. et al (2009): Treephyler: fast taxonomic profiling of metagenomes. Bioinformatics.

EMP107
Genetic heterogeneity of anaerobic rumen fungi as revealed by D1/D2 domain of 28S rDNA sequences

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Anaerobic rumen fungi inhabit the gastrointestinal tract of ruminants and other non-ruminant herbivores. Because of their biotechnological applications, they have been explored by the scientist's world over. They are even regarded as the primary colonizers of ligno-cellulose and most active cellulose degraders in the biological world. Till date, their six genera and 20 species have been described following conventional morphological classification system of using features like growth pattern (moncentric or polycentric), thallus morphology (filamentous or bulbous) and number of flagella per zoospores (monoflagellated or polyflagellated). But, tthese features tends to be pleomorphic, making it essential to use molecular approaches for accurate identification and differentiation of anaerobic fungi and its species. 18S rDNA (SSU) based identification has been termed obsolete for species level identification because of highly conserved and less variable regions, while the internal transcribed spacer (ITS) region based differentiation is also questioned as it is not a single copy region. Moreover, its variability is sometimes not high enough to be able to differentiate at species level. Therefore, the D1/D2 domain of large subunit rDNA (28S) region of various isolates was explored to find its suitability as a potential biomarker for studying genetic heterogeneity of rumen fungi. In present study, rumen fungi were isolated from fistulated buffalo using a serum bottle modification of Hungate roll-tube technique. Various isolates of *Anaeromyces* and *Orpinomyces* were identified on the basis of their morphological features. DNA was isolated using CTAB method. D1/D2 domains of 28S rDNA was amplified using NL1 (5´-GCA TAT CAA TAA GCG GAG GAA AAG-3´) and NL4 (5´-GGT CCG TGT TTC AAG ACG G-3´). After sequencing the PCR products, multiple sequence alignments were performed using BioEdit software. The DNA sequence alignment showed that there were a total of 59 nucleotide variations in both the genera with maximum being T→C and T→A at 11 places; C→T at 7 places; A→T and G→A at 6 places; G→T at 5 places; C→A and T→G at 4 places; A→G at 3 places and C→G at 1 place. Moreover, one addition mutation of

nucleotide A was also found. Sequencing and analysis of other genera is currently under progress.

EMP108
Carbon stable isotope fractionation of chlorinated ethenes and ethanes by *Dehalococcoides* ribotype BTF08 and *Dehalococcoides ethenogenes* 195

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The carbon stable isotope fractionation of vinyl chloride (VC), dichloroethene (DCE) and dichloroethane (DCA) was investigated during reductive dechlorination to ethene. Isotope fractionation terms the change of the given natural stable isotope ratio of a substance, caused by transformation processes, where bonds with lighter isotopomers (e.g. ^{12}C) have a slightly lower bond energy resulting in a faster reaction compared to the heavier isotopomers (e.g. ^{13}C). This leads to an enrichment of heavy isotopes in the residual fraction of the substrate allowing the calculation of an enrichment factor (εC). Given that isotope fractionation is determined by the chemical reaction mechanism, similar degradation pathways and enzymes are expected to result in a similar fractionation. In this study we could demonstrate that *Dehalococcoides* ribotype BTF08 fractionates VC (εC = -28.8 ± 1.5 ‰), 1,1-DCE (εC = -12.4 ± 1.1 ‰) and cis-DCE (εC = -30.5 ± 2.7 ‰) stronger compared to other *Dehalococcoides* strains [1]. Additionally, preliminary experiments showed that DCA carbon isotope fractionation is stronger than DCE fractionation. In summary, the results support that DCE and DCA dehalogenation occur via different pathways in *Dehalococcoides* and that *Dehalococcoides* ribotype BTF08 may contain novel DCE and VC dehalogenases.

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EMP109
Prevalence of *Campylobacter* species in North American wild birds

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Campylobacter bacteria have a significant impact on human health, being responsible for the majority of food borne illnesses and gastroenteritis worldwide. Understanding the prevalence of bacterial pathogens in environmental factors influencing the commercial food industry such as wild birds, may serve as an additional useful tool for examining the spread of disease organisms and therefore further understanding of the overall epidemiology of the organism. The emergence of new infectious diseases in wildlife and the increasing contact between humans and human food supply chains with wildlife has created a need to better understand the role of environmental influences as vectors of pathogenic bacteria such as *Campylobacter jejuni*, not only in wild birds in Europe, but also in wild birds in the United States. Wild birds have been thought to factor into the epidemiology of this bacterium, although few studies have estimated the prevalence of *Campylobacter* in North American wild birds.
This study evaluated the prevalence of *Campylobacter* spp. in gulls (Laridae), shorebirds (Scolopacidae), and migratory and resident waterfowl (Anatidae) through culture and PCR-based methods. Results from culturing methods indicated that 43% (n=65) of *Arenaria interpres* and 28% (n=32) of *Calidris pusilla*, both long distance migratory shorebird species sampled in the Delaware Bay, were positive for *Campylobacter* spp. Only 5% (n=279) of *Branta canadensis* were positive for *Campylobacter* spp. while another North American migratory goose species, *Chen caerulescens,* had a prevalence of 30% (n=111). Of several Laridae species, all of the genus *Larus*, 14% (n=147) were positive for *Campylobacter* spp. including *Campylobacter jejuni*. All samples will be further analyzed through MLST-PCR specific for *C. jejuni* to explore similarities and differences between poultry and human clonal complexes. Wintering waterfowl and gulls often occur on agricultural fields possibly spreading disease organisms. Shorebirds undergo long distance migrations and have the potential of transferring bacteria and antibiotic resistant genes across long distances. With implications of wild birds transmitting the *Campylobacter* bacteria to other avian species, such as poultry, this study provides further insight into *C. jejuni* occurrence within selected wild bird populations in North America, USA.

EMP110
The bacterial community in the natural gas reservoir Altmark (Germany) and the assessment of indigenous microorganisms

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Carbon capture and storage into depleted natural oil and gas fields could be a solution for members of the EU to achieve the aim of decreasing greenhouse gases by 20% until 2020. Therefore, research on putative storage sites like the natural gas field Altmark is strongly needed to support and secure this intention.
RECOBIO-2, part of the BMBF-funded *Geotechnologien* consortium, investigates the presence of microbes in formation waters of the Altmark gas field and their potential role in CO_2 turnover. This gas reservoir is operated by GDF SUEZ E&P Germany GmbH and is located in the southern edge of the Northeast German Basin. The *Rotliegend* formation has an average depth of 3300m, a bottom-hole temperature between 111 and ~120°C, is characterized by high salinity (up to 350g/l) and low concentrations of dissolved sulfate.
Two types of samples were analyzed: formation water collected at the well head (wh) and formation water sampled down hole (dh). Some of the wells were treated frequently with a foaming agent while others are chemically untreated.
Molecular-genetic analyses of formation water of different wells were performed to assess the structure of the microbial community. Despite the extreme environmental conditions, RNA and DNA were successfully obtained and sequenced. Bacterial 16S rRNA gene sequences of formation water (wh and dh) from well Mellin B (Heidberg-Mellin) were affiliated mainly with *Firmicutes* (wh 58%, dh 62%) and *Bacteroidetes* (wh 26%, dh 21%). Especially *Desulfotomaculum* spp., *Thermoanaerobacterium* spp. and bacteria related to uncultured ones from a petroleum reservoir were detected.
The extent of indigenous microbes in samples of three wells was assessed with a special sampling approach. The down hole sampling device was flushed, the water collected and then the formation water was sampled. T-RFLP patterns showed a variation and allowed a differentiation of terminal fragments of putative indigenous bacteria from those of possibly external origin.

EMP111
Long term bioimmobilization and bioremediation at an AMD contaminated site in Eastern Thuringia

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The residual contamination with heavy metals and the low pH affects re-establishing of vegetation in AMD polluted areas. Microbially assisted bioremediation and bioimmobilization strategies were tested with respect to altering element uptake into plant biomass, leading to planting regimes that maximize reduction of the ecotoxicological risk and input into food webs and water ways.
The experimental site is a remediated site, disturbed by several decades of uranium leaching (Eastern Thuringia, Germany). On this site, the effect of 5 cm topsoil or municipal compost addition on plant availability of metals was investigated for several years. The soil was inoculated with mycorrhizal fungi and streptomycetes with plant growth promoting properties. A soil characterization including sequential extraction was performed in the amended as well as in an untreated control plots to investigate possible shifts in metal availability/mobility as an effect of the amendments added. The bioavailable heavy metal fraction was lowered by metal binding to the organic fraction. The inoculation with microorganisms aided the treatment of the soil. Diversity and evenness of the plant community were increased and plant growth was increased with inoculation. These effects were stable

for several years. Thus, microbially aided revitalisation of disturbed soils, as well as addition of soil amendments were used to combine metal immobilization and enhanced biomass production. Future trials will test whether enhanced phytoextraction by remobilization from the organic fraction and uptake into plant biomass under controlled conditions can be devised to promote future possible land-use.

EMP112
Intrinsic biodegradation potential of environmentally significant corrosion inhibitors used in borehole heat exchanger fluids

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1H-benzotriazole, its derivative tolyltriazole, and sodium-2-ethylhexanoate belong to the most frequently used corrosion inhibitors in borehole heat exchanger systems. In case of a leakage, a local groundwater contamination might occur where glycol-based heat transfer fluid containing corrosion inhibitors enters the aquifer down to a depth of 150 meters. Thus, an assessment of the intrinsic biodegradation potential of these corrosion inhibitors is fundamental.
Microcosm experiments were conducted using aquifer material from a depth of 60 meters that was sampled directly during the installation of borehole heat exchangers. Benzotriazole, tolyltriazole, and sodium-2-ethylhexanoate were added as sole carbon sources at initial concentrations of 0.05 mM. Microcosms were incubated under denitrifying, iron-, and sulfate-reducing conditions at the presumed aquifer temperature of 12°C.
Within more than 200 days of incubation, no intrinsic biodegradation potential was observed for benzotriazole and tolyltriazole under the various redox conditions investigated. In contrast, sodium-2-ethylhexanoate was readily utilized by the microorganisms within 65 and 40 days under denitrifying and sulfate-reducing conditions, respectively.
In further experiments, the effect of benzotriazole and tolyltriazole on the biodegradation of ethylene glycol, which is most widely-applied antifreeze agent in borehole heat exchangers, was assessed. Ethylene glycol itself was readily biodegradable under denitrifying and sulfate-reducing conditions.
Addition of benzotriazole affected the initiation of the biodegradation of ethylene glycol. In control experiments, the biodegradation of ethylene glycol started after 2 days, whereas in the presence of benzotriazole the lag period lasted 7 days. More than 98 % of the initial substrate in the control experiments was degraded within eight days. In the presence of the two corrosion inhibitors the degradation of ethylene glycol proceeded at a lower rate and 98 % of the substrate were not degraded until 15 days of incubation.

These findings indicate that benzotriazoles may not only threaten groundwater quality due to their own toxicities but in addition inhibit the biodegradation of other organic compounds.

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EMP113
Biomethylation of metal(loid)s in anaerobic habitats: A novel multi-metal(loid) methylation mechanism directly coupled to methanogenesis

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Biomethylation and -hydrogenation of Group 15 and 16 metals and metalloids by microorganisms are widespread phenomena in anaerobic habitats including sewage sludge, soils and, as recently shown, the gut of mice and man. In spite of the significant impact on metal(loid) mobility and toxicity, little is known about the biochemical mechanisms of these processes. As yet, biochemical details are only available for arsenic, which is methylated by S-adenosylmethionine (SAM) dependent methyltransferases expressed in response to elevated arsenic concentrations. For identification of alternative metal(loid) methylation mechanisms, we focused on *Methanoarchaea*, since, in contrast to other physiological groups, the capability to volatilize a broad spectrum of metal(loid)s (As, Sb, Bi, Te and Se) was observed for almost all methanogens studied.
Here, we studied the methyltransferase MtaA from *Methanosarcina mazei*, which catalyses the methyl group transfer from methylcobalamin to coenzyme M in the course of methylotrophic methanogenesis. We demonstrate that the same metal(loid)s (As, Se, Sb, Bi, Te), which are methylated by *Methanosarcina mazei in vivo*, are also methylated by *in vitro* assays with purified recombinant MtaA, thereby revealing the first mechanism capable of multielement methylation of metal(loid)s. In addition to methylation, formation of hydride As, Se and Sb species was observed. The mechanism of methyl transfer from methylcobalamin to the metal(loid) as well of metal(loid) hydride generation is discussed. Overall, these results can explain why the methylation of a broad range of metal(loid)s is a widespread phenomenon in anaerobic habitats and a general feature of methanogens.

EMP114
Microbial Hitchhikers on Intercontinental Dust - Chadian Origins

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Dust that originates from deserts is now known to be a vehicle for the spread of microbial communities via natural atmospheric pathways. Only those microorganisms that are present in desert soils at the source of dust events can be blown into the stratosphere and cross continents. Molecular signatures of microorganisms (bacteria, algae and fungi) found in desert soil can be used to trace the spread of these communities through natural atmospheric pathways on our planet. Soils of the deserts of the Republic of Chad present the biggest sources of Aeolian dust and are an obvious place to study micro-organisms before they begin their intercontinental travels. Nine sand samples from Bardaï in the desert zone to the north-west of the country and others from the arid Sahelian belt in the Center were collected. Geochemical analysis was performed by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) and Inductively Coupled Plasma - Mass Spectrometry (ICP-MS). Here we document the spectrum of both cultivable micro-organisms as well as those whose presence could only be revealed by metagenomic procedures (cloning and direct high throughput sequencing).

EMP115
Influence on the persistence of antibiotic residues and antibiotic resistance activity in response to manure treatment

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The application of untreated manure, digestate and liquid wastes to agricultural areas provides essential fertilization for crops and pasture in an economically efficient way, yet enabling the entry of veterinary pharmacological trace compounds and antibiotic-resistance-genes (ARGs) into the environment [2]. Due to the constant supply of manure containing antibiotics, its reservoirs act as breeding grounds for antibiotic resistance and multidrug-resistance, promoting pathogenic activity. While the threat of the environmental distribution of antibiotics has been a focal point of investigation in recent years little is known about the long term effects of sub-therapeutic antibiotic levels on microbial communities at the transcriptional level and the distribution and persistence of ARGs [1; 3]. The aim of this ongoing study is the development of manure-treatment-systems to simultaneously degrade antibiotic substances and their respective ARGs prior to land-application and biogas-production. The focus lies on the

tetracycline, sulfonamide and fluoroquinolone families, which are known to be avidly applied in animal husbandry [4]. Known antibiotic resistance genes will be monitored via quantitative real-time-PCR on transcriptional as well as genomic levels to illuminate changes and the persistence of resistance activity driven by the selective pressure of antibiotics present. At the same time 16s rDNA-based sequence analysis of manure samples are preformed to examine microbial diversity changes. Preliminary results reveal variations in the biodiversity patterns of manure samples, with a significant increase (30%) of species distribution in samples with undetectable antibiotic concentrations indicating that antibiotic levels found in conventionally treated manure may have severe ecological consequences. Furthermore, pathogenic activity shows an invariable level of 72 % of the total biodiversity, independent of antibiotic-concentration. This approach will generate an insight into the persistence of ARGs and thus allowing manure-utilisation without the distribution of resistant strains that threaten human health and ecological diversity.

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EMP116

Will not be presented!

EMP117

Agricultural Soil Protists Assimilate Carbon from Supplemental ^{13}C-Cellulose

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Soil prokaryotes are degraders of cellulose in aerated agricultural soils. Certain soil micro eukaryotes (i.e., fungi and protists) may (a) utilize cellulose and its degradation products or (b) graze on cellulose-degrading bacteria. In a preceding study, agricultural soil slurries were supplemented with ^{13}C-cellulose or ^{12}C-cellulose (control). Dissimilation under oxic conditions yielded carbon dioxide, whereas mixed acid and butyrate fermentations and ferric iron reduction were important anaerobic processes under anoxic conditions. *Actinomycetes*, *Planctomycetes*, and a novel family-level taxon within the *Bacteroidetes* were primary consumers under oxic conditions as determined by 16S rRNA-based stable isotope probing (rRNA SIP). In contrast, *Clostridiaceae* and novel family-level taxa of *Bacteroidetes* and *Actinobacteria* were primary consumers under anoxic conditions. In the current study, ^{13}C-labeled protists were analyzed and assessed by (a) terminal fragment length polymorphism (TRFLP) analyses and (b) generating gene libraries of 18S rRNA genes using primers specific for Eukaryotes, *Chrysophyceae* (*Heterokontophyta*), and *Kinetoplastea* (*Kinetoplastida*). Labeled OTUs were detected in oxic treatments, whereas labeling was not apparent in anoxic treatments. Assimiliation of ^{13}C by soil protists might suggest that grazing on cellulose-degrading bacteria was an important pathway of carbon assimilation from cellulose into soil protistan community. However, it is unknown if detritivory or also osmotrophy, i.e. direct assimilation of cellulose and breakdown products, were protistan feeding strategies.

EMP118

Reductive Dechlorination of Chlorinated Benzenes in Anaerobic Enrichment Cultures from Vietnam and Germany

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Chlorinated benzenes are important industrial intermediates and solvents. The intentional or accidental release causes significant concern due to the toxicity and persistence. Among the chlorobenzenes, hexachlorobenzene (HCB) is the most persistent environmental pollutant and is listed as one of the 12 persistent organic pollutants in the Stockholm Convention. A second congener of high concern is 1,3,5-TCB which has been described as the product of HCB degradation under anaerobic conditions and is persistent under aerobic and anaerobic conditions. Only marginal information is available on the degradation of 1,3,5-TCB by anaerobic mixed cultures.

Using inocula from sediments, soils or water originating from different locations in both Vietnam and Germany we obtained mixed cultures dechlorinating trichlorobenzenes (TCBs) (1,2,4-TCB, 1,2,3-TCB and 1,3,5-TCBs) and HCB. HCB was reductively dechlorinated to pentachlorobenzene, which was transformed to a mixture of 1,2,3,5- and 1,2,4,5-tetrachlorobenzenes. The final end-products from HCB were 1,3,5-TCB, 1,3-dichlorobenzene (DCB), 1,4-DCB and monochlorobenzene (MCB). The experiments were repeated with TCBs (1,2,4- and 1,2,3-TCB) and the final end-products of these TCBs were 1,3-DCB, 1,4-DCB and monochlorobenzene. Besides, also 1,2-DCB was produced from TCBs. In the pathway producing 1,3,5-TCB from HCB, only doubly flanked chlorine substituents were removed while in the pathway leading to 1,2-DCB, 1,3-DCB, 1,4-DCB and MCB from HCB and TCBs both doubly and singly flanked chlorine substituents were removed. We are now maintaining these dechlorination pattern and try to link dechlorination pathways with single populations in the mixed consortia. Isolation of bacteria that remove singly flanked chlorine substituents is essential to further understand the biochemical reasons for the reaction specificity of different enzymes.

Hölscher et al. 2010, Environ. Sci. Technol. 44: 8936-8942

EMP119

Investigation of biosynthesis, function and significance of siderophores in *Phaeobacter gallaeciensis*

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Because of the very low solubility of Fe^{3+} in the sea, iron is a limiting factor for microorganisms, not only for phytoplankton and cyanobacteria, but also for heterotrophic bacteria. To be able to compete for iron, some bacteria produce siderophores, which are strong soluble Fe^{3+} chelating ligands. The role of siderophores is to scavenge iron from the environment and make the mineral available to the cell. Representatives of the *Roseobacter* group (*Alphaproteobacteria*) are very successful in many marine habitats and show a high metabolic versatility, however, production of siderophores has not been reported yet for Roseobacters. In the genome of *Phaeobacter gallaeciensis*, a representative of the *Roseobacter* group, we found genes coding for a putative iron-siderophore uptake system. Production of siderophores by *P. gallaeciensis* was subsequently confirmed by a chrome azurol S (CAS) assay. Construction of knock out mutants to study genetic and regulatory aspects of the siderophore biosynthesis in *P. gallaeciensis* is in progress. On the one hand we focus on the investigation of the biosynthetic pathway, on the other hand we characterize the function and importance of the siderophore for the organism. Furthermore, *Phaeobacter gallaeciensis* produces acylated homoserine lactones (AHLs), a class of signalling molecules involved in bacterial quorum sensing. Quorum sensing systems were also reported to have influence on siderophore production. To study a possible correlation, we constructed an AHL-deficient mutant in order to compare the expression of genes involved in siderophore production in the wild-type and the mutant strain.

EMP121

Detection of specific epibacterial communities affiliated to the marine Roseobacter group reflects adaptation to various macroalgae

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Bacteria of the *Roseobacter* clade are abundant and widely spread in many marine ecosystems and were also detected on various macroalgae. Marine macroalgae are known to be covered by a dense microbial biofilm with a variety of different species. The microbial communities are specific for each algal species. In order to investigate whether organisms of the *Roseobacter* clade are present on many different macroalgae, we sampled a variety of

species of marine macroalgae of the German Wadden Sea and the Spanish Atlantic coast of Galicia. The presence and diversity of Roseobacters on the different algae was analysed by using a *Roseobacter* clade-specific 16S ribosomal RNA gene-based PCR-DGGE approach. Our results revealed presence of a variety of Roseobacter on all investigated algal species. Furthermore, we detected different compositions of Roseobacters on all different macroalgae. This indicates a species specific relationship between these bacteria and the algae. In addition to the use of molecular biological methods, new strains affiliated to the *Roseobacter*-clade were isolated from the algae for further analysis of the relationship between the algae and the bacteria. Since production of secondary metabolites was reported previously for Roseobacters obtained from macroalgae, the isolates were tested for production of antibiotic compounds and compared to isolates obtained from the water column.

EMP122
A simple time-resolved microdilution assay for determination of dynamic microbial growth inhibition by metal nanoparticles

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Engineered metal- and metal-oxide nanoparticles are increasingly used in products for everyday life and comprise additives that exhibit advantageous antimicrobial properties. Once released into the environment, it is expected that several types of metal nanoparticles could possess adverse effects on microbes of exceeding environmental importance. However, existing standard ecotoxicological methods are only of limited applicability for studying the whole spectrum of potential nanoparticle impacts on microbial life.
Agar diffusion tests could be successfully adapted to test the microbial susceptibility to noble metal nanoparticles (Pd, Pt) but testing Ag(0) nanoparticles led to methodological difficulties and required the addition of dispersive and stabilizing agents such as polysorbate detergents. Nanoparticle transport, biosorption, toxic effects and microbial resistance mechanisms are subject to temporal relatedness and will thus largely affect microbial growth dynamics. Therefore, a common microdilution assay was modified to allow simultaneous cultivation and real-time analysis of microbial growth inhibition. The automated assay in 96-well microtiter plates combines high temporal resolution with the analysis of many replicates. It comprises analysis by MATLAB which is used to numerically determine the maximum slope of Gompertz functions fitted to microbial growth curves. Test organisms including *Cupriavidus necator* H16, *E. coli*, and *Pseudomonas putida* exhibited different susceptibility to stable dispersions of metal and metal-oxide nanoparticles (Ag, Pd, Ni, ZnO, TiO_2, CuO, ZrO_2, CeO_2), with Ag(0) nanoparticles (D90 < 15 nm) as most effective against Gram-negative bacteria. Ag(0) concentrations above 0.008 % (w/v) demonstrated complete and irreversible inhibition of microbial growth, whereas extended lag phases and partial growth inhibition was observed at Ag(0) concentrations between 0.001 and 0.008 %. In contrast, *Bacillus* spp. revealed up to ten-fold lower sensitivity against Ag(0) nanoparticles. This effect was independent from primary particle size distribution suggesting interference by nanoparticle reaggregation in growth media, or cross effects induced by Ag^+ ions released from the surface of the tested materials.
Extending our study to complex microbial communities from activated sludge requires the application of molecular fingerprinting methods and state-of-the-art metabolic-pattern analysis by proton transfer reaction mass spectrometry (PTR-MS). These techniques are used to follow the temporal dynamics of community structure and function and will contribute to a better understanding of antimicrobial activity of metal nanoparticles and their fate in the environment.

EMP123
High CO_2 concentrations negativly effect methanogenesis and sulfate reduction in gas fields of the North German Plain

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In consequence of their global warming potential, large-scale solutions are needed to reduce the emission of greenhouse gases such as CO_2 or CH_4 . The Carbon Capture and Storage (CCS) technique offers one option to reduce emissions. Favourable CO_2 storage sites are depleted gas and oil fields. Our study is focusing on the direct influence of high CO_2 concentrations on the autochthonous microbial population and environmental parameters in such potential storage sites.
The investigated reservoir formation (Schneeren) is operated by Gaz de France Suez E&P DEUTSCHLAND GmbH. The conditions in the reservoir differed between the studied wells A and B in various geochemical and microbiological parameters. Based on these results our study included both cultivation and molecular biological approaches.
The two production fluids (wells A and B) differed in the dominating microbial activity for indigenous fluids and substrate amended enrichments. Methanogenis was strongly induced after the addition of various substrates with higher rates for fluids of well A. On the other hand were the highest induced sulfate reduction activity detected in hydrogen amended fluids of well B. Results of the molecular biological analysis of the original fluids supported the activity data for both fluids. The abundance of archaeal 16S rDNA and *mrcA* was several magnitudes higher in fluids of well A whereas well B was dominated by *Bacteria*.
Incubations with high CO_2 concentrations showed a significant decrease of methane and sulfide production with increasing CO_2 levels. In a second step enrichments from the reservoir fluids and pure cultures of reference organisms were incubated under *in situ* pressure and temperature with elevated CO_2. During the short term incubation these experiment showed no detectable sulfate reduction activity while the total cell number was stable. Further molecular based analysis revealed witch parts of the Schneeren community can survive high CO_2 partial pressures in the incubations. In conclusion this study of simulated CCS operations will provide information about possible biogeochemical and microbiological changes during the storage of CO_2.

EMP124
Microbial induced mineral precipitation and corrosion in geothermal plants

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The development of renewable energy sources such as geothermal energy is of increasing importance to secure a sustainable energy supply and a reduction of greenhouse gas emission to the atmosphere at the same time. For an efficient and permanent reliable use of geothermal energy the interruption of operation due to malfunction of the facility has to be prevented.
Up to now only a few studies focus on microbial induced processes that could influence the operational reliability of geothermal plants. A geothermally used groundwater system was investigated under microbial, geochemical, mineralogical and petrological aspects. The monitored groundwater system is located in the North German Basin, Germany. To characterize the microbial biocenosis of a seasonal heat storage fluid and filter samples were taken from regularly and shift as well as disturbed plant operation and analyzed based on 16S rDNA. Among fingerprinting methods (SSCP, DGGE) for the characterization of the microbial biocenosis, FISH will be applied for the quantification of microorganisms and the determination of their metabolic activity. The identification of microorganisms enables the correlation to metabolic classes and provides information about biochemical processes in the used groundwater system.
First results of analyses of the influence of the operational mode on the number of cells and the metabolic activity will be presented. Indicator organisms, which can be useful for an early detection of plant failures, will be identified, if possible.

Our goal is to enhance process understanding particularly related to scaling and corrosion processes of engineered geothermal systems to contribute to the optimization of plant reliability.

EMP125
Short term response of a pristine indoor aquifer system to a simulated toluene spill

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The intrinsic potential of groundwater ecosystems to recover from hydrocarbon contamination is of prime concern when it comes to protection and sustainable management of groundwater resources. However it needs in-depth understanding of biological, geochemical and hydrological processes at pristine and contaminated sites to substitute descriptive research by assessment of approaches with predictive power.
A pristine sandy indoor aquifer has been designed mimicking oligotrophic groundwater aquifer which allows a thorough system characterisation at non-impacted conditions followed by a spatio-temporal highly resolved investigation of system response upon toluene injection and during its recovery.
Although there is a pile of lab studies on natural attenuation of aromatic hydrocarbons, none have comprehensively addressed the following concerns: (i) what is the time scale for an ecosystem reaction (efficient biodegradation) towards organic contamination in case of a pristine aquifer? (ii) does the indigenous microbial diversity add pronounced resilience to the system and can microbial patterns be used as indicators for ecosystem status assessment? (iii) is there a quantitative correlation between degraders abundance and a distribution of respective degradation genes and processes? (iv) does the ecosystem return to its original status after removal of contamination and how fast?
First obtained results, prove an immediate response of the indoor groundwater ecosystem to toluene introduction. Screening of bacterial community composition via T-RFLP fingerprinting revealed a decreasing diversity inside and at the fringes of the toluene plume. This was accompanied by increasing total cell counts, higher ATP values and increased bacterial carbon production rates. A relative maximum of genes encoding for toluene degradation shortly hints at fast establishment of specific degradation lineages in the intrinsic groundwater (and sediment) community. Already after 4 weeks of constant toluene injection a net decrease of toluene, rapid oxygen depletion in the center of the plume and disappearance of sensitive members of the community was observed.
The indoor aquifer provides an ideal playground to assess the impact of toluene contamination and the natural attenuation potential of groundwater ecosystems.

EMP126
Fungal and green algal microbial communities on natural stone surfaces

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Microbial biofilms, consisting of green algae, filamentous fungi and bacterial organisms cover solid surfaces, such as plastic material, glass and natural stone. In the present study, biofilm samples from diverse dimension stone surfaces where used to analyze the diversity of bacteria, green algae and filamentous fungi in a comparative approach. Isolated organisms were used to unravel their strategies for adhesion to stone surfaces and for endolithic growth.
The overall diversity of certain groups markedly differs with respect to certain features of the stone surface. Analysis of cloned sequences obtained from environmental DNA revealed that surfaces covered with thin gypsum crusts are preferentially colonized by unicellular cyanobacteria. On other surfaces (sandstone, limestone), green algal species dominate.
The diversity of filamentous fungi rather depends on exposure of the stone surface to sunlight: From W-exposed, shaded surfaces, free living ascomycetes, mainly melanized black fungi, could be retrieved. An ESE, sun-exposed surface was barely colonized; the retrieved organisms were mainly lichen ascomycetes and the lichen alga *Trebouxia*.

Hallmann, C., Rüdrich, J., Enseleit, M., Friedl, T., Hoppert, M. (2010) Microbial diversity on a marble monument: a case study. Environ Earth Sci - DOI 10.1007/s12665-010-0772-3

FBV001
Grey mould isolates from German strawberry fields reveal a new type of multidrug resistance and evidence for a novel taxon next to *Botrytis cinerea*

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Botrytis cinerea is a destructive pathogen of many fruit and and vegetable crops worldwide, which needs to be controlled by fungicide treatments in commercial cultures. We have recently described the wide-spread appearance of grey mould strains in vineyards with reduced sensitivities to different fungicides (MDR phenotypes), due to increased drug efflux activity caused by the overexpression of two genes encoding ABC- and MFS-type transporters. We have extended our analysis to strawberry fields, which receive many fungicide treatments during flowering. A high proportion of grey mould isolates from strawberries showed high frequencies of resistance to all currently used fungicides. We also detected a new MDR phenotype ($MDR1^h$), with higher resistance levels than MDR1. This phenotype is correlated to even higher constitutive levels of the *atrB* ABC transporter gene. $MDR1^h$ isolates were found to be genetically distinct, showing significant sequence divergence compared to known *B. cinerea* strains. We are currently analyzing the taxonomic status of these isolates and the mutations leading to $MDR1^h$.

FBV002
Mutations and migration of *Botrytis cinerea* field strains with multidrug resistance phenotypes in French and German vineyards

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Botrytis cinerea is a destructive pathogen of many fruit and and vegetable crops worldwide, which needs to be controlled by fungicide treatments. In French and German vineyards, *B. cinerea* strains with multiple fungicide resistance (MDR) phenotypes have been observed with increasing frequencies. MDR results from mutations that lead to constitutive overexpression of genes encoding drug efflux transporters. In MDR1 strains, several point mutations in a transcription factor encoding gene (mrr1) have been identified that lead to constitutive activation of the ABC transporter gene atrB. In MDR2 strains, overexpression of the MFS transporter gene *mfsM2* has been found to result from two rearrangements in the *mfsM2* promoter caused by insertion of a retroelement (RE)-derived sequence. MDR2 strains containing the type A rearrangement are widely distributed in French and German vineyards, while strains with type B rearrangement have been found only in the Champagne. MDR2 strains harbouring either *mfsM2* type A or type B mutations show the same resistance phenotypes, and similar overexpression of *mfsM2*. Population genetic analyses were conducted that support the hypothesis that the two MDR2-related mutations have only occurred once, and are responsible for the appearance and subsequent spread of all known MDR2 strains in French and German wine-growing regions.

FBV003

Molecular and chemical characterization of secondary metabolite gene clusters in *Fusarium fujikuroi*

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The filamentous fungus *F. fujikuroi* is known to produce a variety of several secondary metabolites such as the plant hormones gibberellins, polyketide pigments such as bikaverin and fusarubin, and mycotoxins like beauvericin, fusarin C and moniliformin (MON) which cause enormous economical losses in trade of crops.
In order to reduce the health risk of these mycotoxins in food, feed and biotechnologically produced gibberellin preparations, identification of the involved gene clusters is of great importance.
The recently sequenced genome of *F. fujikuroi* contains 17 polyketide synthases (PKS). So far we know the genes which encode the polyketide synthases of bikaverin, fusarin C, fumonisin and fusarubin. Currently we are working on the elucidation of the biosynthetic pathway of fusarin C using the deletion mutants of the involved gene cluster which recently have been identified in *Fusarium* ssp. Until now only the hybrid polyketide synthase/nonribosomal peptide synthetase-encoding gene (PKS/NRPS) has been identified in *F. venetatum*.
Further on the identification of the biosynthetic genes for the mycotoxin MON is of great interest. Up to now none of the MON biosynthetic genes is known in any *Fusarium* ssp. Therefore we are generating deletion mutants for putative PKS genes in a MON-producing *F. fujikuroi* strain, because a PKS pathway is suggested in the literature.
Besides' we study the influence of the regulation mechanism for different pathway genes (the role of nitrogen and pH), as well as the impact of global regulators on their expression (e.g. velvet, laeA and histone-modifying enzymes).
To investigate products of the remaining PKS with unknown functions the establishment of a method for LC-MS (comparison of the product spectra of the deletion mutants with the wildtype) is on its way.

FBV004

Preventing Fusarium Head Blight of Wheat and Cob Rot of Maize by Inhibition of Fungal Deoxyhypusine Synthase

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Upon posttranslational activation, the eukaryotic initiation factor-5A (eIF-5A) transports a subset of mRNAs out of the nucleus to the ribosomes for translation. Activation of the protein is an evolutionary highly conserved process which is unique to eIF-5A: the conversion of a lysine to a hypusine. Instrumental for the synthesis of hypusine is the first of two enzymatic reactions mediated by deoxyhypusine synthase (DHS). We show that DHS of wheat and the pathogenic fungus *Fusarium graminearum*, which causes one of the most destructive crop diseases worldwide, are transcriptionally upregulated during their pathogenic interaction. Although DHS of wheat, fungus, and human can be equally inhibited by the inhibitor CNI-1493 *in vitro*, application during infection of wheat and maize flowers results in strong inhibition of the pathogen without interference with kernel development. Our studies provide a novel strategy to selectively inhibit fungal growth, without affecting plant growth. We identified fungal DHS as a target for the development of new inhibitors, for which CNI-1493 may serve as a lead substance.

FBV005

The mitogen-activated protein kinase HOG1 in *Fusarium graminearum* is involved in osmoregulation, sexual reproduction and virulence.

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Fusarium graminearum is an important ascomycetous plant pathogen and the causal agent of Fusarium head blight disease in small grain cereals and of cob rot disease of maize. Infection with *F. graminearum* leads to yield losses and mycotoxin contamination. Among the mycotoxins produced by the fungus, the trichothecene deoxynivalenol (DON) was shown to be important for virulence in wheat. The trichothecene production is influenced by the osmotic environment. In this regard we analyse the *F. graminearum* orthologue of the *Saccharomyces cerevisiae* hog1 mitogen-activated protein kinase (MAPK). The HOG1 deletion mutants show increased sensitivity towards osmotic treatments. The mutants show a reduced growth rate on agar plates supplemented with 0.8 M NaCl. On this medium conidial germination is severely impaired. Germ tubes emerging from conidia are swollen and contain multiple nuclei. Furthermore, sexual reproduction is harmed in the deletion mutants. They completely fail to produce perithecia and ascospores on wheat nodes and carot agar. The *in planta* DON production is nearly abolished in the deletion mutants. However, we observed an even higher DON concentration in the deletion mutants when analyzed under *in vitro* induction conditions. The HOG1 deletion mutants are completely apathogenic towards wheat and maize. Except for the point-inoculated spikelet, no disease symptoms are detectable. In order to analyze the infection pattern of the mutants in more detail, we constitutively express the fluorescent protein dsRED in these strains and in the wild type. Using these strains we are able to monitor the formation of infection structures on the surface of the spikelet and to follow the infection process in the plant.

FBV006

Will be presented as poster with the ID FBP045!

FBV007

Cell wall thickness and composition in the yeasts *Saccharomyces cerevisiae* and *Kluyveromyces lactis* adapt to growth conditions

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The cell wall is an essential part of fungal cells, which provides protection against adverse environmental conditions and determines cell morphology. In yeasts, it is made up of two layers. The inner one appears electron transparent in transmission electron microscopy (TEM) and is composed of β-1,3-glucan, β-1,6-glucan and a minor amount of chitin. The outer - more electron dense - layer consists of mannoproteins. Both, the polysaccharide and the protein composition of the cell wall are constantly remodelled as a result of normal growth and are also dependent on the environmental conditions, such as carbon sources or the presence of damaging agents. This remodelling is triggered by the cell wall integrity pathway, which detects perturbances at the cell surface, triggers an intracellular, highly conserved MAPK cascade and leads to proper cellular responses by activation of transcription factors.
We will report on our results regarding a comparison of cell walls from two different ascomycetous yeast species: The baker's yeast *S. cerevisiae* and the milk yeast *K. lactis*. Both the cell wall proteome and the overall thickness of the cell wall, which corresponds to its polysaccharide composition, vary in response to the carbon soure used for growth (i.e. glucose *versus* ethanol).
Another conserved protein kinase complex so far only reported to the regulate carbohydrate metabolism also seems to influence cell wall thickness and composition in *S. cerevisiae*. We will present our preliminary data with regard to this regulation.

FBV008

The AUACCC-binding protein Khd4 regulates cell morphology and pathogenicity in *Ustilago maydis*

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In the plant-pathogen *Ustilago maydis* evidence is accumulating that post-transcriptional processes play a major role in regulating cell morphology and pathogenicity. Key factors of the post-transcriptional machinery are RNA-binding proteins, which recognize specific motifs within target transcripts to regulate for example translation, localization, or mRNA-stability. We are working with the RNA-binding protein Khd4 that contains at least five K homology domains. Deletion of *khd4* leads to severe consequences: disturbed cell shape, abnormal cell wall composition, cytokinesis defect, and strongly reduced pathogenicity. Interestingly, the KH domains 3 and 4, which recognize the motif AUACCC, are required for Khd4 function since mutations in the conserved structural motif G-X-X-G lead to the *khd4*

deletion phenotype. Furthermore, the motif AUACCC is necessary and sufficient for binding and is most likely a regulatory element since it accumulates in untranslated regions. An independent mRNA expression profiling approach revealed that the binding motif is significantly enriched in transcripts showing altered expression levels in *khd4*Δ strains. Since the vast majority of potential Khd4 target mRNAs exhibit increased amounts in deletion mutants, Khd4 might promote mRNA instability. Consequently, microscopic studies with the RNA-helicase Dhh1, a component of processing bodies, that are known sites of mRNA degradation, revealed co-localization of Khd4 and Dhh1. These findings suggest that Khd4 might function in mRNA-stability processes and is important for the post-transcriptional regulation of cell morphology and pathogenicity in *U. maydis*.

FBV009
Molecular basis of photoconidiation in the filamentous fungus *Trichoderma atroviride*.

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Trichoderma is a common soil fungus used as a photomorphogenetic model due to its ability to conidiate upon exposure to light. In total darkness, *T. atroviride* grows indefinitely as a mycelium provided that nutrients are not limiting. However, a brief pulse of blue light given to a radially growing colony induces synchronous sporulation. Photoconidiation in *Trichoderma* is controlled by the orthologs of the *N. crassa* white-collar genes (blr1 and blr2), that form the photreceptor complex. Recently, we have applied high-throughput sequencing technology to the study of the *Trichoderma atroviride* transcriptome. We obtained RNA samples from the wild type strain grown in the dark or after exposure to a pulse of white or blue-light, as well as from a photoreceptor mutant (Δblr-1) exposed to white light. We identified over 300 light responsive genes, both induced and repressed, the majority of them *blr1* dependent. However, there is an important set of genes that is induced independently of this photoreceptor. Among the genes identified there are transcription factors, DNA-repair enzymes, and a set chaperons, including heat shock proteins, suggesting that light is perceived as a stress signal by *Trichoderma*. We have obtained gene disruption mutants of several of the transcription factors, and other key genes. Using this strategy we have obtained mutants that do not conidiate in response to light, as well as mutants that do not require this stimulus to conidiate.

FBV010
Using codon-improved GFP for imaging gene expression during germination and host penetration of *Botrytis cinerea* conidia

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Germination of spores is a fundamental event in fungal life, represented by the initiation of growth from a dormant state. In plant pathogens, germination immediately precedes host penetration, and therefore is of crucial importance for the success of infection. We have performed transcriptome studies to follow gene expression changes during germination and differentiation of *Botrytis cinerea* conidia. Massive changes in gene expression were observed already after 1 hour, before germ tube emergence. The genes that were specifically upregulated during germination (1-4 h.p.i.), were found to be enriched in genes encoding secreted proteins, indicating a strong secretory activity during the early stages of development. In a *bmp1* MAP kinase mutant, which is essential for germination on a hydrophobic surface and host penetration, upregulation of these genes was not observed. Using a codon-improved version of the *egfp* gene, strong GFP fluorescence could be detected for the first time in *B. cinerea*. Promoter-GFP reporter strains confirmed germination-specific expression for several germination genes. In particular, we found that the expression of several cutinase genes was regulated both in a developmental and in a substrate (cutin monomers or waxes) dependent manner. These data indicate a very early molecular communication between pathogen and host which starts during or even before germ tube emergence.

FBV011
Characterization of Small GTPase Complexes and their Effects on Polar Growth during Infection of *Claviceps purpurea*

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The biotrophic plant pathogen *Claviceps purpurea* which infects monocotyledonous plants, among them important crops like rye, is an interesting model organism for research in plant-pathogen interactions. The strict polar growth in *C. purpurea*‘s early infection stages in rye ovaries is of particular interest, as the fungus is not recognized as a pathogen possibly due to its pollen tube-like growth. Small GTPases and their effectors are known to be involved in polarity. Therefore, the investigation of the effects of these factors is crucial for a better understanding of polar growth in filamentous fungi.
Knockout strains of the small GTPases Rac and Cdc42 and of the p21 activated kinase (PAK) Cla4 as a GTPase downstream effector have been generated. The deletion strains of the small GTPases showed inverse phenotypes with regard to sporulation and growth patterns. In contrast to that the deletion strain of *cla4* greatly resembled the phenotype of the *rac* knockout, thus indicating towards an involvement of Cla4 and Rac in the same pathway [1, 2]. For a better understanding of the dynamics in the GTPase cycles, the genes of two guanine nucleotide exchange factors (GEFs), Cdc24 and Dock180, which are predicted to be specific for Cdc42 or Rac, were identified in the genome. Knockout approaches of these genes have been started and interaction studies are being carried out. No direct interaction of Cdc24 and Cdc42 could be observed, which indicates the need for a scaffold protein to allow the reaction between a GTPase and its corresponding GEF. First hints pointing to an interaction of the scaffold protein Bem1 with Cdc24 could be observed in a yeast two hybrid assay, reinforcing this particular hypothesis. As a second candidate, the gene for the scaffold protein Far1 was identified in the genome and is being investigated to elucidate its role in polar growth and formation of GTPase complexes. Furthermore, the sequence of a second PAK, Ste20, which is putatively involved in the Cdc42 pathway, was identified in the genome and is currently being analyzed.
The results obtained by these approaches will result in a clearer picture of cellular processes and complex compositions during infection of *C. purpurea* and can give useful hints for a better understanding of polar growth in this fungus.

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FBV012
Interaction between *Streptomycetes* and *Aspergillus nidulans*

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Microorganisms as bacteria and fungi produce many important low-molecular weight molecules that show different biological activities. Genome mining of fungal genomes indicated that their potential to produce these compounds designated secondary metabolites is greatly underestimated. However, most of the fungal secondary metabolism gene clusters are silent under laboratory conditions. Therefore, a major challenge in this emerging area is to understand the physiological conditions under which these compounds are produced. Results in this area will lead to the discovery of new bioactive compounds and to new insights in fundamental aspects of communication between microorganisms.
To address these questions the important model fungus Aspergillus nidulans was coincubated with 58 different Streptomycetes. With one particular species, a specific interaction was shown. For the first time, using microarray analyses at the molecular level it was demonstrated that this

interaction leads to the specific activation of two distinct silent secondary metabolism gene clusters. Electron microscopy confirmed the intimate interaction of the fungus and the bacterium. Full genome arrays of A. nidulans were applied to elucidate the whole genome response to the streptomycete. Data on the molecular regulation of the involved secondary metabolism gene clusters will be presented.

FBV013
Analysis of the Mating-type loci from the homothallic Ascomycete *Eupenicillium crustaceum*

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The homothallic *Eupenicillium crustaceum* Ludwig is very closely related to the penicillin-producer *Penicillium chrysogenum*, which is supposed to reproduce only asexually. However, recently strains of *P. chrysogenum* have been shown to carry either the mating type (*MAT*) locus *MAT1-1* or *MAT1-2* suggesting a heterothallic breeding system (Hoff et al. 2008). To analyze the molecular basis of homothallism in *E. crustaceum*, we cloned and sequenced its *MAT* sequences. Two *MAT* loci, *MAT1-1* and *MAT1-2*, reside in the genome of *E. crustaceum*. *MAT1-1* is flanked by conserved *apn2* and *sla2* genes and encodes a homologue of the alpha-box domain protein MAT1-1-1, while *MAT1-2* carries the HMG domain gene *MAT1-2-1* and is flanked by a degenerated *sla2* gene and an intact homologue of the *P. chrysogenum* ORF *Pc20g08960*. To determine functionality of the *E. crustaceum MAT* genes, we demonstrate their transcriptional expression during vegetative development. Furthermore, the alpha-box domain sequence of MAT1-1-1 and the HMG domain sequence of MAT1-2-1 were used to determine the phylogenetic relationship with other ascomycetes. Phylogenetic trees confirmed strong relationships between the homothallic *E. crustaceum* and the supposedly heterothallic *P. chrysogenum*.

[1] Hoff, B. et al (2008): Eighty years after its discovery, Fleming's *Penicillium* strain discloses the secret of its sex. Eukaryotic Cell 7: 465-470.

FBV014
Antifungal and Antibacterial activity of marine actinomycetes strains isolated from east and west coastal regions of India

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Exploration of secondary metabolites from microorganisms paved a way for the identification of new antibiotics. Among the different classes of microorganism, marine actinomycetes attract researchers having economically valuable secondary metabolic compound of biological significance. Trichophyton rubrum is the most common dermatophyte species and the most frequent cause of fungal skin infection in humans worldwide. It is the organism responsible for all type of tinea infection. Jock itch, athlete's foot and ringworm are collectively said to be tinea. Symptoms of these infections vary depending on where they appear on the body. It is the major concern because feet and nail infections caused by this organism is extremely difficult to cure.
In the present study a total of 124 marine actinomycetes were isolated from marine environment on Starch Casein Agar medium from different locations of east and west coastal regions of India. All these isolates were tested against T. rubrum. Based on screening results, two potential actinomycetes were selected and tested for their antifungal activity against dermatophyte fungi T. mentagrophytes, against human bacterial pathogens Staphylococcus aureus, Bacillus subtilis, Salmonella typhi, Klebsiella pneumoniae, Enterococcus faecalis, Proteus vulgaris and against the yeast Candida albicans. These isolates showed remarkable antibacterial and antifungal activity. Based on physiological, biochemical characterisation, these strains belong to the genus Streptomyces. According to the physiological study, the strains had maximum growth rate at a NaCl concentration of 1g/L. These strains produced antifungal metabolite intracellularly under submerged fermentation conditions. The fermentation parameters such as substrate concentration, pH, fermentation period and inoculam size were optimized by response surface methodology (RSM).
The antibiotic substance was extracted with methanol and purified through silica gel column chromatography in chloroform: methanol (19:1) step gradient.
The UV visible spectrum of this active compound suggested being alkaloidal nature. The NMR and X-ray crystallography studies of this compound are now in progress.

FBV015
Infection structures and mycotoxin induction of *Fusarium graminearum* on wheat florets

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Questions: The mycotoxin producing fungal pathogen *Fusarium graminearum* is the causal agent of Fusarium head blight (FHB) of small grain cereals in fields worldwide. Although *F. graminearum* is highly investigated by means of molecular genetics, detailed studies about hyphal development during initial infection stages are rare. In addition, the role of mycotoxins during initial infection stages of FHB is still unknown.
Methods: We investigated the infection strategy of the fungus on different floral organs of wheat under real time conditions by constitutive expression of the *dsRed* reporter gene in a *TRI5prom*::*GFP* mutant. Additionally, a green fluorescent protein (GFP) coupled *TRI5* promoter allowed visualisation of trichothecene induction during infection. A tissue specific infection behaviour and *TRI5* induction were tested by using different floral organs of wheat. Through combination of bioimaging and electron microscopy infection structures were identified and characterised. In addition, the role of trichothecene production for initial infection was clarified by using a Δ*TRI5-GFP* reporter strain.
Results: The present investigation demonstrates the formation of foot structures and compound appressoria by *F. graminearum* in addition to short infection hyphae. All infection structures developed from epiphytic runner hyphae. Compound appressoria including lobate appressoria and infection cushions were observed on inoculated caryopses, paleas, lemmas, and glumes of susceptible and resistant wheat cultivars. Subcuticular growth of the fungus originates from lobate appressoria and infection cushions. A specific trichothecene induction in infection structures was demonstrated by different imaging techniques. Interestingly, a Δ*TRI5-GFP* mutant formed the same infection structures and exhibited a similar symptom development compared to the wild type and the *TRI5prom*::*GFP* mutant.
Conclusions: The evidence for the formation of specialised infection structures by *F. graminearum* during infection on wheat florets reveals that the penetration strategy of this fungus is more complex than postulated to date. Monitoring of *TRI5*-induction demonstrates a specific induction of trichothecenes in infection structures. A trichothecene deficient Δ*TRI5-GFP* mutant exhibits the same mode of infection and leads to wild type-like disease symptoms on inoculated glumes and caryopses. We conclude that trichothecene biosynthesis is induced in infection structures, but is neither necessary for their development nor for formation of primary symptoms on wheat.

FBV016
The Role of the autophagy related genes *Smatg4* and *Smatg8* in the sexual development of *Sordaria macrospora*

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In filamentous ascomycetes, autophagy is involved in several developmental processes. Nevertheless, until now little is known about its role in fruiting-body development. We therefore isolated genes of the homothallic ascomycete *Sordaria macrospora* with sequence similarity to the *Saccharomyces cerevisiae* autophagy-related genes *ATG4* and *ATG8* encoding a cystein protease and an ubiquitin like protein which is a structural component of the autophagosome. This is the first characterization of an *ATG4* and *ATG8* homologue in *S. macrospora*. We were able to generate homokaryotic knockout, as well as complementation mutants in *S. macrospora*. The D*Smatg4* and D*Smatg8* deletion mutants were unable to form perithecia and are therefore sterile. By reintroducing *Smatg4* and *Smatg8* ectopically into the deletion strains the deletion mutant phenotype was restored and the complemented strains were able to produce perithecia again. Our results indicate that *Smatg4* and *Smatg8* are directly or indirectly involved in perithecia formation. Furthermore, we were able to localize SmATG8 via N-terminal GFP fusion indicating that SmATG8-GFP is localized to the autophagosome and is degraded in the vacuole along with

the autophagosome and its cargo. Localization experiments of SmATG4 with a C-terminal GFP fusion showed a cytoplasmatic localization of SmATG4.

FBV017
Characterization of a putative α-carbonic anhydrase from the filamentous ascomycete *Sordaria macrospora*

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Carbonic Anhydrase (CA) catalyzes the hydration of carbon dioxide to bicarbonate and protons. CAs have evolved in all three domains of life. Based on their amino acid sequence and structure, they can be divided into five distinct groups (α,β,γ,δ,ξ) which share no sequence similarity and have supposable evolved independently. All known fungal CAs belong either to the α- or to the β-class and to date, only β-CAs have been characterized in fungi (Elleuche and Pöggeler 2009). Therefore, we investigated in this study, the role of a α-CA in the filamentous ascomycete *Sordaria macrospora*, termed CAS4. The *S. macrospora cas4* gene encodes a putative protein of 368 amino acids with a predicted molecular mass of 39.6 kDa. cDNA of the *S. macrospora cas4* gene fused to either RGS-His-tag or GST-tag was heterologously expressed in *E. coli*. SDS-PAGE and Western-blot analyses with anti-RGS-His and anti-GST antibodies revealed protein bands with apparent molecular weights consistent with the calculated molecular weights of the *S. macrospora* CAS4 protein. CAS4 exhibit a signal peptide for the endoplasmic reticulum and is therefore predicted to be secreted. Using Western-Blot analysis we were able to show secretion of a Flag-tagged-CAS4. To better understand the role of the *S. macrospora* CAS4, we generated a Δcas4 deletion mutant. The characterization of the Δcas4 mutant revealed a reduced vegetative growth rate compared to the wild type. Under stress conditions the mutant also shows a slight delay on fruiting body production. In addition, the expression pattern of cas4 was analyzed by semi-quantitative RT-PCR.

FBV018
Characterization, purification and cloning of the O-Methyltransferase of *Alternaria alternata*

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Black-moulds of the genus *Alternaria* contaminate many foodstuffs and agricultural products. In addition to the economical damage these fungi can produce harmful secondary metabolites, the *Alternaria* toxins. Some of these mycotoxins such as alternariol (AOH), alternariolmonomethylether (AME), altenuene (ALT) are polyketides and AOH is produced via the polyketide pathway. AOH is than methylated by the alternariol-*o*-methyltransferase, transferring a methyl group from SAM to AOH to yield AME. The enzyme was partially purified and characterized, but the sequence is still unknown [1, 2].
As *Alternaria alternata* is not sequenced yet, putative methyltransferases were identified by BLAST-analysis in the genome of the close relative *A. brassicicola* and the sequences were used to clone several SAM dependent methyltransferases of *Alternaria alternata*. Three partial and one total sequence were cloned.
With the active expression of the identified genes being not easy, the alternariol-*o*-methyltransferase of *Alternaria alternata* was also characterized in crude extracts and partially purified. An SAM dependent activity-test was developed to identify the enzyme. The products were analysed by HPLC. With the N-terminal sequence of the enzyme it should be possible to determine the gene.

[1] Gatenbeck and Hermodsson (1965): Enzymic Sythesis of Aromatic Product Alternariol.
[2] Stinson and Moreau (1986): Partial Purification and some Properties of an Alternariol-*o*-Methyltransferase from *Alternaria tenuis.*

FBV019
Approaches for directed strain improvement targeting enhanced biosynthesis of gibberellic acid in *Fusarium fujikuroi*

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The filamentous fungus *Fusarium fujikuroi* is known to produce high amounts of different secondary metabolites such as the red pigment bikaverin, the mycotoxin fusarin C and the phytohormone gibberellic acid (GA). Particularly gibberellins exhibit a great biotechnological impact as application of GAs in higher plants induces early flower bud formation and shoot elongation as well as an increased fruit size and enhanced yields of crops such as seedless grapes and corn. Therefore, each year about ten tons of gibberellins are consumed by the agricultural industry as plant growth regulators.
Since the knowledge about biosynthesis pathways is accessible to a greater extent, the rice pathogen *F. fujikuroi* constitutes a capable species for GA production by fermentation. To increase GA yields directed genetic modifications of pathway genes of competing secondary metabolites were performed. These modifications lead to a higher metabolite flux into GA biosynthesis and thereby enhanced GA production rates compared to the wild type. In addition, regulation of key enzymes of the precursor providing primary metabolism has to be investigated to circumvent negative feedback regulation by different intermediates or end products. By understanding enzyme regulation on transcriptional and protein level both gene expression and enzyme activity will be enhanced significantly. These regulatory mechanisms shall be for example elucidated by identification of positively or negatively acting transcription factors.
To finally combine all approaches in one strain new selection markers or possibilities for re-using common markers have to be established for *F. fujikuroi*.

FBV020
New insights in the regulation of mycotoxin production in the plant pathogen *F. graminearum*

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The fungal pathogen *Fusarium graminearum* is the causal agent of Fusarium head blight in small grain cereals and of cob rot disease of maize. The devastating effect is due to yield losses and mycotoxin contamination. Among the mycotoxins produced by the fungus, the trichothecene deoxynivalenol (DON) was shown to be important for virulence in wheat. The regulation of DON-production during plant infection and in axenic culture is still not known in detail. Using qRT-PCR and Elisa-based DON measurements we analyse the influence of different nitrogen sources and plant substances on a) DON production and b) on genes that play a certain role in nitrogen signaling. These analyses are accompanied by fluorescence measurements using a reporter strain that expresses the green fluorescent protein GFP under the control of the trichothecene synthase (Tri5) promoter. Using this strain it is possible to directly monitor Tri5 induction under different growth conditions. We show a DON-inducing effect of ammonium ions and plant components. In addition, we started to functionally analyse nitrogen signaling regulator proteins like the GATA-transcription factor AreA and the bZIP-transcription factor MeaB in order to assess their function in the regulation of toxin production and virulence.

FBV021
Molecular analysis of polyketide synthase genes involved in secondary metabolism of *Alternaria alternata*

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Filamentous fungi produce a diverse array of secondary metabolites - small molecules that are not necessary for normal growth or development. Classes of fungal secondary metabolites include polyketides, non-ribosomal peptides terpenes indole terpenes etc. Fungal PKSs are responsible for the biosynthesis of mycotoxins and other secondary metabolite.

There are several polyketide synthase genes are involved in the production of secondary metabolites in *Alternaria alternata* including Alternaiol (AOH) biosynthesis. Alternariol (AOH) is one of the main mycotoxins formed in various foods and feeds contaminated by the mold *A. Alternata*. Alternariol shows cytotoxic, foetotoxic, and teratogenic effects. Since alternariol and other mycotoxins have deleterious properties to humans and animals, efforts have been directed toward the understanding of the molecular mechanisms leading to its biosynthesis. However, till date very little is known about the molecular biology of alternariol synthesis.
The work here describes the identification and characterization of the genes involved in the alternariol biosynthetic pathway. In order to understand the biosynthetic pathway of alternariol or other toxins in *A. alternata,* putative polyketide synthase genes has been identified. The identified PKS genes are located in clusters. Selected genes have been disrupted/deleted to study their involvement in toxin production. The characterization of the toxin profile in certain mutants strains by thin layer chromatography and/or HPLC will help to assign functions to the different PKS proteins. Because gene deletion occurred to be rather difficult in *A. alternata*, RNAi mediated silencing for four different PKS genes has also been performed for identifying in which secondary metabolite biosynthesis they are involved. The metabolic profiling of secondary metabolites is under way.

FBV022

Analysis of the *Aspergillus fumigatus* proteome reveals metabolic changes and the activation of the pseurotin A biosynthesis gene cluster in response to hypoxia

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Aspergillus fumigatus is the most important airborne fungal pathogen and the main causative agent of the opportunistic, often lethal infection invasive aspergillosis. To colonize the human lungs, this saprophytic fungus has to adjust its physiology to the host's environment including the adaptation to hypoxia, which represents an important virulence attribute. Therefore, we intended to obtain a comprehensive overview about this process on the proteome level. To ensure highly reproducible growth conditions, an oxygen-controlled chemostat cultivation was established. Two-dimensional gel electrophoresis analysis of mycelial and mitochondrial proteins as well as two-dimensional Blue Native/SDS-gel separation of mitochondrial membrane proteins led to the identification of 117 proteins with an altered abundance under hypoxic in comparison to normoxic conditions. This proteome analysis revealed an increased activity of the glycolytic pathway, the TCA-cycle, and especially respiration and amino acid metabolism. Consistently, hypoxia elevated the cellular contents in heme, iron, copper and zinc. Furthermore, hypoxia induced biosynthesis of the secondary metabolite pseurotin A as demonstrated at proteomic, transcriptional and metabolite levels. The observed and so far not reported stimulation of the biosynthesis of a secondary metabolite by oxygen depletion may also affect the survival of *A. fumigatus* in hypoxic niches of the human host. Among the proteins so far not implicated in hypoxia adaptation, an NO-detoxifying flavohemoprotein was one of the most highly up-regulated proteins which indicates a link between hypoxia and the generation of nitrosative stress in *A. fumigatus*.

FBV023

Fungal systems - Tools for the milligram- to gram-scale preparation of an environmentally relevant metabolite of fenoprofen

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Since the ubiquitous distribution of pharmaceutical residues in surface aquifers becomes more and more obvious, questions concerning toxic effects on human health and the ecosystem are arising. Measured trace concentrations of individual drugs were found to be several orders of magnitude below the acute-effect level and toxic action towards members of the bioscenosis is at least very unlikely. However, effects caused by long-term exposure, by synergetic effects of complex drug mixtures, and by metabolites of microbial transformation are poorly investigated. In contrast to the metabolism of drugs in humans, little knowledge exists on transformation mechanisms of most pharmaceuticals by microorganisms. Usually the detection and identification of a microbial intermediate gives a first hint on a transformation mechanism. However, such investigations are considerably facilitated by available references for those compounds.
Bioconversion of pharmaceuticals by filamentous fungi may, in certain cases, serve as a strategy to obtain such compounds. The present work deals with the preparation of 4′-hydroxyfenoprofen (3-(4-hydroxyphenoxy)-α-methylbenzeneacetic acid) from fenoprofen by *Epicoccum nigrum* DSM838 and *Cunninghamella elegans* DSM1908. This metabolite, which is known to be a major intermediate during fenoprofen phase-I metabolism in humans, was also found in this study to be formed during the aerobic biotransformation of the non-steroidal anti-inflammatory drug by water and sediment from a river.
Transformation studies of fenoprofen with both filamentous fungi showed a complete (co)metabolic conversion in the concentration range of 160 µM and 500 µM under aerobic conditions. Under the conditions investigated, transformation of 0.48 mmol fenoprofen in a 3 L fermenter yielded 76.7 mg of 4′-hydroxyfenoprofen, corresponding to a 62.5 % theoretical yield. Thus, *C. elegans* is the first reported biological system for the fermentative regiospecific hydroxylation of fenoprofen.

FBV024

Nitrogen metabolism of wood decomposing basidiomycetes and their interaction with diazotrophs as revealed by IRMS

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Isotope ratio mass spectrometry (IRMS) is an advanced method to investigate carbon, nitrogen, oxygen, sulphur and hydrogen in organic samples. In particular the N-content, its isotope signature and the C/N ratio reveal important facts of nutrient cycling, niche separation and ecological food webs. In this study the characteristics of nitrogen exchange of wood decomposing microorganisms were investigated. It was revealed that the growth of the white rotting fungus *Trametes versicolor* is enhanced after the addition of ammonia or urea, while the brown rotting fungus *Oligoporus placenta* is not accelerated. In addition, a mutualistic interaction with atmospheric N_2-assimilating (diazotrophic) bacteria was investigated. Cultivation experiments under an atmosphere of $^{15}N_2$ and O_2 and subsequent IRMS analysis of the dry biomass of the diazotrophs *Azotobacter croococcum*, *Beijerinckia acida* and *Novosphingobium nitrogenifigens* revealed that they assimilated up to 12 % of their nitrogen by fixed N_2. The experiments reflected nitrogen availability as a prerequisite for efficient growth for wood decomposing fungi and diazotrophs. Co-cultivation experiments of both revealed that depending on the growth characteristics and bacterial N_2 assimilation activity nitrogen is transferred from bacteria into basidiomycetal biomass. In conclusion, a first indication of an interaction between wood decomposing basidiomycetes and diazotrophs was obtained which is a novel pathway of fungal nitrogen acquisition.

FBV025
Orphan GPCRs in Schizophyllum commune

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The filamentous fungus S. commune has been a model organism for sexual development of basidiomycetes since the early 20th century. Numerous studies revealed the importance of two gene loci, A and B, responsible for mating and sexual development. While A codes homeodomain transcription factors, B codes for a pheromone/receptor system. Both occur in multiallelic, associated subloci leading to a large number of different specificities in nature (Aalpha 9, Abeta 32, Balpha 9, Bbeta 9), which then control compatibility or abortion of mating. The B-receptors (STE3-like, 7 transmembrane domains, G-protein coupled) recognize pheromones of non-self specificity, inducing a signal transduction pathway and specific gene regulation. After sequencing of strain H4-8, new developments in research have occured. E.g., there are four new STE3-like GPCRs, homologous to the known Balpha and Bbeta specific ones. Their function is unknown, because a B-locus deletion strain without any interactions seen in B-dependent development still contains those four GPCRs, which obviously do not respond to any pheromone. However, first results indicate their importance since sequence identity between unrelated strains was found arguing for conservation of these genes. Overexpression is performed to give insights into the function of this new class of pheromone receptor-like genes.

FBP001
Will not be presented!

FBP002
Will not be presented!

FBP003
Protein-dependent interactions among *Streptomyces hyphae* and biotechnological implications

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Streptomycetes produce many metabolites with medical and biotechnological applications. During fermentations, their hyphae build aggregates, a process in which the newly identified protein HyaS plays an important role. The corresponding *hya*S gene is present within in all investigated *Streptomyces* species. The HyaS protein is dominantly associated to the substrate-hyphae. This WT strain builds cylindrically shaped clumps of densely packed substrate-hyphae, often fusing to higher aggregates (pellets), which remain stably associated during shaking. Investigations by immuno-electronmicroscopy suggest that HyaS induces tight fusion-like contacts among substrate-hyphae. Biochemical studies indicate that the C-terminal region of HyaS has amine oxidase activity. Furthermore, the level of undecylprodigiosin, a red pigment with antibiotic activity, is influenced by the engineered *hya*S-subtype within a strain. A second protein named HycS and its corresponding gene were identified. Biochemical studies revealed that HyaS and HycS are strongly interacting. These data present the first molecular basis for future manipulation of pellets, and concomitant production of secondary metabolites during biotechnological processes.

FBP004
In situ localization of a novel protein provoking aggregation among hyphae

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The *Streptomyces* protein HycS has been found to play a role in mediating interactions among hyphae of *Streptomyces lividans* as well as with selected fungal strains. The *hyc*S gene and designed mutated genes were cloned into *Streptomyces* strains lacking a functional *hyc*S gene and analysed as to aggregate-formation. Based on these results, functional domains and revelant amino acid residues of the HycS protein were deduced. Furthermore, the *hyc*S gene and its mutated variants were fused with the *egfp* gene. The subsequently obtained transformants of selected *Streptomyces* strains were investigated by fluorescence microscopy as to the location of each designed fusion protein during different stages of development. The results of the studies led to new insights on the role of the HycS protein. The biotechnological implications will be outlined.

FBP005
Antioxidant and antibacterial properties of the extracts from four *Pleurotus ostreatus* strains

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The aim of this work was focused on the obtaining of *Pleurotus ostreatus* mycelium by fermentation in liquid medium, in order to realise freeze-dried biomass and extracts with high antioxidant and antimicrobial activity. The tests were realised at 25^0C, in 300 ml Erlenmeyer flasks. The most effective carbon source and the optimum agitation speed were determined, in order to cultivate the two strains in a medium containing 2% malt extract and 2% peptone.
For all four strains, the optimum agitation speed was established at 150 rpm. The most effective carbon source for *Pleurotus ostreatus* EVFB1 was glucose (10%), for *Pleurotus ostreatus* EVFB3 was glucose (15%), for *Pleurotus ostreatus* EVFB4 was lactose (15%) and for *Pleurotus ostreatus* EVFB5 was sucrose (10%). The obtained biomass was freeze-dried and was submitted to ethanol and pure methanol extraction. It resulted that all four *Pleurotus ostreatus* strains had an antioxidant activity by 15% higher on average when ethanol was used as solvent. The phenols quantity was similar, notwithstanding the solvent used, for *Pleurotus ostreatus* EVFB1 strain being by 25% higher as compared to all *Pleurotus ostreatus* strains used, namely 68,6 mg/g gallic acid. The results were also comparable in the case of reducing power, the ethanolic extracts being the most effective. The four *Pleurotus* strains proved narrow antibacterial activity against Gram-negative and Gram-positive bacteria tested.

[1] Vamanu, E. and N. S. Vamanu (2010): The obtaining of an antioxidant based on a *Rosmarinus officinalis* freeze-dried extract, International Journal of Pharmacology, 6, 4, 387 - 392.

FBP006
Properties of the ND5 subunit of the mitochondrial complex I (NADH:ubiquinone oxidoreductase) from *Yarrowia lipolytica*

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The first complex in the mitochondrial phosphorylation system is the electrogenic NADH:ubiquinone oxidoreductase (complex I). The complex consists of 45 subunits and especially the subunit ND5 is considered to be part of the membrane-bound transporter module. The bacterial homolog of ND5, the subunit NuoL of complex NDH-1, actually acts as transporter for Na^+ and K^+ in vesicles from the endoplasmatic reticulum in *S. cerevisiae,* which lacks an endogenous complex I [1]. Furthermore the expression of human ND5 in *S. cerevisiae* leads to an increased resistance at high external concentrations of Na^+ or K^+ [2]. This suggests that ND5 acts as a cation transporter independently from other complex I subunits. Here we investigate the properties of the ND5 homolog from the yeast *Yarrowia lipolytica.* This very hydrophobic protein was present in ER membranes, or directed to the mitochondrium of *S. cerevisiae*. Compared to human ND5, higher expression yields were observed. ND5 was solubilized with Zwittergent 3-12 and enriched by a Ni^+-sepharose chromatographic step. Proteolytic digestion of native ER vesicles containing ND5 showed that the N-terminus is oriented towards the external lumen of the vesicles, paving the way for cation transport studies. Mutations in ND5 observed in patients suffering from neurodegenerative diseases were introduced in the *Y. lipolytica* ND5 to study their effect on the transport properties of ND5 *in vivo* and *in vitro*.

[1] Gemperli, A. C. et al (2007): Transport of Na(+) and K (+) by an antiporter-related subunit from the Escherichia coli NADH dehydrogenase I produced in Saccharomyces cerevisiae. Arch Microbiol 188(5): 509-521.

[2] Steffen, W. et al. (2010): Organelle-specific expression of subunit ND5 of human complex I (NADH dehydrogenase) alters cation homeostasis in Saccharomyces cerevisiae. FEMS Yeast Res 10(6): 648-659.

FBP007
Endocytosis and toxicity of ricin A in yeast

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The plant toxin ricin represents one of the most powerful poisons of biological heritage. This heterodimeric glycoprotein belongs to the class of A/B toxins. It consists of two polypeptide chains amongst which the B-chain represents the cell surface binding domain mediating toxin uptake by the mammalian target cell. This domain is linked via a single disulfide bond to the A-chain (RTA) that catalyzes the N-glycosidic cleavage of a specific adenine residue in the sarcin/ricin loop of 28S ribosomal RNA. After endocytotic uptake and retrograde transport, ricin enters the endoplasmatic reticulum from where it is retrotranslocated into the cytosol, most likely by using the Sec61p translocon for ER exit. Subsequently, the B-chain is polyubiquitinated and proteasomaly degraded. It is asumed that only a limited number of A-chain molecules are able to escape degradation finally causing cell death by inhibiting protein synthesis.
In contrast to mammalian cells, yeast is not killed by external application of ricin. Following expression of biologically active ricin A subunit variants carrying a C-terminal $His_{(6)}$-tag and different ER retention signals in *E. coli*, we analyzed the cytotoxic effect of the purified constructs after external application against yeast spheroplasts by flow cytometry and oxygen-sensor microtiter plate measurements. We also fused ricin A to the b-subunit of the viral yeast A/B toxin K28 in order to enable the chimeric ricinA/K28β fusion protein to be taken up by yeast via receptor-mediated endocytosis. To do so, we constructed different fusion proteins of K28β and ricin A and expressed them in *E. coli*. After successful expression and purification we used these toxin fusions to study the intracellular transport of ricin A in yeast as model organism.

FBP008
Process optimization of alternariol production with the filamentous fungus *Alternaria alternata*

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Black-moulds of the genus *Alternaria* contaminate many foodstuffs and agricultural products. In addition to the economical damage these fungi can produce harmful secondary metabolites, the *Alternaria* toxins. Some of these mycotoxins such as alternariol (AOH), alternariolmonomethylether (AME), altenuene (ALT) and tenuazonic acid (TA) have been described as cytotoxic, genotoxic and mutagenic *in vivo* and *in vitro*. Due to the fact that mycotoxins could be detect in many foodstuffs and these fungi growing even at low temperatures it is necessary to produce the mycotoxins in high amounts for the elucidation of the genotoxic, cytotoxic and mutagenic potential. For better scalability and evaluation of the parameters influencing growth and toxin production a fluid submerse cultivation was chosen.
In a first approach alternariol production was performed in a small scale bioreactor (1.5 l) in a semi-synthetic medium with glucose as carbon source and a mixture of ammonium and nitrate as nitrogen source. As a result 4 mg/l alternariol can be produced. Several fermentations showed that nitrogen has an important regulatory effect since alternariol production starts after complete nitrogen consumption. To investigate the effect of nitrogen and carbon source different substances are tested in shaking flask experiments to optimize the production medium and to find an economic alternative to glucose.
Furthermore the influence of the aeration rate on alternariol production was determined. Therefore different fermentations in the bioreactor were performed with decreasing aeration or decreasing oxygen concentration to observe the effect on alternariol production.

FBP009
Molecular analysis of secondary metabolite biosynthesis in Alternaria alternata

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Secondary metabolites are organic compounds that are not directly involved in normal growth, development or reproduction of organisms. In the mold Alternaria alternata a major group of different secondary metabolites are mycotoxins with heterogenous structures. Important and in food and feed frequently found mycotoxins produced by A. alternata are the polyketides alternariol (AOH), alternariol-monomehtylether (AME) and altenuen (ALT). AOH shows cytotoxic, foetotoxic and teratogenic effects. The polyketide-biosynthesis requires polyketide synthases, multi-domain enzymes, separated into groups dependent on the degree of reduction of their product. One example for a non-reduced or aromatic polyketide is melanin, a pigment found in most organisms. It is known that genes involved in the biosynthesis of polyketides are organized in gene clusters.
This work describes the identification and characterization of genes involved in the biosynthesis of secondary metabolites. In the A. alternata genome eleven putative polyketide synthases and regulators located within the corresponding gene clusters have been identified. One shows high similarity to melanin biosynthesis gene clusters of other organisms. Down regulation of the regulator within this cluster using the siRNA-silencing method led to a whitish phenotype, and surprisingly had a strong impact on polar growth. Additionally the formation of conidia is strongly reduced. This suggests that the regulator may not only control melanin synthesis but also other cellular processes. The analysis of four other polyketide synthases is under way.

FBP010
Yeast-based protein delivery to mammalian phagocytic cells is increased by coexpression of bacterial listeriolysin

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Yeast-mediated protein delivery to mammalian antigen-presenting cells is a powerful approach for inducing cell-mediated immune responses. We show that coexpression of the pore-forming protein listeriolysin O from *Listeria monocytogenes* leads to improved translocation of a proteinaceous antigen and subsequent activation of specific T lymphocytes. As the resulting yeast carrier is self-attenuated and killed after antigen delivery without exhibiting any toxic effect on antigen-presenting cells, this novel carrier system suggests itself as promising approach for the development of yeast-based live vaccines.

FBP011
Regio- and enantioselective hydroxylation of various alkanes catalyzed by secreted fungal peroxygenase

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Selective hydroxylation of non-activated aliphatic hydrocarbons is a particular challenging reaction in organic synthesis. However, numerous microorganisms are known to be capable of oxidizing and even utilizing *n*-alkanes as carbon source. They use specific intracellular enzymes such as methane monooxygenase (MMO) or certain cytochrome P450 monooxygenases (P450s) to transform the inert alkanes into the corresponding alkanols, which are further oxidized to their ketones or via aldehydes to fatty acids.
Using such enzymes *in vitro* for biocatalytic oxygen transfer reactions would offer an interesting alternative to achieve selective hydroxylation. However, these biocatalysts are intracellular and less stable enzymes, which are hardly suitable for application in isolated form.
Here we report on a secreted peroxygenase from the agaric fungus *Agrocybe aegerita*, which catalyzes the H_2O_2-dependent monooxygenation of several

linear, branched and cyclic alkanes. Linear hydrocarbons from C3 to C16 become hydroxylated at the 2- and 3-position. Branched alkanes were hydroxylated like linear ones, if there is a free hydrogen to abstract. In a second hydroxylation step the corresponding ketones are formed.
Unlike intracellular MMO and P450s, *A. aegerita* peroxygenase is highly stable and does not need complex cofactors and electron transport proteins (NAD[P]H, flavin reductases). Even in organic solvents such as n-hexane or acetone solution up to 60% nearly no loss in activity can be observed within two hours.
Beyond that we could show, that the hydroxylation of pentane, hexane, heptane and octane is enantioselctive with an enatiomeric excess between 36% for (*S*)-pentanol and 99.9% for (*R*)-3-octanol.

FBP012
Mating type - specific proteins from the zygomycete *Mucor mucedo*

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During their sexual interactions, zygomycetous fungi communicate via an elaborate series of carotene-derived compounds, namely trisporic acid and its biosynthetic progenitors. The number of proteins affected by sexual stimulation, however, seems to be low.
In this project, genes that are exclusively expressed in one of the mating types during sexual and asexual development and whose proteins are used to induce sexual structures in the complementary partner are identified and characterized.
2D-gel electrophoresis is used as a tool to identify different spot-patterns in the complementary mating types. The following developmental stages are analysed: substrate mycelia (no zygophores, no asexual structures), trisporic acid induced mycelia, harvested 18h after induction and uninduced and mycelia as controls. All stages are investigated in the (+)-type and the (-)-type as well as in (±)-types cultivated together. In addition, cell wall proteins of (+)-, (-)- and (±)-cultures grown in liquid medium have been isolated and investigated on 2D-gels.
Spots that were expressed in only one of the mating types were chosen and analysed by MALDI-TOF. For the resulting sequences degenerated primers were constructed. Fragments resulting from PCR were cloned into a vector and multiplied in *E.coli*. Sequencing the plasmid inserts was used to confirm whether the right insert was ligated into the vector.
To confirm whether the chosen genes are transcribed differentially, Northern Blots will be performed and antibodies against the proteins will be made in order to analyze expression levels during growth and differentiation. At the present state of the project, the low amount of differentially expressed proteins confirms previous data found in the zygomycete *Absidia glauca* [1, 2].

[1] Teepe, H. et al: FEBS - Letters 234, 100-106.
[2] Vetter, M. et al: Microbiol. Res. 149, 17-22.

FBP013
Molecular analysis of gene expression in *Mucor mucedo:* Manipulation by transformation with antisense morpholino oligonucleotides

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crgA in *Mucor mucedo* and other zygomycetes codes for a negatively acting transcriptional regulator. Thus, negative regulation of crgA gene in *Mucor mucedo*, using morpholino oligonucleotides (MO), has a positive effect on light-regulated carotenogenesis. This is the first demonstration of using MO to down-regulate a gene in fungus. The crgA gene from *Mucor mucedo* was cloned and sequenced. The MO were designed using the sequenced crgA gene to suppress expression of the crgA gene, resulting in additional accumulation of carotene.
This system has been used as a proof-of-principle for the morpholino-oligonucleotide approach in mucoralean fungi. We now concentrate on down-regulation of the ku70 gene in *Mucor mucedo* in order to achieve stable integrative transformation events in this organism. For establishing the technique, we make use of the cloned leuA gene that we intend to introduce into a leuA mutant. Gene and mutant are available for *Mucor circinelloides*. A silent mutation with a unique XbaI restriction site was created in the leucine gene of plasmid pEUKA4. The KU 70 MO was designed and used for transformation of protoplasts of the *Mucor circinelloides* leucine auxotroph together with the above mutated plasmid. This approach allows easy screening of transformants in minimal medium without leucine. Transformation can further be confirmed by XbaI digestion of the transformant DNA followed by Southern blot analysis.

[1] Navarro, E. et al (2000): S.Eur J Biochem. 2000 Feb; 267(3):800-7.

FBP014
Molecular analysis of horizontal gene transfer between two mucoralean fungi: *Parasitella parasitica* and *Absidia glauca*

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Parasitella parasitica, a facultative parasite of zygomycetous fungi, transfers nuclei and thus DNA to its host *Absidia glauca*. Interspecific recombinants (para-recombinants) contain both, parasite and host DNA information [1]. The mechanism of this transfer, as well as the stability and fate of the transferred DNA is not clear. In this respect we used an adenine auxotrophic *P. parasitica* strain for infection of a methionine auxotrophic *A. glauca* host strain to analyze prototrophic recombinants and the direction of the gene transfer.
The mutant strains were characterized at the molecular level. The mycelium of the *P. parasitica ade*- mutant shows an orange-red color similar to *ade1* or *ade2* mutants of baker's yeast. DNA sequence analysis of the *ade2* gene encoding the phosphoribosyl-aminoimidazole carboxylase of the *P. parasitica* mutant strain shows the interruption of the open reading frame by a stop codon as the result of a single point mutation.
Feeding experiments with different methionine precursors of the *A. glauca* mutant reveal growth on cystathionine or homocysteine but no growth with homoserine complementation. The cystathionine-gamma-synthase (CGS) or the homoserine-acetyl-transferase (HAT) may thus be blocked in function. The genes were cloned and sequenced. The mutant *A. glauca* HAT gene shows an insertion of a new DNA element which interrupts the open reading frame. Two putative HAT genes of *P. parasitica* were cloned and sequenced.
For four successive sporulation cycles of para-recombinants, the percentages of complemented, prototrophic phenotypes were determined. In most cases the para-recombinants show an *Absidia* morphology.
Hybridization analysis of the para-recombinant DNA with the *A. glauca* HAT gene probe shows no homologous recombination at the HAT gene locus. No hybridization signals could be detected with the two *P. parasitica* HAT probes, indicating a copy number far below single copy or the complementation by an unknown third HAT gene. To analyze the complementation ability of the isolated HAT genes, transformation experiments were started with the *A. glauca* mutant strain using vectors containing the *A. glauca* wild type HAT gene as well with the *P. parasitella* genes. Prototrophic transformants with the *A. glauca* HAT gene were isolated.

[1] Wöstemeyer et al (2002): in Horizontal gene transfer (eds. Syvanen, Kado) 21; pp. 241-247.

FBP015
Interplay between protein degradation, oxidative stress response, polar growth and virulence in the pathogen *Aspergillus fumigatus*

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The ability of eukaryotic cells to rapidly adapt to environmental changes is mainly achieved by a tightly controlled protein turnover. A conserved mechanism for controlled protein degradation is the ubiquitin proteasome system. Target proteins are attached to ubiquitin within the ubiquitin-protein ligase (E3) and therefore marked for degradation via the 26S-proteasome. The largest group of E3-enzymes is the SCF Cullin1 Ring ligases (CRL), which are multisubunit enzymes. The F-box subunit functions as a substrate adaptor and therefore is responsible for the substrate specificity of the E3 enzyme. The exchange of F-box proteins requires the COP9 signalosome CSN. Defects in CSN result in increased oxidative stress, impairment of polar growth and development, and a misregulated secondary metabolism in the mold *Aspergillus nidulans* [1, 2]. We have analyzed the genes for the

three F-box proteins Fbx15, Fbx23 and Fbx29 in the opportunistic pathogen *Aspergillus fumigatus*. Deletion of these genes results in defects in polarized growth during oxidative stress. Further analysis revealed that these genes are required for proper growth under amino acid starvation conditions induced by 3-amino-triazole or 5-methyl-tryptophan, which causes histidine- and tryptophan-starvation by false feedback inhibition. The *A. nidulans sconB*-homolog in *A. fumigatus*, which is the F-box protein encoding gene involved in the regulation of cysteine synthesis pathway of *A. nidulans*, is essential for *A. fumigatus*. We could further show that the gene for the F-box protein Fbx15 is required for virulence of *A. fumigatus* in a murine model. Functional GFP-tagged versions of Fbx15 and Fbx25 are localized in the nucleus. Future studies aim to identify the potential targets of these F-box proteins.
This work is supported by the Deutsche Forschungsgemeinschaft, DFG Research Unit 1334.

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[2] Braus G. H. et al (2010): Fungal development and the COP9 signalosome. Curr. Opin. Microbiol. 13: 1-5.

FBP016
Identification of regulatory proteins involved in sexual development in *Mucor mucedo*

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Sexual development in mucoralean fungi is regulated by retinoid-like beta-carotene derivatives, the trisporoids. Trisporoids serve as pheromones in partner recognition and possibly as internal transcription regulators. Numerous studies deal with their complex, co-operative synthesis. Some of the biosynthesis enzymes have been studied in detail, both, at the enzymatic level and at the level of their genes, but their control and especially the trisporoid signal perception events are essentially unknown.
We have now started to search for regulatory proteins involved in sexual development of *Mucor mucedo*. Towards this goal, we have made use of commercially available antibodies against mammalian retinoid binding transcription factors and other regulatory proteins. Using such antibodies, we identified a putative retinoid binding protein, cross-reacting with an antibody against cellular retinaldehyde-binding protein CRALBP, and a putative homeobox protein. We identified and cloned the putative *CRALBP* gene using PCR and inverse PCR, as well as Southern hybridization. As analytical tool, CRALBP was overexpressed in *Escherichia coli* for investigating the role of the putative CRALBP in early sexual development.

FBP017
Development of a tool for genetic manipulation of the zygomycete *Mucor mucedo*

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Genetic analysis of *Mucor mucedo*, a zygomycete model organism for studying sexual communication and action of the trisporoid pheromone system, is hampered by a strong tendency towards autonomous replication of introduced plasmids instead of stable integration. We are working on development of reliable tools for genetic manipulation based on interference with the DNA double strand break-repair system.
In several fungi, targeted gene disruption via homologous recombination is used for analysis of gene function. Although integration of exogenous DNA at homologous sites in the genome occurs easily in *Saccharomyces cerevisiae*, and is at least possible in some filamentous fungi, it is rare in those fungi where DNA integration occurs predominantly by non-homologous end joining (NHEJ), leading for DNA integration at ectopic sites in the genome. Direct ligation of DNA strands in NHEJ is mediated by a DNA-dependent protein kinase, a DNA ligase complex, and the Ku70-80 heterodimer. Homologoues of Ku70 and Ku80 have been identified in many organisms. Recent studies in filamentous fungi have shown increased gene targeting frequencies in Ku-deficient mutants, indicating that Ku-disruption strains are efficient recipients for gene targeting. We are searching for a *M. mucedo* homologoues to the human *KU70* and *KU80* genes using PCR, inverse PCR, and Southern hybridization.

FBP018
Do NOX enzymes and GSA alpha subunits participate in identical signaling pathways in *Sordaria macrospora*?

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Filamentous fungi in general can undergo sexual and asexual reproduction. Both developmental processes are rather complex and involve regulation of gene expression, specialization of cell types and intercellular communication. In filamentous ascomycetes, asexual development leads to the production of conidia, nonmotile asexual propagules that are separated from specialized sporogenous cells. In contrast, sexual spores are formed in multicellular fruiting bodies. The proper development of these fruiting bodies with mature sexual spores is crucial for sexual development of ascomycetes. To explain how this complex process takes place and is regulated is the aim of our work.
The ascomycete *Sordaria macrospora* is an excellent model organism to study the complex development from vegetative hyphae to sexual structures like fruiting bodies. This is due to the fact that no asexual reproduction takes place and no mating partner is needed. In the last few years, important players involved in sexual development in *S. macrospora* could be identified and characterized, indicating a complex regulatory protein network. Components of this protein network are three G-protein alpha subunits (GSA) which participate in two different signaling pathways leading to sexual development: GSA1 and GSA2 are involved in the formation of mature fruiting bodies, whereas GSA3 is important for spore germination. Similar results could be obtained for the NAD(P)H oxidases NOXA, NOXB and their regulator NOXR in other fungi. Our current work is now focused on the connection of NOX and GSA proteins in the regulation of sexual development.

FBP019

Will be presented as oral presentation with the ID FBV025!

FBP020
Proteomic profiling of the short-term response of *Aspergillus fumigatus* to hypoxic growth conditions

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The filamentous fungus *Aspergillus fumigatus* is an opportunistic airborne pathogen causing systemic infections in immunocompromised patients. It is obligate aerobe and requires molecular oxygen for growth. However, during the infection process *A. fumigatus* has to adapt quickly to very low oxygen concentrations when it grows in inflammatory, necrotic tissue. Recently, it was shown that hypoxia is involved in virulence of *A. fumigatus* [1]. In our lab, the metabolic long-term response of this fungus has recently been analyzed by using an oxygen-controlled chemostat. Still little is known about the short-term adaptive mechanisms of *A. fumigatus* to low oxygen concentrations. To gain more insights, we aimed to investigate the immediate response of *A. fumigatus* after oxygen depletion on the protein level by proteomic approaches. *A. fumigatus* was cultivated as a batch culture in a 3 L bioreactor. After pre-cultivation of *A. fumigatus* at 21 % (vol/vol) molecular oxygen concentration, the oxygen supply was shifted to 0.21 % (vol/vol) and several samples were taken during a 24 hour period of hypoxia. Cytosolic protein levels were analyzed by 2D-DIGE gel electrophoresis and differentially regulated proteins were identified by MALDI-TOF/TOF-analysis. Significant changes in the amino acid, carbohydrate and energy metabolism were observed within 24 hours of hypoxic growth. Glycolytic enzymes and proteins involved in amino acid metabolism were up-regulated. Furthermore, there was an increased production of proteins involved in respiration, electron transport and a general stress response. In contrast, enzymes which catalyze steps in sulfur metabolism and the biosynthesis of fatty acids were down-regulated. Furthermore, proteins of the pentose phosphate pathway (PPP) and the TCA cycle were down-regulated during the short-term response, as well. Strikingly, we determined also a strong up-regulation of the NO-detoxifying flavohemoprotein FHp under hypoxic conditions. In summary, hypoxia has a strong influence on the metabolic regulation of *A. fumigatus* and the

character of the long- and short-term response to hypoxia differs only partially. In future experiments we will analyze the function of FHp in the context of adaptation to hypoxia.

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FBP021
The identification and quantification of sphingolipid biosynthesis proteins in the yeast *Pichia ciferrii* using mass spectrometry

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Sphingolipids are amphiphatic molecules which can be found in all eukaryotic organisms. This complex group of metabolites is involved in numerous biological processes, such as cell growth [1], heat stress response [2] and protein turnover [3]. Although the biosynthesis of sphingolipids has been described in *Saccharomyces cerevisae* [4], this biosynthesis pathway remains to be fully elucidated in *Pichia ciferii*.
Interestingly, and in contrast to other yeast strains, *P. ciferrii* is characterized by the excretion of acetylated sphingoid bases, mainly tetraacetyl phytosphingosine (TAPS) [5]. Therefore, it was the aim of our current study to compare a TAPS-excreting with a non-excreting strain. We were able to identify 1693 proteins with at least two peptides which are 20% of the whole *Pichia ciferrii*-proteome, over 500 of which passed our conservative selection criteria for quantification.
In addition we identified key enzymes of the fatty acid biosynthesis pathway and of the sphingolipid pathway (i.e. serine palmitoyltransferase and sphinganine hydroxylase), which is primarily localized in the endoplasmic reticulum.

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FBP022
Natural products from marine fungi for the treatment of cancer

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Despite marine fungi are a potent group of secondary metabolite producers, they are not well characterised and underutilised in terms biotechnological application. Here, we demonstrate the sustainable exploitation of marine natural resources providing appropriate culture conditions for the group of marine fungi, thus enabling efficient production of marine natural products in the laboratory and also in large scale cultures, avoiding harm to the natural environment. In the focus are new anti-cancer compounds. These compounds will be characterised to the stage of *in vivo* proof of concept ready to enter further drug development in order to valorise the results of the project.
Two approaches are used to gain effective producer strains:
a) Candidate strains originating from our unique strain collection of marine fungi are characterised and optimised using molecular methods.
b) New fungi are isolated from unique habitats, i.e. tropical coral reefs, endemic macroalgae and sponges from the Mediterranean. Culture conditions for these new isolates are optimised for the production of new anti-cancer metabolites.
We develop a process concept for these compounds providing the technological basis for a sustainable use of marine microbial products as result of Blue Biotech. Therefore, we will explore the potential of marine fungi as excellent sources for useful new natural compounds along the added-value chain from the marine habitat to the drug candidate and process concept.

FBP023
Uptake of Various Yeast Genera by Antigen-Presenting Cells and Influence of Subcellular Antigen Localization on T Cell Activation

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The use of yeast cells as vehicles for proteinaceous antigens is a very promising vaccination approach. Using the biotechnologically well-accepted yeast genera *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Kluyveromyces lactis*, and *Pichia pastoris* we investigated the interaction kinetics between these yeasts and phagocytic cells as well as the involvement of dectin-1 and mannose receptor in phagocytosis. Further, we analyzed whether these recombinant yeast genera expressing an intracellular, extracellular or surface-displayed ovalbumin (ova) derivative were able to activate ova-specific CD8 T lymphocytes. We found that the kinetic patterns of yeast uptake by phagocytic cells varied between the different yeasts. Additionally, the subcellular localization of the ova antigen influenced the rate of T cell activation. These results suggest that each yeast genus has its particularities regarding recognition by phagocytes and its potential as antigen delivery vehicle which have to be considered in vaccination approaches.

FBP024
Phenotypical and transcriptional analysis of photoconidiation in mutants of the RNAi machinery of *Trichoderma atroviride*

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Trichoderma atroviride is one of the most used biocontrol agent due to its mycoparasitic activity. Conidia is useful as inocula in the field and greenhouse, therefore the understanding of he switch that determines the entry to conidiation is the great interest, not only due to its biotechnological relevance but also as an example of fungal development.
In *Trichoderma atroviride,* conidiation is induced by light and the possible participation of small RNAs in this process has not been explored,as well the role of the proteins involved in their biogenesis and function, such as Dicer (Dcr), RNA dependent RNA polymerase (RdRP) and Argonaute (Ago).
The *T. atroviride* genome encodes two *dicer* homologues. We have obtained singles and double mutants of them. Photoconidiation is altered in Δ*dcr2* and double mutants. In contrast with the wild type they do not respond to constant exposure to white light. In addition, we carried out high-throughput mRNA sequencing by SOLiD of samples from wild type, Δ*dcr1, Δdcr2, Δdcr1Δdcr2* strains after 60 h of exposure to white light.
1655 genes are differentially expressed in the mutants strains, as compared to wild type. Two genes that are up-regulated in Δ*dcr2* and Δ*dcr1Δdcr2* are *ago1* and *rdr3*. When exposed to constant white light Δ*rdr3* is altered in photoconidiation, but Δ*ago1* is not.
These data suggest that the RNAi machinery, hence sRNAs, are involved in the regulation of development in this *Trichoderma.*

FBP025
Purification and partial characterization of the main pectin lyase from *Aspergillus giganteus* grown in orange waste

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Pectin lyase (PL) is a pectin despolimerizing enzyme that has wide possibilities of industrial application mainly in processing of textile fibers, coffee and tea fermentation, vegetable-oil extraction and treatment of paper pulp. The purification of pectinolytic enzymes makes possible to know details about the molecule struture and its biochemical properties. Orange waste is an agro-industrial residue abundantly produced in Brazil by the

orange juice industry and its utilization in the PL production leads to an increase in yield with a reduction in the process cost; moreover, it adds value to the waste from the orange juice industry. The goal of this work was to determine an efficient methodology to isolate the major extracellular pectin lyase produced by *Aspergillus giganteus* and to characterize its enzymatic activity. The main PL was purified after three steps: 1. DEAE-Sephadex A-50 column equilibrated with imidazole-NaOH buffer pH 6.0, proteins were eluted with a linear gradient from 0 to 1.0 M NaCl; 2. CM-Sephadex C-50 column equilibrated with sodium acetate buffer pH 5.5, proteins were eluted with a linear gradient from 0 to 0.5 M NaCl; 3. Sephadex G-100 column equilibrated with ammonium acetate pH 6.8. After the three steps the PL presented eletrophoretic homogeneity and purification fold of 47.8 with recovery of 12.4%. The purified PL has a molecular weight of 55 kDa. The enzyme presented its higher activity when incubated in pH 8.5 and 50ºC. In the absence of substrate the PL is reasonably stable at 40ºC, keeping 70% of its activity after 15 min, but at 50ºC the enzyme loses its activity fast with a half-live of 9 min, although the *A. giganteus* PL are more stable than the commercially available enzymes Rapidase C80® (DSM) and Pectinase CCM® (Novozymes). The best condition to stock the enzyme at 4ºC is in acid and neutral solution. The PL degrades better citrus pectins with 72 and 34% of esterification, but is also able to degrade apple pectin and high esterified citrus pectin. The kinetics parameters, measured on citrus pectin 72% esterified, were K_m 4.8 mg.mL^{-1}, V_{max} 1,129.8 U.mg^{-1}.min^{-1} and K_{cat} 770 s^{-1}.

[1] Hoondal, G.S. et al (2002): Appl Microbiol. Biotechnol., 59, 409-418.
[2] Ortega, N. et al (2004): Int. J. Food. Sci. Technol., 39, 631-639.

FBP026
The role of BEM-1 as possible regulator of NOX-1 and NOX-2in*Neurospora crassa*

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Reactive oxygen species (ROS) play essential roles in cell differentiation in microbial eukaryotes (Lara-Ortíz *et al*., 2003, Aguirre *et al*., 2005, Cano-Domínguez *et al*., 2008). The NADPH oxidase (NOX) enzyme complex catalyzes the production of superoxide by transferring electrons from NADPH to O_2. The phagocytic NOX consists of the membrane-associated catalytic core gp91^{phox} and p22^{phox} subunits (cytochrome *b558*). The assembly of the cytosolic regulatory proteins p47^{phox}, p40^{phox}, p67^{phox} and Rac2 with cytochrome b_{558} results in NOX activation. *Neurospora crassa* contains two NADPH oxidase genes (*nox-1* and *nox-2*), which encode proteins that are homologous to phagocyte Nox2 (gp91^{phox}). We reported that deletion of *nox-1* results in mutants unable to differentiate sexual fruiting bodies and show reduction of growth and asexual development. The inactivation of *nox-2* only affects the germination of the sexual spores (ascospores). *N. crassa* NOX activity requires other proteins like the p67^{phox} ortologue NOR-1, and possibly other proteins like BEM-1 which was proposed as a functional homologue of p40^{phox} (Kawahara and Lambeth, 2007)). BEM-1 contains two amino-terminal Src homology 3 (SH3) domains and carboxy-terminal PX and PB1 domains. *N. crassa* mutants lacking BEM-1 (bud emergence protein in *S. cerevisiae*) show a decrease in radial growth but were are able to develop normal sexual fruiting bodies, while most ascospores from *Δbem-1* homozygous crosses failed to germinate. This results suggests that BEM-1 is required for full NOX-1 activity during vegetative growth and for NOX-2 activity during ascospore germination but is fully dispensable for NOX-1 function during sexual development. To test whether NOR-1 and BEM-1 interact and regulate polar growth, we have generated *nor-1::gfp* and *bem-1::rfp* fusions and will determine their localization.

FBP027
Antarctic fungi as a potential bioresource of cold-active antioxidant enzyme catalase

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In recent years, a lot of evidence has accumulates revealing that the cold-adapted microorganisms possess enormous biotechnological potential, offering a range of economic and environmental advantages over previously used temperate producers. An important component of this potential are cold-active (CA) enzymes, which remain active at low temperatures and have correspondingly low temperature activity optima. Catalase (CAT, EC 1.11.1.6) is one of the key antioxidant enzymes involved in aerobic cell response against oxidative stress by scavenging of H2O2. Moreover, CAT can be very useful in medicine, food, pharmaceutical and textile industry. In the present study, psychrophilic and mesophilic filamentous fungi were isolated from samples collected in the vicinity of the permanent Bulgarian Antarctic base „St. Kliment Ohridski" on Livingston Island. The pure cultures from 55 isolates were identified at least to genus level and screened for ability to produce CA CAT. All tested strains demonstrated enzymatic potential. Among screened isolates 25 strains produced high level of CAT. The best producers belong to genera Penicillium, Cladosporium, Aspergillus, Geomyces, Lecanicillium, Epicoccum.. Psychrotrophic strains Cladosporium oxysporum 251, Penicilium dierckxii 246, Penicillium italicum 232 and Aspergillus sp. 266 gave the highest activity. The physiological characteristics of flask and bioreactor cultures were assessed to understand optimal growth conditions. The results indicated that various factors including carbon and nitrogen source, air, pH, and inoculum size influence enzyme synthesis. Optimum pH and temperature for crude CAT were determined.

Acknowledgments: This work was supported by the National ScientiWc Fund of the Ministry of Education and Science, Bulgaria (grants DO02-172/08 and BG051PO001-3.3.04/32), which is greatly acknowledged.

FBP028
Analysis of the diversity and biodegradation possibilities of fungi in wastewater biocoenoses

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Fungi are ubiquitous in the environment and play an important role in a variety of different ecosystems, e.g. wastewater biocoenoses. As decomposers of many micro- as well as macro-pollutants, they represent an essential component in the „living" part of activated sludge. Nevertheless, our understanding of the wastewater fungal diversity and their exact functions in these biocoenoses remains uncertain. An attempt to gain insight into the abundance and biodegradation abilities of wastewater fungi was the aim of this work. To shed light on some of these questions, culture-based methods were combined with the following molecular techniques: denaturing gradient gel electrophoresis (DGGE), PCR, DNA sequencing and the fluorescent-in-situ-hybridization-method (FISH). Different wastewater treatment plant (WWTP) samples were collected, enriched with the desired compound (the antibiotic Sulfamethoxazole) and tested for the occurrence of fungi. The result was 12 different species of fungi in pure cultures able to grow on agar plates containing Sulfamethoxazole as the sole C and N source. Visible growth occurred within 3-5 days after inoculation, which, even compared to bacteria, is quite fast concerning that the nutrient source is an antibiotic. Chemical analysis, carried out by GC-MS/MS, will hopefully provide information about the extend to which Sulfamethoxazole is used and thus degraded, and if this compound is not fully mineralized, what „byproducts" are formed. Another approach that will be taken is PCR-DGGE. This cultivation-independent method will allow for the characterization and comparison of wastewater fungal biocoenoses without the „great plate count anomaly" problem. This method reveals the community's diversity and allows for comparison of the „original" fungal species from activated sludge with the cultured ones. The final task will be to identify the metabolic (end)-products and hopefully link them with the producing species.

FBP029
Exploration of rCciAPO1 from Coprinopsis cinerea: First recombinant aromatic peroxygenase

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The gene CC1G_08427, heterologously expressed in A. oryzae by Novozymes A/S codes for a aromatic peroxygenase (APO): rCciAPO1. Spectroscopic studies with CO-bound protein indicate the constituation of a heme-thiolate enzyme. The observed ability to hydroxylate naphthalene to 1-naphthol as major reaction product and the N-oxygenation of pyridine are

typical for wild type APOs from Agrocybe aegerita and Coprinellus radians. Although rCciAPO1 showed no halogenating activity, it was able to oxidize bromide and iodide to tribromide and triiodide, respectively. The physicochemical characterization of this novel enzyme lead to following results: The molecular mass is about 44 kDa with a broad range of variation due to non-uniform glycosylation with 14-44 % sugar content and a MW of 38 kDa for the enzymatically deglycosylated protein. With a pH optimum of 7.0, a temperature optimum above 60 °C and a high tolerance towars organic cosolvents during reactions more typical properties of previously studied APOs were met.

FBP030
Growth-dependent secretome of *Candida utilis*

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Recently, the food yeast *Candida utilis* has emerged as an excellent host for production of heterologous proteins [1]. Since secretion of the recombinant product is advantageous for its purification, we characterized the secreted proteome of *C. utilis*. Cells were cultivated to the exponential or the stationary growth phase and the proteins in the media were identified by mass spectrometry. In parallel, a draft genome sequence of *C. utilis* strain DSM 2361 was determined by massively parallel sequencing. Comparisons of protein and coding sequences established that *C. utilis* is not a member of the CUG clade of *Candida* species. In total, we identified 37 proteins in the culture solution, 17 of which were exclusively present in the stationary phase, whereas 3 proteins were specific for the exponential growth phase. Identified proteins represented mostly carbohydrate-active enzymes associated with cell wall organization, while no proteolytic enzymes and only a few cytoplasmic proteins were detected. Remarkably, cultivation in xylose-based medium generated a protein pattern that diverged significantly from glucose-grown cells, containing the invertase Suc2 as the major extracellular protein, particularly in its highly glycosylated S-form. Furthermore, cultivation without ammonium sulfate induced the secretion of the asparaginase Asp3. Comparisons of the secretomes of *C. utilis, K. lactis*, *P. pastoris* and the human fungal pathogen *C. albicans* revealed a conserved set of 10-12 secretory proteins [2, 3, 4].

[1] Ikushima, S. et al (2009): Biosci Biotechnol Biochem 73:1818-24.
[2] Sorgo, A. G. et al (2010): Yeast 27:661-72.
[3] Mattanovich, D. et al (2009): Microb Cell Fact 8:29.
[4] Swaim, C. L. et al (2008): Proteomics 8:2714-23.

FBP031
Regulation of ABC transporter genes in *Botrytis cinerea*

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ABC-type drug efflux transporters use the energy from ATP hydrolysis for transmembrane substrate translocation, and contribute to chemical stress tolerance of cells. In fungi, they have been shown to mediate the efflux of mating hormones, self-produced toxins, antibiotics, plant toxins and a variety of artificial drugs including fungicides. We have identified the Mrr1 transcription factor which activates AtrB, a major ABC transporter that partly controls tolerance of *B. cinerea* against various drugs. Point mutations leading to activation of Mrr1 were found in natural *B. cinerea* populations, leading to multidrug resistance (MDR) phenotypes. Using an *atrB*-GFP reporter strain, the *atrB* inducing activity of various drugs was analysed. By *atrB* promoter deletions, we have determined the Mrr1 binding site. Similarly, we have identified a transcription factor binding site in the promoter of *atrD* encoding another ABC transporter involved in the efflux of azole fungicides. It is our goal to unravel the mechanisms of drug activation of transcription factors that control ABC transporters, and to understand their contribution to the tolerance of chemical stress and plant defence mechanisms.

FBP032

Will not be presented!

FBP033
The regulation of ergot alkaloid biosynthesis in *Claviceps purpurea*

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Claviceps purpurea is an ubiquitous phytopathogenic ascomycete which produces the secondary metabolites ergot alkaloids (EA). The altogether 14 genes encoding the specific enzymes for the biosynthesis of EA are clustered. To date the molecular mechanisms of cluster regulation in *C. purpurea* are unknown. No transcription factor gene has been found within the cluster region involved in the synthesis of EA. It is only known to date that the EA in *C. purpurea* wild-type are produced during the ripening of the sclerotium and not in axenic cultures. Mutant strains producing alkaloids in submersed cultures require under specific conditions: (a) tryptophan as inducer and precursor, (b) a high osmotic value, (c) a low phosphate level. The alkaloid biosynthesis was speculated to be regulated by changes in the chromatin organization, a hypothesis checked by the cultivation of *C. purpurea* in the presence of either inhibitors of histone deacetylases (HDACis) or histone acetyltransferases (HATis) [1]. Other global regulators which are involved in the regulation of secondary metabolism and development like VeA and LaeA are discovered in *Aspergillus nidulans* [2,3]. We are investigating whether *C. purpurea* biosynthesis of ergot alkaloids could be regulated through factors homologous to VeA and LaeA. We have identified and sequenced homologues of *veA* and *laeA* in *C. purpurea* (*Cpvel1* and *Cplae1*) and have started a functional analysis including heterologous complementation in a ΔveA mutant of *Fusarium fujikuroi* and the deletion of *veA* and *laeA* in *C. purpurea*

[1] Lorenz et al (2009): Phytochemistry 70:1822-1832.
[2] Kim et al (2002): Fungal Genet Biol, 37:72-80.
[2] Bok et al (2004): Eukaryotic Cell, 3:527-535.

FBP034
Overexpression of alpha-ketoglutarate dehydrogenase in *Yarrowia lipolytica* and its effect on production of organic acids

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The yeast *Yarrowia* (*Y.*) *lipolytica* is one of the most intensively studied 'non-conventional' yeast species. Its ability to secrete various organic acids, like pyruvic (PA), citric (CA), isocitric (ICA) and alpha-ketoglutaric (KGA) acids, in large amounts is of interest for biotechnological applications. We have studied the effect of the alpha-ketoglutarate dehydrogenase (KGDH) complex on the production process of alpha-ketoglutarate (KGA). Being well studied in *Saccharomyces cerevisiae* this enzyme complex consists of three subunits: alpha-ketoglutarate dehydrogenase (Kgd1), dihydrolipoyl transsuccinylase (Kgd2) and lipoamide dehydrogenase (Lpd1). Here we report the effect of overexpression of these subunits encoding genes and resulting increase of specific KGDH activity on organic acid production under several conditions of growth limitation and an excess of carbon source in *Y. lipolytica*. The constructed strain containing multiple copies of all three KGDH genes showed a reduced production of KGA and an elevated production of PA under conditions of KGA production. However, an increased activity of the KGDH complex had no influence on organic acid production under citric acid production conditions.

FBP035

Activation of a silent secondary metabolite gene cluster in *Aspergillus fumigatus* by co-cultivation with *Streptomyces rapamycinicus*

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Aspergillus fumigatus is the most important air-borne human fungal pathogen. Its genome exhibits far more gene clusters predicted to encode secondary metabolites than compounds known. Because these unidentified metabolites could have interesting biological activity and could also serve as drug candidates, it is crucial to activate these often silent gene clusters. Recently, we were able to mimic physiological conditions under which one of these gene clusters is very likely active [1]. During these investigations we discovered the principle that silent gene clusters in the filamentous fungus *Aspergillus nidulans* are activated by a distinct bacterium, i.e., *Streptomyces rapamycinicus*, which resulted in the formation of orsellinic and lecanoric acid. As reported here, interestingly, this streptomycete has the potential to induce silent gene clusters in other fungi. As an example, we discuss data obtained by co-culturing the human pathogen *A. fumigatus* with the same *Streptomyces rapamycinicus* that leads to induction of silent gene clusters and the production of novel metabolites.

[1] Schroeckh et al (2009): Intimate bacterial-fungal interaction triggers biosynthesis of archetypal polyketides in *Aspergillus nidulans*. PNAS. 106(34): 14558-14563.

FBP036

Identification of conidia-associated surface proteins in the human pathogenic fungus *Aspergillus fumigatus*

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The saprophytic fungus *Aspergillus fumigatus* is one of the most important human pathogenic fungi that causes severe invasive lung infections in immunocompromised patients. The asexual reproduction of *A. fumigatus* leads to the formation of conidia which are released into the atmosphere. Based on their small size of 2 to 3 µm in diameter, they are inhaled by humans and can reach the lung alveoli. Hence, conidia are the fungal entity which have the initial contact with the host's immune system. Besides cell wall polysaccharides the conidial surface proteins are the first molecular structures which are recognised by the host's immune system. To characterise the composition of the *Aspergillus fumigatus* conidial surface proteome, we released surface proteins, especially glycosylphosphatidylinositol-anchored proteins (GPI) by HF-pyridine extraction and subsequent LC-MS/MS analysis. We identified 210 different proteins, of which 50 showed a signal peptide for secretion and 9 proteins a GPI anchor attachment signal. The most abundant surface proteins of conidia of the WT strain ATCC 46645 represented the hydrophobin protein RodA and a hypothetical protein. To elucidate the role of the conidial melanin layer on the composition of the conidial surface proteome we also investigated spores of the *pksP* mutant (Jahn *et al*., 1997), which produces white, melanin-free conidia and which is drastically reduced in virulence. Using spectral counts for peptide quantification we detected four GPI-anchored proteins that were missing in HF-pyridine extracts of the *pksP* mutant: an extracellular matrix protein, an antigenic cell wall galactomannoprotein, the glutaminase GtaA and the 1,3-β-glucanosyltransferase Gel1. The HF-pyridine extract of the mutant strain contained an increased amount of cytoplasmic proteins, e. g. ribosomal proteins, which might indicate a higher metabolic activity and therefore a reduction in dormancy.

[1] Jahn, B. et al (1997): Isolation and characterization of a pigmentless-conidium mutant of *Aspergillus fumigatus* with altered conidial surface and reduced virulence. Infect Immun 65: 5110-5117.

FBP037

Chitin deacetylase from *Podospora anserina* with two chitin binding domains

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Chitin deacetylases (CDAs) convert the biopolymer chitin into chitosan. CDAs can e.g. be found in plant pathogenic fungi which have been shown to change their cell wall chitin into chitosan upon penetrating the host tissue. Interestingly, some species, e.g. *Fusarium graminearum* and *Podospora anserina*, harbor genes which consist of the catalytic deacetylase domain and also one or more chitin binding domains (CBDs).The latter are known to help chitinases to act on insoluble chitin polymers. In assuming a similar function in CDAs, we decided to heterologously express such a gene to purify the corresponding protein and to characterize its enzymatic properties. So far, the activity of an enzyme containing the CDA domain and also one or more CBDs is not described. Chemically produced chitosans possess only random patterns of acetylation (PAs), but enzymatically deacetylated chitosan may have non-random PAs We want to analyze the activity and specificity of these enzymes and their catalysis products, because the CBD may influence the mode of action and therefore also the biological activities of the produced chitosans.
One putative *P. anserina* CDA is predicted to contain a CBD and CDA domain and is very similar to the group of known CDAs, mostly related to the *Colletotrichum lindemuthianum* CDA in the catalytic domain. Domain identification by *hidden Markov models* of the PFAM database shows one N-terminal and one C-terminal CBD. Because the two chitin binding domains are different in sequence and length they may have different substrate affinities and/or specificities. We want to analyze the function of the different domains by synthesis of the full length protein and the truncated protein lacking one or both chitin binding domains followed by analysis of substrate activity and specificity. The CDA gene is synthesized and optimized for expression in *Hansenula polymorpha*.

FBP038

Pyranose-2-oxidase production by the white rot fungus *Pycnoporus cinnabarinus*: characterization of the enzyme and a putative gene

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Peroxidases and laccase are involved in lignocellulose degradation by direct or indirect (via mediators) action. Peroxidases depend on the provision of peroxides as a co-substrate. Therefore lignin degrading fungi need mechanisms for the formation peroxides. Besides glyoxal-oxidase, aryl alcohol oxidase and glucose 1-oxidase Pyranose 2-oxidase (POx, pyranose: oxygen 2-oxidoreductase EC 1.1.3.10) is a possible candidate for this function. The presence of POx is relatively widespread among wood-degrading basidiomycetes but the enzyme has only been isolated from a limited number of fungal species and only a few genes and c-DNA sequences are known.
In the presence of molecular oxygen the enzyme catalyse the oxidation of several aldopyranoses at carbon-2 and sometimes but in lesser extent at carbon-3. Besides oxygen the reduction of some different quinones has also been shown. Therefore three possible functions of the enzyme are proposed: 1. formation of H_2O_2 for ligninolytic peroxidases 2. reduction of quinones instead of oxygen and 3. involvement in the biosynthesis of cortalcerone an antibiotic of fungal origin.
Pycnoporus cinnabarinus has not jet been shown to produce this enzyme. For this organism we could show the formation of manganese peroxidase as the only known ligninolytic peroxidase, therefore a H_2O_2 generating enzyme system should be present.
POx production is correlated with idiophasic growth and seems not to be extracellularly. A correlation of H_2O_2 in the culture supernatant with POx activity in the cells is not clear indicating that this enzyme should not be the only H_2O_2 generating activity under the conditions tested.
POx has been isolated, purified to apparent homogeneity and characterized biochemically. Besides D-glucose other pyranoses (e.g. L-sorbose, D-xylose, cellobiose) can be oxidized. The protein is a homotetramer with a molecular mass of about 244 kDa containing flavin.
Using PCR with degenerated primers leading to partial sequences followed by a genome walking protocol with gene specific primers two open reading frames could be detected cloned and sequenced. A protein model (POX1) derived from one of the gene sequences (*POX1*) using AUGUSTUS

software consists of 661 amino acids on 15 exons. By ESI-MS-analysis of the purified enzyme 9 peptides could be found covering 39% of the protein. POX1 is the only gene product expressed under the conditions tested. A proposed *POX2* gene and protein (POX2) shows some significant differences to POX1 and to other published c-DNA- and protein sequences. *POX2* expression could not be shown and its physiological role is still unknown.

FBP039
Protoplast analysis of the fungus *Ashbya gossypii* revealed a correlation between differentiation and vitamin over-production

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Ashbya gossypii, a filamentous hemiascomycete, is known to produce 100mg vitamin B_2 (riboflavin) per g biomass. Regulation of this overproduction is evident since less than 1% is produced at constant dilution rate in chemostatic culture [1]. The molecular mechanism controling that difference in productivity is a regulation of the genes *RIB1, RIB2 and RIB3*, encoding the enzymes of the biosynthesis pathway. Evidence for induction under nutritional stress was given by increasing RT-PCR signals compared with constitutively expressed *TEF* or *ACT1*. Additionally, *RIB3*, encoding the first enzyme, showed increased initiation of transcription. This was shown by fusion of its promoter with *lacZ*. On enzyme level, an increase of specific activity of dihydroxy butanone phosphate synthase, encoded by *RIB3*, was determined in the transition from growht to the production phase [2].
The measured changes were only partly consistant with the cell morphologies observed by microscopy. As soon as growth rate declined a significant part of the cells formed spores, a second part accumulated riboflavin, resulting in a green fluorescence, and a third part appeared hyaline. In the mycelium a quantification of cell types was impossible. Therefore a conversion to protoplasts by digestion of the cell walls was performed. Up to 10^9 protoplast were liberated per millilitre. This was deteced by FACS analysis. Furthermore flow cytometry distinguished 50% needle-shaped spores from spherical protoplasts. Up to 80% of the latter emitted green light when excited at 488 nm indicating riboflavin accumulation.
By FACS sorting more than 10^6 riboflavin accumulating protoplasts were separated from hyaline protoplasts. In a typical crude extract more than 90 mU beta-galactosidase activity was determined per mg protein. In contrast less than 5 mU were detectable in hyaline protoplast showing no riboflavin accumulation. This difference allows the conclusion that riboflavin over-production is limited to a part of the cells. To achieve full over-production potential differentiation not into four but into a single cell type which overproduces and stores riboflavin might become a promissing approach.

[1] Stahmann, K.-P. (2010): Production of vitamin B2 and a polyunsaturated fatty acid by fungi pp 231-246 In: *Industrial Applications* Martin Hofrichter (ed) Vol. X der Serie *The Mycota* K. Esser (ed) Springer, Heidelberg.
[2] Schlösser, T. et al (2007): Growth stress triggers riboflavin overproduction in *Ashbya gossypii*. Applied Microbiology and Biotechnology 76(3): 569-578.

FBP040
Genetic Characterisation of MCF A95, a Micro-colonial Fungus that Colonises Bare Rocks

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Melanised microcolonial fungi (MCF) colonize bare rock surfaces in deserts and other arid areas and are unequaled among eukaryotic organisms in their ability to withstand extreme heat, dessication and UV radiation. These organisms are crucial in the establishment of subaerial rock biofilms and, as such, set the stage for a variety of interactions important for mineral/material stability and rock weathering. MCF are a taxonomically diverse group of ascomycetes that possess simplified stress-protective morphologies. Partly as a result of their peculiar compact colonial structure and protective cell walls, MCF are able to survive a broad spectrum of physical stresses including temperature, salt, UV-irradiation and desiccation. These survival specialists exist because of multiple secondary metabolic products supporting their stress tolerance - melanins, carotenoids, mycosporines and compatible solutes. A meristematic black yeast species, *Sarcinomyces petricola* (A95), was isolated from the sun exposed marble monument in Athens (Greece). As many rock-inhabiting fungi, A95 is positioned in the early diverging lineages of Chaetothyriales, which were shown to be ancestral to opportunistic pathogens and lichens. A95 is a relatively fast-growing strain which was identified as a suitable model organism for genetic analysis. For its stress tolerance A95 relies on a broad spectrum of stress protection mechanisms typical for MCF. The whole genome sequence of *Sarcinomyces petricola* (454 and Illumina methods) is currently underway. Different methods have been tested to establish a transformation protocol for A95. A commonly used method using the binary Ti vector system of *Agrobacterium tumefaciens* was employed (De Groot et al., 1998). The stress- tolerant morphology of the black yeast, especially the thickness of their cell wall and melanization makes the DNA transfer from *A. tumefaciens* to A95 a complicated task. Several methods to circumvent this problem were tested. By Microprojectile Bombardment small gold particles were coated with DNA and directly transferred into the nucleus. Other approaches are the transformation of protoplasts or increasing the receptivity of A95 to *A. tumefaciens* transformation by mechanical or chemical weakening of the cell wall.

[1] De Groot, M.J.A. et al (1998): *Agrobacterium tumefaciens* - mediated transformation of filamentous fungi. Nature Biotechnology 16: 839-842.

FBP041
Regioselective hydroxylation of diverse flavonoids by an aromatic peroxygenase

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Selective transfer of oxygen functionalities to non- or little activated carbon atoms (e.g. aromatic rings) is a challenging problem for chemical synthesis. Biotransformations based on the activity of oxidoreductases would offer an elegant alternative. Here we report that fungal peroxygenase from *Agrocybe aegerita* (*Aae*APO) can selectively hydroxylate a variety of flavonoids (plant ingredients with various biological functions, e.g. as strong antioxidants).
The results showed that the hydroxylation reactions proceed rapidly and regioselectively yielding C6-hydroxylated reaction products of diverse flavonoids such as flavone, flavanone, apigenin, luteolin as well as daidzein.
Studies using ^{18}O-enriched hydrogen peroxide ($H_2{}^{18}O_2$) as co-substrate revealed that the oxygen incorporated into the reaction product in fact derived form the peroxide, which points to a true peroxygenase mechanism. Thus, mass spectral analysis of the metabolite formed during the *Aae*APO-catalyzed hydroxylation of daidzein in the presence of $H_2{}^{18}O_2$ in place of H_2O_2 showed a shift of the principal $[M+H]^+$ ion from m/z 271 to m/z 273 in case of 6-hydroxydaidzein (demethyltexasin).
Interestingly, flavonoid glycosides, especially multiple glycosilated compounds such as rutin, are not subject of peroxygenase attack, very probably due to stearic hindrance.
Our results raise the possibility that fungal peroxygenases may be useful for versatile, cost-effective, and scalable syntheses of hydroxylated flavonoids.

FBP042
Studies of wood degradation by wood-decay fungi with a new experimental setup

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Wood is a hard, fibrous tissue found in many plants. It is a natural composite of cellulose fibers embedded in a matrix of hemicelluloses and lignin, which is consisting mainly of carbon-carbon linked and ether linked phenylpropane building blocks. Lignin is a natural barrier against microbial attack and is modified only by radicalic mechanisms catalysed by peroxidases (manganese peroxidase, lignin peroxidase), phenoloxidase (laccase) or hydroxylic radicals (produced by the Fenton's reaction). Wood-decay fungi are known as the most efficient wood degraders. A flexibel experimental approach was set up to investigate the spatiotemporal degradation of

lignocellulose and the secretion of extracellular oxidoreductases in wood chips. Fungi of different eco-physical groups were tested for their ability to grow on beech wood chips: i) classic white-rot (e.g. *Pycnoporus cinnabarinus*), ii) „unspecific" wood-rot (e.g. *Agrocybe parasitica*) or iii) brown-rot (*Fomitopsis pinicola*). Silicon tubes (ø 12 mm) were packed with beech wood chips and subsequently moisturized with beech wood extract. Inoculation and continuous low aeration were carried out unidirectional. After one month growth period silicon tubes were harvested and segmented. These segments were analyzed for Klason lignin content, organic acids produced by the fungi and ligninolytic enzyme activities were determined. No ligninolytic enzyme activities were detectable in cultures of *Fomitopsis pinicola*, this is typically for brown-rotters although growth was observed. In contrast all white-rotters produced manganese-oxidizing enzymes and laccase of different ratios. Highest enzyme activities were detected in their respective last segment (up to 1000 mU g^{-1} and 560 mU g^{-1} respectively). Likewise residual lignin content increased from point of inoculation up to the end point of growth.

FBP043
Application of streptavidin-based affinity chromatography combined with high-sensitive mass spectrometry for the identification of putative regulatory factors of Cephalosporin C biosynthesis

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Primary and secondary metabolism in filamentous fungi is usually controlled by a network of transcription factors that act as activators or repressors on gene expression. In order to isolate proteins from whole extract that bind specifically to promoter sequences, we developed a reliable method using a Streptavidin-based affinity chromatography and biotinylated DNA-Fragments combined with high-sensitive mass spectrometry.
The filamentous fungus *Acremonium chrysogenum* is the main producer of cephalosprin C. The biosynthesis of this beta lactam antibiotic is catalyzed by at least seven enzymes, two of which have expandase / hydroxylase and acetyltransferase activity [1]. These proteins are encoded by the *cefEF* and *cefG* genes, whose expression is driven by a strong 939 bp promoter. Using the biotinylated cefEF/cefG-promoter as a bait sequence, we developed an isolation procedure to isolate DNA binding proteins from *A. chrysogenum*. As a proof of principle the known promoter binding protein CPCR1 which is involved in the regulation of cephalosporin biosynthesis was identified [2].

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FBP044
Nourseothricin-based expression vectors for the production of heterologous proteins in the yeast *Candida utilis*

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For more than six decades *Candida utilis* represents an industrially important yeast, being classified as GRAS (*generally recognized as safe*). This yeast assimilates and easily adapts to a number of different carbon and nitrogen sources. Furthermore, growth of *C. utilis* is hardly affected by extremes in pH, and being Crabtree-negative it does not produce ethanol in aerated cultures, which limits growth in other yeast species. Initially, *C. utilis* was used as a food yeast to produce single cell protein in high quality from cheap, biomass-derived waste substrates. Recent reports, which describe the production of several heterologous proteins in *C. utilis*, e. g. the sweetener monellin from *D. cumminsii* [1], α-amylase from *S. solfataricus* [2] and xylanase from *S. olivaceoviridis* [3], have suggested that *C. utilis* is an efficient host for the high-level protion of recombinant proteins and may become an alternative to more established yeast expression hosts.
Here, we report on the development of a set of novel expression vectors for heterologous gene expression in *C. utilis*. These vectors contain the autonomously replicating sequence (*ARS*) of *C. albicans* for plasmid maintenance and the *SAT1* gene for selection on nourseothricin-containing media. Furthermore, we inserted a number of strong promoters of *C. utilis* genes, including *TDH3*, *PCK1* and *PMA1*, which can be used to trigger heterologous gene expression. Promoter efficiencies were investigated by fluorescence quantification, ONPG assays and immunoblot analysis using the two reporter proteins green fluorescent protein (GFP) and β-galactosidase (LacZ).

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FBP045
The multifunctional roles of chitinases in mycoparasitism and cell wall remodelling in the fungus Trichoderma

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The potential biological functions of fungal chitinases cover a plethora of different aspects including cell wall remodeling during the fungal life cycle and degradation of exogenous chitin as nutrient source. Genome analysis of the mycoparasitic fungi Trichoderma atroviride and T. virens showed that these fungi have an expanded arsenal of chitin degrading enzymes. They are especially enriched in the only recently described subgroup C chitinases that contain multiple carbohydrate-binding domains (CBM18, CBM50 (LysM)), which possibly enables them to efficiently degrade insoluble substrates such as fungal cell walls. Methods: In order to elucidate the involvement of subgroup C chitinases in exogenous chitin degradation and/or fungal cell wall remodelling and recycling in T. atroviride and T. virens, we performed transcriptional profiling of the genes by RT-PCR and analysed the modular architectures and structural features of the substrate binding sites of the proteins with 3D modelling. Results: In T. atroviride, interestingly only few subgroup C chitinase genes were found to be inducible by chitin. Instead they were induced by the complex carbohydrate structures of the fungal cell walls. Further, the transcriptional profiles of tac2 and tac6 indicated a role of the respective proteins in fungal cell wall remodeling during hyphal growth. Analysis of the catalytic core of TAC6 revealed an alteration in the active site which could imply specific substrate adaption or lack of the chitinolytic activity with residual strong binding affinity to the substrate. In T. virens the transcriptional profiles of subgroup C chitinases are strongly different from T. atroviride and suggest even more versatile roles of these proteins. Some of the genes are expressed during germination, growth and branching and in parallel a few of those are also induced during mycoparasitism. Further, in contrast to T. atroviride, the majority of the subgroup C chitinase genes in T. virens can be induced by exogenous chitin. Conclusion: Our results provide first insights into fungal subgroup C chitinases and show diversified roles of these chitinases in degradation of exogenous chitinous carbon sources and fungal cell wall remodelling/recycling.

FGV001
Genome and proteome of *Desulfobacula toluolica* Tol2, a sulfate-reducing aromatic compound degrader

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Sulfate-reducing bacteria (SRB) are keyplayers in the marine carbon cycle, in particular members of the *Desulfobacteriaceae*. These metabolically versatile SRB are abundant in marine sediments, where they are involved in complete oxidation of organic compounds. *Desulfobacula toluolica* Tol2, a representative of this family, is able to anaerobically degrade several aromatic compounds, including oil-derived toluene and *p*-cresol, as well as a multitude of aliphatic alcohols and carboxylic acids. The complete genome sequence of strain Tol2 is the second of an aromatic hydrocarbon-degrading SRB. The 5.2 Mb chromosome belongs to the largest presently known genomes of SRB, encoding 4382 open reading frames. The metabolic

versatility of strain Tol2 is reflected by the large number of genes related to catabolic functions, e.g. >100 genes were assigned to aromatic compound degradation pathways. Complete oxidation of the organic substrates is achieved via the Wood-Ljungdahl pathway. The large number (>400) of genes related to signal transduction suggests a fine tuned regulatory response of strain Tol2 to changing environmental conditions. Considering the large number of mobile genetic elements (>160 transposase related genes), a high degree of genome plasticity has to be assumed like previously observed for its close relative *Desulfobacterium autotrophicum* HRM2. The genome based functional assignment was supported by comprehensive differential proteomic analysis, allowing for a corroborated reconstruction of the catabolic network of strain Tol2. While toluene degradation involves *bss* and *bbs* gene products, analogous to the pathway of denitrifiers, benzoate degradation involves *bam* gene products as described for *Geobacter metallireducens* GS-15, although not all components of the strain GS-15 *bam* gene clusters are present in the chromosome of strain Tol2. Genome inspired, phenylalanine was identified as so far unknown growth substrate of strain Tol2. Based on the proteomic data, a degradation pathway differing from denitrifiers can be proposed. Overall, the formation of pathway related proteins is rather specific, demonstrating the regulatory adaptability of strain Tol2.

FGV002
Genome mining of anti-inflammatory *B. bifidum* S17 reveals multiple loci potentially involved in host-microbe interactions

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Bifidobacteria represent an important group of intestinal bacteria and some members are reported to suppress inflammation *in vitro* and in animal models of chronic intestinal inflammation. This makes them interesting alternatives for the treatment of intestinal inflammatory disorders.
B. bifidum S17 was shown to strongly adhere to intestinal epithelial cells (IECs) and to display potent anti-inflammatory activity both *in vitro* and *in vivo*. We thus sequenced and annotated the genome of this interesting probiotic candidate strain (accession number CP002220). The complete genomic information of the *B. bifidum* S17 is contained on a single circular chromosome of 2,186,882 bp with an average GC content of 62%. A total of 1,782 protein coding genes, 53 tRNA genes for all amino acids, and three *rrn* operons were identified. To 67% of the genes a function could be assigned based on similarities of the deduced amino acid sequence with proteins of known function. Approximately 10% of all genes are devoted to the carbohydrate metabolism which allow *B. bifidum* S17 to metabolize a wide range of substrates including human milk oligosaccharides, host derived mucins and different polyols (e.g. sorbitol). Mobilome analysis revealed the presence of a CRISPR system, which shares high similarity to the CRISPR genes found in several Lactobacillus species and is thus presumably horizontally acquired.
S17 is able to strongly adhere to intestine epithelial cells. In line with this observation 3 clusters of cell-wall associated proteins with their corresponding sortases were found. Moreover, 25 proteins with domains involved in adhesion to extracellular matrix and host-derived glycans were identified.
The striking feature of *B. bifidum* S17 is its ability to significantly antagonize intestinal inflammation *in vivo* suggesting a powerful immunomodulatory capacities of this strain. Possible candidates contributing to this effect were found in the genome of B. bifidum S17. These include a myosin cross-reactive protein, lactocepin, as well as several potentially glycosilated serin-rich proteins. Moreover two proteins with domains involved in inhibition of macrophage migration and activation were identified indicating a possible cross-talk of *B. bifidum* S17 with the host immune system via these proteins.

FGP001
Complementation Studies to Identify Novel Thiol-Disulfide Oxidoreductases

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Thiol-disulfide oxidoreductases play an important role in different cellular processes such as redox signaling and protein folding. We are interested in the identification and characterization of novel thiol-disulfide oxidoreductases from metagenomic datasets. We plan to concentrate on sequence data which was obtained in the Global Ocean Sampling project, the largest metagenomic project to date. To find new thiol-disulfide oxidoreductases, we plan to use the power of *Escherichia coli* genetics. *E. coli* hast wo distinct cellular compartments, the cytoplasm and the periplasm. In theses compartments reside thiol-disulfide oxidoreductases with specific and opposite functions. Within the periplasm, the oxidase DsbA is responsible fort he oxidation of protein thiols, while the reductases TrxB and Gor keep protein thiols reduced in the cytoplasm. We will exploit the fact that the phenotypes of null-mutants in genes encoding those proteins can be complemented by oxidases and reductases, respectively. We successfully constructed two complementation plasmids, one with an OmpA signal sequence for periplasmic destination of the protein of interest (pPC) and one for the cytoplasmic destination without any leader sequence (pCC). Phenotypic experiments and protein expression tests revealed correct functionality of both vector systems. In the future both contructs can be used to characterize metagenome derived potential oxidoreductases.

FGP002
Efficient, global scale quantification of absolute protein amounts by integration of targeted mass spectrometry and 2-D gel-based proteomics

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Systems biology moved more and more in the focus of the life science research. For mathematical modeling and simulation of biological processes knowledge on absolute protein concentrations is mandatory. A new approach for the absolute quantification of proteins at a global scale has been developed and its applicability demonstrated using glucose starvation of the Gram-positive model bacterium *Bacillus subtilis* and the pathogen *Staphylococcus aureus* as proof of principle examples. For this purpose a subset of proteins was initially absolutely quantified by employing a targeted mass spectrometric method and isotopically labeled internal standard peptides. Known concentrations of these anchor proteins were then used to calibrate a 2-D gel allowing a calculation of the absolute amount of all detectable proteins in the 2-D gel. With this technique we were able to absolutely quantify more than 400 cytosolic proteins in a pH-range from 4-7 providing protein concentrations of central metabolic enzymes. This new strategy is fast, cost-effective and applicable to any cell type, and thus of value for a broad community of labs with experience in 2-D gel based proteomics and interest in quantitative approaches.

FGP003
Characterization of the response of *Staphylococcus aureus* to the host cell environment: Enrichment and analysis of secreted *S. aureus* proteins by isolation of phagosomes

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S. aureus is a pathogen that causes a broad range of human diseases [1]. Its virulence is predominantly caused by secretion of various virulence factors like superantigens, hemolytic toxins, adhesins and enzymes which are all known to interfere with host cell signaling or survival. Although *S. aureus* has been widely recognized as an extracellular pathogen there is growing evidence that *S. aureus* can also invade into and persist in non-professional phagocytic cells [2]. However, the study of adaptation of *S. aureus* upon internalization by proteomic approaches is severely compromised by the very low number of bacteria recoverable from host cells. Recently, we introduced a newly developed workflow that combines a pulse-chase SILAC approach [3], GFP supported enrichment of bacterial proteins by fluorescence activated cell sorting (FACS) and gel-free mass spectrometry analysis (MS) for monitoring of the proteome of *S. aureus* RN1HG internalized by human epithelial cells [4]. Using this workflow we identified

about 600 *S. aureus* proteins from $3x10^6$ to $6x10^6$ bacteria. However, secreted proteins which are of particular interest in the interplay between *S. aureus* and its host were not covered by this approach. In order to study the role of secreted bacterial proteins after internalization by human epithelial cells, we adapted our established workflow to allow detection of secreted staphylococcal proteins. Since *S. aureus* was shown to be located in phagosomes [5], we isolated these phagosomes by density gradient centrifugation and analyzed the proteome of the internalized bacterial pathogen at three time points after internalization. Beside the identification and quantitation of more than 500 intracellular proteins with this approach, about 25 secreted virulence factors were monitored which could not be captured by earlier workflows.
Moreover, selected proteins were also quantified with the aid of synthetic heavy-isotope labeled peptides or proteins, which were added as external standards to each sample.
Thus, high precision MS approaches combined with phagosome enrichment techniques provide new insights into the virulence factors repertoire of internalized *S. aureus* and thus its interaction with its host.

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FGP004
Biosynthesis of the siderophore rhodochelin requires the coordinated expression of three independent gene clusters in *Rhodococcus jostii* RHA1

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The biosynthesis and the secretion of siderophores is one of the the main iron-mobilizing strategies used by microorganisms to cope with iron-limiting conditions [1]. Here, we report the isolation, the structural characterization and the genetic analysis of the biosynthetic origin of rhodochelin, a unique mixed-type catecholate-hydroxamate siderophore isolated from *Rhodococcus jostii* RHA1, which is assembled through an NRPS-dependent pathway [2]. Rhodochelin structural elucidation was accomplished via MS^n- and NMR-analysis and revealed the tetrapeptide to contain an unusual ester bond between an L-δ-N-formyl-δ-N-hydroxyornithine moiety and the sidechain of a threonine residue. Gene deletions within three putative biosynthetic gene clusters abolish rhodochelin production, proving that the ORFs responsible for rhodochelin biosynthesis are located in different chromosomal loci. These results give detailed insights into natural product biosynthesis and represent the first example of NRPS crosstalk involving three separate genomic regions.

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FGP005
In vivo mobilization of fosmid (meta)genomic libraries

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Currently, genomic libraries are most often produced in *E. coli* and they lack appropriate mobilizing and selection elements which would allow their transfer to other host organisms. The Cre/*loxP* system has been reported to be very effective for site-specific insertion of controlling elements into large-insert genomic libraries from artificial chromosomes (BACs/PACs) for eukaryotic cells [1, 2]. To our knowledge, there are no Cre/*loxP* -based fosmid modification systems established for bacterial hosts other than *E. coli*. Here, we report the Cre/*loxP* mediated modification of (meta)genomic fosmid libraries by *in vivo* recombination and their mobilization into the extremely thermophilic bacterium *Thermus thermophilus* HB27.
In summary, *E.coli* EPI300 clones carrying pCC1FOS (meta)genomic fosmid libraries from *Spirochaeta thermophila* were successfully modified *in vivo* by co-transforming a Cre recombinase expression vector and the corresponding suicide plasmid. The integration into the fosmid was specific to the single *loxP* site, hence false recombination, deletion or undesired modifications were not observed. The pCC1FOS vectors were transferred into *T. thermophilus* HB27 via natural competence, resulting in chromosomal integrants via homologous recombination that were stably maintained with antibiotic selection at 60°C. Furthermore, we currently evaluate the use of the Cre/*loxP* system for the mobilization of genomic libraries in *Bacillus* as a gram positive model host.
We speculate that large-insert DNA fragments with an existing *loxP* site can be mobilized in a variety of bacterial host organisms. With this specific and efficient *in vivo* recombination system, additional cloning procedures can be omitted and no further modifications of the DNA libraries are needed. In future, we apply the Cre/*loxP* system in order to screen and identify novel genes from (meta)genomes in *Thermus thermophilus* as an alternative expression host.

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FGP006
Functional analysis of the *Synechocystis* sp. PCC 6803 *ycf34* gene product, an ortholog of a conserved chloroplast open reading frame

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Cyanobacteria are the ancestors of the chloroplasts due to an endosymbiotic event that occurred 2 billions years ago. For nearly all proteins that are encoded by chloroplast genomes orthologs exist in cyanobacteria. Plant and algal chloroplast genomes still mainly contain genes involved in photosynthesis and housekeeping of the organelle. The remaining genes include open reading frames of unknown function and have been designated *ycf* for hypothetical chloroplast open reading frame. No final conclusion can be illustrated about these *ycfs* without functional analysis of the resulting gene products. Ycf34 (locus *ssr1425*) is a hypothetical open reading frame that is conserved in all cyanobacterial lines and in the chloroplast genomes of *Cyanophora paradoxa*, red algae and some brown algae harboring red algal derived plastids . No orthologs of this gene are found in the nuclear genome of higher plants. We report here on the phenotypical and functional analysis of the cyanobacterial *ycf*34 gene product using a mutant of *Synechocystis* sp. PCC 6803 lacking the gene product. We show that Ycf34 is a new small protein tightly bound to the thylakoid membrane. It is possibly involved in the adaptation of the cyanobacterial light harvesting antenna, the phycobilisomes, to different light conditions. The mutant has a significantly reduced level of phycocyanin as revealed by 77K fluorescence spectroscopy under light conditions, which require changes in the composition of the phycobilisomes. The wild-type phenotype was restored by expression of an epitope-tagged Ycf34 fusion protein. Genetically engineered strains of *Synechocystis* expressing the FLAG-tagged Ycf34 fusion protein were used for the localisation of Ycf34. A GST fusion of Ycf34 was used for heterologous expression in *E.coli* and the purified Ycf34 protein was used for different activity assays which will be shown.

FGP007
Directed and undirected mutagenesis in *Bacillus licheniformis*

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Bacillus licheniformis is an organism of great scientific and biotechnological potential. For further improvement of this strain we established and developed methods for markerless deletions and insertions in *B. licheniformis*. Especially for the introduction of markerless insertions in the genome, DNA transfer is a central problem due to the larger vector size.

Therefore we developed a conjugative vector system for markerless deletions and insertions. These vectors were exemplarily used for the deletion of genes involved in C2 metabolism and methylcitrate cycle, as well as for the deletion of genomic regions. Next to genome reduction the vector system can be used for markerless insertion of target genes and gene clusters in defined chromosomal loci.
For further metabolic studies and definition of a core genome of *B. licheniformis* we are working on the establishment of an undirected transposon mutagenesis method for this organism. We use the *mariner* transposon Himar1 that integrates at TA sites of the chromosome and is a good candidate for random integration.

FGP008
A novel family of carbohydrate-binding modules revealed by the genome sequence of *Spirochaeta thermophila* DSM 6192

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Spirochaeta thermophila is a thermophilic, free-living and cellulolytic anaerobe [1]. The genome sequence data for this organism has revealed a high density of genes encoding enzymes from more than 30 glycoside hydrolase families and a non-cellulosomal enzyme system for (hemi)cellulose degradation [2]. A novel GH-associated module of unknown function was detected in the genome of *S. thermophila* DSM 6192. In this organism, the module was found as a highly conserved C-terminal part in seven different glycoside hydrolase ORFs. Very few sequences with detectable homology to module X could be found in the publicly available databases. The module was present (in the same context) in the draft genome of the other sequenced *S. thermophila* strain, DSM 6578, but was not found in the genomes of the other *Spirochaeta* species for which draft genome sequences are available, e. g. *S. africana*, *S. caldaria*, *S. coccoides* and *S. smaragdinae* (http://img.jgi.doe.gov/m). Significant similarity to module X was detected in the draft genomes of *Cytophaga fermentans* DSM 9555 and of the clostridial species *Mahella australiensis* DSM 15567, both phylogenetically unrelated to *S. thermophila* and non-cellulolytic, but inhabiting similar environments.
In order to be able to get information about the modules' functions, we expressed and purified one isolated recombinant module X. Binding experiments showed that it represents a novel carbohydrate-binding module which binds to microcrystalline cellulose and is highly specific for this substrate. The novel CBM does not show any detectable amino acid sequence similarity to known modules. It is therefore proposed to represent a new CBM family.

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FGP009
Comparative analysis of oxidative stress damage on the proteome level in *Corynebacterium glutamicum*

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Since oxygen accumulated in the atmosphere and is utilized in cellular processes, e.g. in the respiratory chain, microorganisms are exposed to oxidative stress. The continuous formation of reactive oxygen species (ROS) including hydrogen peroxide (H_2O_2), superoxide (O_2^-) or the hydroxyl radical (HO^-) leads to damage in different macromolecules like DNA, lipids and particularly proteins. The response towards oxidative stress was investigated in several bacteria including *E. coli* or *B. subtilis* mainly at the transcriptome level, revealing different regulatory networks [1, 2] but global proteome studies focusing on particular modifications are lacking. Interestingly, even less is known about the effect of ROS on the Gram-positive biotechnological amino acid producer *C. glutamicum*, although the oxygen distribution in fermentation processes is a critical parameter effecting cellular physiology.
To address potential H_2O_2 dependent protein modifications, a mutant lacking catalase - no longer able to decompose H_2O_2 - was employed as a tool, with wild type serving as control. By *in vitro* studies of posttranslational modifications (PTM) using Oxyblot™ as well as LC-MS/MS a variety of different oxidative modifications, an increased number of PTMs as well as, interestingly, a considerable difference between the cytoplasmic and membrane fraction were found. Subsequently, *in vivo* studies using controlled bioreactors confirmed that high levels of modification occur depending on an oxygen excess in wild type cells as well. Mostly, an increase in methionine, proline, leucine and histidine oxidations as well as in kynurenines, targeting enzymes of the central metabolism as well as the oxidative stress response was indicated by proteome analyses. We are developing a toolbox to quantify these modifications in a shotgun proteomic approach, applying stable isotope labelling, high accuracy mass spectrometry and the rich internet application QuPE [3]. This will be used to unravel oxidative damage under different growth conditions and its strain dependency. Ultimately, we want to apply the knowledge about ROS targets and the particular oxidative modifications to improve the stress resistance of *C. glutamicum*.

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[3] Albaum, S.P et al (2009): Bioinformatics. 25 (23): 3128-3134.

FGP010
Genomic Potential and Virulence Mechanisms of the Honey Bee Larva Killer *Paenibacillus larvae*

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Honey bees are among the most important livestock due to their role in pollination of many crops, fruit, and wild flowers [4]. Nowadays, 90% of commercial pollination is performed by managed honey bees. The demand for this service is growing faster than the global stock of domesticated bees [1, 2], which might lead to an imbalance of supply and demand in the near future. Therefore, honey bee health is of crucial importance not only for apiculture but also for agriculture.
Paenibacillus larvae, a Gram-positive bacterial honey bee pathogen, causes American Foulbrood (AFB), which is the most serious infectious disease of honey bees. Outbreaks of American Foulbrood in Europe are caused by two different *P. larvae* genotypes, ERIC I and ERIC II, which differ in virulence [3]. The complete genome size of *P. larvae* strain 04-309 (ERIC II) is 4,046,334 bp and consists of 4,057 predicted and manually corrected protein-coding genes. Strikingly, it encodes a large number of virulence-associated proteins (toxins, hemolysins, proteases) and contains a wide array of large multimodular enzymes producing nonribosomal peptides or polyketides (NRPS, PKS). These proteins are likely to play a key role in virulence of *P. larvae*. The draft genome sequence of *P. larvae* strain 08-100 (ERIC I) comprises 4,5 Mbp and consists of more than 4,800 putative protein-encoding genes. Comparative genomics of these two *P. larvae* pathotypes revealed the acquisition of virulence factors by horizontal gene transfer and provided new insights into the evolution and pathogenicity. Moreover, newly identified putative insecticidal proteins may be effective alternatives for the biological control of AFB disease worldwide.

[1] Aizen, M. et al (2008): Long-term global trends in crop yield and production reveal no current pollination shortage but increasing pollinator dependency. Curr. Biol. 18, 1572-1575.
[2] Aizen, M.A. and L.D. Harder (2009): The global stock of domesticated honey bees is growing slower than agricultural demand for pollination. Curr. Biol. 19, 915-918.
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FGP011
Functional genome analysis of *Geobacillus sp*. HH01, an organism that secrets a thermostable lipase

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The genus *Geobacillus* comprises thermophilic bacteria. As members of the *Bacillaceae Geobacilli* are Gram-positive, endospore-forming rods that live facultative aerob. *Geobacillus spp.* have been isolated from oilfields as well as from geothermal volcanic environments or hay compost and diverse other habitats. Strains of the genus have been found to utilize a broad range of (polymeric) carbon sources i.e. polysaccharides, proteins and *n*-alkanes. Some strains of *Geobacilli* secret proteases and lipases to degrade their polymeric substrate extracellularly and are therefore of high interest for industrial applications.
Here we present a functional genome analysis of *Geobacillus sp.* HH01 isolated from soil. The genome size and the GC content are approximately 3.5 Mb and 52%, respectively. The initial assembly resulted in 182 contigs with an average coverage of 13. Currently different PCR-based techniques are employed to close the remaining gaps and to resolve misassembled regions. Gene prediction, annotation and genome comparison are performed as described in Liesegang *et al*.
The focus of the analysis is on putative industrial interesting features. The genome will be examined for secretion systems, genetic accessibility, secondary metabolism (PKS/NRPS cluster), and especially on exoenzymes such as lipases, proteases and amylases.

[1] Liesegang, H. et al: Complete genome sequence of Methanothermobacter marburgensis, a methanoarchaeon model organism. J Bacteriol 192: 5850-5851.

FGP012
Functional genome analysis of the purine-utilizing bacterium *Clostridium acidiurici*

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Clostridium acidiurici is a purine-utilizing organism. It is able to grow with uric acid and xanthine as sole carbon, nitrogen and energy source. The major fermentation products from these substrates are ammonia, carbon dioxide and acetic acid. *C. acidiurici* is unable to degrade complex nitrogen-containing substrates such as tryptone or yeast extract [1].
Raw sequencing of the *C. acidiurici* genome was done by the Goettingen Genomics Laboratory employing the 454 GS FLX XLR Titanium pyrosequencing technology. Sequences were assembled into contigs using the Newbler assembly tool from Roche. To close remaining gaps and to identify misassembled regions caused by repetitive sequences, different PCR-based techniques are currently employed. The estimated genome size and the GC content are 3 Mb and 29.74%, respectively.
To elucidate the genome features and the unique metabolism of *C. acidiurici* annotation and genome comparisons are performed.
Automatic annotation indicated the existence of common pathways like glycolysis/gluconeogenesis and their specific enzymes. However, *C. acidiurici* did not show any growth on other substrates than purines, including C5- and C6-sugars or amino acids. Further manual annotations revealed an incomplete phosphotransferase system, which might be the reason for the organism's inability to use sugars as substrates.
Further growth tests shall reveal the stress response on salts, heavy metals and antibiotics, which were predicted by automatic and manual annotation.

[1] Vogels, G. D. and C. van der Drift (1976): Degradation of Purines and Pyrimidines by Microorganisms. Bacteriol. Rev. 40(2): 403-468.

FGP013
Proteomic and transcriptomic elucidation of mutant *Ralstonia eutropha* G+1 with regard to glucose utilization

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Taking advantage of the available genome sequence of *R. eutropha* H16, glucose uptake in the UV generated glucose-utilizing mutant *R. eutropha* G+1 was investigated by transcriptomic and proteomic analyses. Data revealed clear evidence that glucose is unspecifically transported by a not strictly specific N-acetyl glucosamine phosphotransferase system (PTS)-type transport system, which is overexpressed probably due to a derepression of the encoding *nag* operon by an identified insertion mutation in gene H16_A0310 (*nagR*) in this mutant. Phosphorylation of glucose is subsequently mediated by NagF (cytosolic PTS component EIIA-HPr-EI) or GlK (glucokinase), respectively. The inability of the defined deletion mutant *R. eutropha* G+1 Δ*nagFEC* to utilize glucose strongly confirms this finding. In addition, secondary effects of glucose, which is now intracellulary available as carbon source, on the metabolism of the mutant cells in the stationary growth phase occurred: Intracellular glucose degradation is stimulated by stronger expression of enzymes involved in the 2-keto-3-deoxygluconate 6-phosphate (KDPG) pathway and subsequent reactions yielding pyruvate. The intermediate phosphoenolpyruvate (PEP) in turn supports further glucose uptake by the Nag-PTS. Pyruvate is then decarboxylated by the pyruvate dehydrogenase multienzyme complex to acetyl CoA, which is directed to poly(3-hydroxybutyrate), PHB, which is synthesized in greater extent as indicated by the upregulation of various enzymes of PHB metabolism. The larger amounts of NADPH required for PHB synthesis are delivered by significantly increased quantities of proton-translocating NAD(P) transhydrogenases. This current study successfully combined transcriptomic and proteomic investigations to unravel the phenotype of this hitherto undefined glucose-utilizing mutant.

FGP014
Genome-analysis of *Clostridium saccharoperbutylacetonicum*

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Clostridium saccharoperbutylacetonicum strain N1-4, is known as a butanol-hyperproducing bacterium. Various organic compounds are fermented, such as glucose, fructose, saccharose, xylose and cellobiose, but also sorbitol, dulcitol and inositol. The industrial strains of *C. saccharoperbutylacetonicum* are used in the fermentation processes for the production of the solvents acetone, butanol, and ethanol from a variety of sugar- and starch-based substrates.
The economics of butanol production is primarily affected by raw materials used, yields and concentrations of solvents as well as productivity. One of the most important economic factors in solvent fermentation is the cost of substrate. Thus, the availability of an inexpensive raw material is essential if solvent fermentation is to become economically viable.
C. saccharoperbutylacetonicum N1-4 is a hyperamylolytic strain and capable of producing solvents efficiently from cassava starch and cassava chips which represents an alternative cheap carbon source for fermentation processes.
To extend our knowledge on the biochemistry and physiology of this interesting organism, we completely sequenced the genome of *C. saccharoperbutylacetonicum* N1-4. The strain has two replicons, a chromosome with the size of 6.5 Mb and a megaplasmid of 135 kb; the G+C content of the DNA is 29.53 mol%. Some features of this organism apparent from the genome sequence will be reported.

FGP015
Comparative genomics and transcriptomics of *Propionibacterium acnes*

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The anaerobic Gram-positive bacterium *Propionibacterium acnes* is a human skin commensal, but is occasionally associated with inflammatory diseases. Recent work has indicated that evolutionary distinct lineages of *P. acnes* play etiologic roles in disease while others are associated with health. To shed light on the molecular basis for differential strain properties, we carried out genomic and transcriptomic analysis of distinct *P. acnes* strains. We sequenced the genome of the *P. acnes* strain 266, a type I-1a sequence type (ST) 18 strain. Comparative genome analysis of strain 266 and four other *P. acnes* strains revealed that overall genome plasticity is relatively low; however, a number of island-like genomic regions, encoding a variety of putative virulence-associated and fitness traits, differ between phylotypes. Comparative transcriptome analysis revealed that 225 genes of strain KPA171202 (type I-2, ST34) were differentially transcribed in strain 266 during exponential growth. 47% of these genes belong to the strain-specific gene content of strain KPA171202, indicating that strain-specific functions are utilized. Next, we studied differential expression during exponential and stationary growth phases. Genes encoding components of the energy-conserving respiratory chain as well as secreted and virulence-associated factors were transcribed during the exponential phase, while the stationary growth phase was characterized by up-regulation of genes involved in the stress response and amino acid metabolism. Taken together, our data highlight the genomic basis for strain diversity and identify, for the first time, the transcribed part of the genome, underling the important role active growth plays in the inflammatory activity of *P. acnes*. We argue that the disease-causing potential of different *P. acnes* strains is not only determined by variable genome content but also, and to a greater degree, by variable transcriptomes.

FGP016
Deletion analysis reveals essential genes within the genomic magnetosome island of *Magnetospirillum gryphiswaldense*

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The magnetotactic bacterium *M. gryphiswaldense* synthesizes intracellular membrane-enclosed crystals, which consist of the ferrimagnetic mineral magnetite (Fe_3O_4) referred to as magnetosomes. The biomineralization of magnetosomes is controlled by a specific set of genes, which are located within the conserved magnetosome island (MAI). Beside the *mam* and *mms* genes, encoding magnetosome proteins, the 130-kb region contains in addition numerous genes for transposases, pseudogenes and hypothetical genes of unknown functions. In order to reveal putative functions in magnetosome formation, deletion of the *mms6*-, *mamGFDC*-, and *mamXY* operons lead to severe defects in morphology, size and chain assembly of magnetite crystals. However, even multiple deletions including various combinations of the *mamXY*- and *mamGFDC* operons did not entirely abolish biomineralization, although only tiny and irregular crystallites were formed. In contrast, deletion of the 16 kb *mamAB* operon resulted in the complete loss of magnetosomes. This suggests that while several regions within the MAI are irrelevant for magnetosome formation, other have accessory functions, and only the *mamAB* operon harbors genes that are absolutely essential for magnetosome formation. In conclusion, our approach will help determining the minimal gene set required for magnetosome synthesis and is promising for future „synthetic biology" approaches.

FGP017
Functional Networks of Light Controlled Processes: Identification of Regulatory Factors

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In previous research, many responses to external and internal stimuli have been identified to regulate gene expression in the industrial penicillin producer *Penicillium chrysogenum*. Light for instance acts as a major carrier of information, but in case of *P. chrysogenum* there is little known about the effect of illumination on regulatory networks. It has been shown, that light has an effect on morphology and secondary metabolite production, although only few regulators have been found so far on the molecular level. To identify light induced regulatory responses, and the proteins involved, we used microarray-analysis as an experimental approach. The expression levels of cultures grown in constant (white) light were compared to those of cultures grown in darkness for the same time period, thus we were able to identify genes differently regulated due to illumination. We first looked at the intersection between genes newly found in this approach and sets of genes from previous microarray experiments to reduce the number of candidate genes for further analysis. In these experiments expression levels were compared using wild type, and disruption strains with deleted genes encoding core elements of the *velvet* complex [1]. To identify light induced regulatory factors we screened candidate genes for putative transcription factors. Subsequently we have generated deletion strains using the FLP/*FRT* recombination system [2] for further characterisation of selected putative transcription factors.

[1] Hoff, B. et al (2010): Two components of a *velvet*-like complex control hyphal morphogenesis, conidiophore development, and penicillin biosynthesis in *Penicillium chrysogenum*. Eukaryot Cell 9: 1236-50
[2] Kopke, K. et al (2010): Application of the *Saccharomyces cerevisiae* FLP/*FRT* recombination system in filamentous fungi for marker recycling and construction of knockout strains devoid of heterologous genes. Appl Environ Microbiol 76: 4664-74

FGP018
PcchiBI is a target gene of PcVelA in producer strains of *P. chrysogenum*

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Fungal cell walls are highly dynamic structures with a wide range of essential roles in fungal development and interaction with the environment. One main component of fungal cell walls is chitin, a β-(1,4)-linked homopolymer of *N*-acetyl-D-glucosamine (GlcNAc) subunits. To maintain the plasticity of the cell wall, fungi possess a multiplicity of cell wall modifying enzymes, for example hydrolases involved in the degradation of cell wall components. Chitinases (EC 3.2.1.14) hydrolyze chitin randomly at internal sites to generate low molecular mass chitooligomers and can be found in a wide range of organisms. In fungi, only chitinases of glycosyl hydrolase family 18 (GH18) with morphogenetic, autolytic and nutritional roles are described. According to the CAZy-database, 8 ORFs encoding putative chitinases can be found in the genome of *Penicillium chrysogenum*. Recently we have reported data from microarray analysis showing that genes involved in chitin catabolism are strongly downregulated in a ΔPcVelA mutant of *P. chrysogenum* lacking the global regulator protein PcVelA, a homologue of the VeA protein from *Aspergillus nidulans*. In order to analyze the biological function of a target gene of PcVelA encoding a putative class V chitinase, a disruption strain was generated. The sum of our analysis indicates functional similarities and differences of this chitinase in comparison to homologous proteins from different *Aspergillus* species, illustrating the plasticity of class V chitinases in filamentous fungi.

FMV001
Influence of osmotic and pH stress on the alternariol biosynthesis in *Alternaria alternata*

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Mycotoxin producing *Alternaria* moulds are ubiquitously present and commonly found on fruits, vegetables and grains. Some *Alternaria* species have teratogenic, mutagenic or cytotoxic potential due to their production of mycotoxins such like alternariol, alternariol methyl ether and tenuazonic acid. To date, only limited knowledge is available about the regulation of the synthesis of *Alternaria* toxins, especially under food relevant conditions. In foods fungi are exposed to osmotic stress due to a high concentration of different solutes, and especially in fruits and vegetables fungi encounter acid environments. PacC is a key element in pH gene regulation. At alkaline pH-values, activation of PacC leads to suppression of acidity-expressed genes and at acid pH-values, alkaline-expressed genes are suppressed by inactive PacC. In the current analysis it could be demonstrated that the alternariol biosynthesis is consistent during a wide pH range, whereas osmotic components like NaCl show a deep impact on the alternariol biosynthesis resulting in complete inhibition already at low concentrations. High osmolarity in the environment is usually transmitted to the transcriptional level of downstream regulated genes by the HOG signal cascade, which is a MAP kinase transduction pathway. The inhibition of alternariol biosynthesis by changes in the osmolarity of the substrate might be regulated by this high osmolarity cascade. It therefore became essential to analyse the HOG1 signal transduction pathway of *A. alternata* in more detail. Expression of the MAP kinase genes *hog*1 and *pbs*2 and phosphorylation of the HOG1 protein were analyzed. A clear correlation between HOG1 phosphorylation and alternariol biosynthesis could be established. In addition, knock-down of *hog*1 by protoplast transformation lead to an non-toxic phenotype with reduced HOG1 phosphorylation. However, a combined alkaline and osmotic stress situation induced the alternariol biosynthesis of the transformant, which leads to the conclusion that *hog*1 is not involved into alternariol biosynthesis under alkaline conditions, but *pacC*. This assumption could be confirmed by a *pacC*-knock-down transformant being incapable of alternariol biosynthesis exclusively at alkaline conditions.

FMV002
Differential proteomic expression of enterohaemorrhagic *E. coli* O157:H7 EDL933 grown in intestinal simulating media

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Enterohaemorrhagic *E. coli* (EHEC) are serious causative agents of food-borne infections and can cause a broad range of intestinal and extraintestinal diseases. The survival of EHEC in the food chain and in the gut of the human host may be linked to stress resistance and is dependent of the nutrients available. In different environment EHEC may adapt their proteomic appearance, this leads to a shift in metabolism and maybe increased virulence. However, these mechanisms are largely undescribed.
To test the metabolic properties of EHEC O157:H7 EDL933 grown in the intestine, we used the simulating ileal environment medium (SIEM) and the simulating colon environment medium (SCEM) and compared the growth in these media with that in the rich medium tryptic soy broth (TSB). By the use of 2D-Gelelectrophoresis we determined differentially expressed cytosolic proteins with the least amount of 2 fold higher expression. After growth in different media the proteins were evaluated by Delta2D-Analysing Software and identified via MALDI-TOF-Analysis. Beside proteins involved in metabolic pathways, we found overexpressed flagellin and the autoinducer-2 synthase LuxS, which mediates cell-cell communication during quorum sensing, as well as many stressinduced proteins like chaperons and a glutamate decarboxylase enzyme. Further we could find the global regulator Hns, responsible for stress adaption and regulation LEE-proteins. These first results points to increased pathogenicity after growth in different media exhibit different nutrient supply and interfering substances like bile salt and enzymes.
To investigate the carbonflux in *E. coli* EDL933 we established cultures in SIEM, SCEM and TSB containing ubiquitous $^{13}C_6$-Glucose. In cooperation with the working group of Dr. Eisenreich we could show different incorporation rates of labelled carbohydrates respecting the synthesised aminoacids. Because of the acquired label at very high rates especially alanine and serine seems to be important for growth under aerobic conditions in intestinal simulating media. The results of these experiments have shown that growth of EHEC O157:H7 EDL933 under certain environmental conditions favors expression of virulence-associated proteins. Further investigations are necessary to describe those metabolic pathways in more detail.

FMV003
Characterisation of the incorporation of *Listeria monocytogenes* in a raw milk-biofilm

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A successive establishment of biofilms derived from the microbial community of raw milk is found on devices of milk production without sufficient cleaning. Such microbial communities establish biofilm matrices that enable incorporation of pathogens like *Listeria monocytogenes* and as a consequence a continuous contamination of food processing plants. *L. monocytogenes* was frequently found in raw milk and non-pasteurized raw milk products and as part of a biofilm community in milk meters and bulk milk tanks. *Listeria*-contaminated products are known to cause listeriosis, a severe infection with high mortality for persons at risk, such as pregnant women, elderly or children.
The aim of this project was to identify at which temporal stage of biofilm formation members of *L. monocytogenes* settle best and if there was an interaction with the microbial community of the raw milk. Quantification of settled *L. monocytogenes* in raw milk-biofilm was carried out by fluorescence in situ hybridization (FISH). Microbial interaction on population level was determined by terminal restriction fragment length polymorphism analysis (T-RFLP) while on polymer / physiological level Fourier-Transform Infrared Spectroscopy (FTIR) was employed. The results obtained from the experiments revealed that an early addition of *L. monocytogenes* to raw milk caused a fast and dense biofilm formation and an enriched attachment of milk compounds. The later *L. monocytogenes* was added to the raw milk the lower were their attached cell numbers. Furthermore *L. monocytogenes* interacted with the raw milk-biofilm community depending on their temporal addition. Particularly in the early stage of raw milk-biofilm formation *L. monocytogenes* was a strong competitor.

FMV004
Survival of *Listeria monocytogenes* in lubricants applied in the food industry

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Listeria monocytogenes is a food-borne pathogen that is quite frequently associated with fish products. Due to its tenacity and psychrotrophic growth it may persist on the food-processing equipment and machinery for long periods. The lubricants applied in these machines have been hypothesized as possible reservoirs and sources of *L. monocytogenes* due to their extended service life. In this project we surveyed the survival of three *L. monocytogenes* strains in four H1-lubricants applied in the food, especially fish, industry. For the determination of the viable counts the native lubricants were first suspended in sterile glycerol, and then dilution series were carried out in physiological sodium chloride solution. The samples were spread-plated on Brain Heart Infusion agar and incubated under aerobic conditions. None of the native lubricants contained *Listeria*. Then the lubricants were contaminated with the *L. monocytogenes* strains and their survival was monitored when the lubricants were stored at 37°C. The viable counts of the *L. monocytogenes* strains decreased within 14 days, and the reduction rates were found to depend on the lubricant as well as on the strain. Interestingly, the isolate from smoked salmon tolerated this environment better than the reference and the type strain. The viable counts of all strains were reduced by 99.995% within seven days after the inoculation. The water content of the lubricant (0, 1 and 5%) had no influence on the results. Thus we conclude that the investigated strains cannot survive in the H1-lubricants.

FMV005

The impact of vacuum foil packaging on the quality characteristics of the surface smear microflora of semi-hard smear cheese

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Due to increasing export markets and advancing consumer behavior, cheeses are increasingly sold as vacuum foil pre-packed cheese wheels or portions found in self-service shelves. In recent years, it emerged that vacuum foil packaging influence on the quality characteristics of smear-ripened semi-hard cheese. Hitherto, neither the cause for this phenomenon could be determined, nor are alternative strategies available that prevent the changes in the quality characteristics of the surface smear. We therefore reassess the hypothesis whether microorganisms of the aerobic surface smear ecosystem may contribute to this phenomen by a change in composition and/or metabolism of the surface smear microbiota, due to the switch to anaerobic conditions caused by vacuum foil packaging.
The identification of volatile compounds accumulated within the vacuum foil packs was performed by gas chromatography, in order to provide an indication on the type of microorganisms that might be responsible for the alterations in the quality characteristics of the surface smear. Furthermore, surface smear samples of semi-hard cheeses were investigated by a combination of culture-dependent and culture-independent molecular techniques in order to analyze the surface smear microbiota prior to and after vacuum foil packaging, both, on a phylogenetic and a metabolic level. In storage experiments with vacuum foil packaged cheeses, the succession of the smear microorganisms was monitored under storage conditions. For this purpose, cell counts for relevant bacterial groups were determined on a variety of selective and non-selective culture media. Moreover, isolated pure cultures of bacteria and fungi were identified by 16S rDNA or ITS-2 rDNA sequencing. TGGE, TRFLP, ITS-2 FLP and qPCR were applied in order to analyze the composition of the cheese surface microflora in terms of bacterial and fungal populations using culture-independent techniques.
Preliminary results revealed only slight differences in the overall composition of the surface microflora of un-packed versus vacuum foil packed smear cheeses. Typical smear bacteria such as *Staphylococcus* spp., *Corynebacterium casei*, *Microbacterium gubbeenense*, *Brevibacterium* sp. and *Arthrobacter casei*, as well as typical fungal isolates like *Candida* spp., *Yarrowia lipolytica* and *Debaryomyces hansenii* were identified in the surface microflora of the cheeses, both prior to and after vacuum foil packaging.

FMV006

Granulation of lactic acid bacteria using the fluidized bed technology

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The application of lactic acid bacteria has an economic significance as probiotic and conserving additives for food industry. Therefore a crucial point in the commercialization of lactic acid bacteria is the development of a storable culture which retains the viability of the primary culture. For preserving bioactive materials freeze and spray drying are established methods.
Freeze drying is a gentle but long term and expensive method which is uneconomical. The benefit of spray drying is the formation of free flowing particles in a short time but it is disadvantageous that the required high temperatures reduce the viability of mesophilic microorganisms. The fluidized bed drying technology is an alternative, gentle and cost saving method for the preservation of microorganisms. The present study analyses the influence of the fluidized bed drying technology on cell viability and storage stability of the model strain *Lactobacillus plantarum*. Different carrier materials were tested and recent studies have shown that the usage of maltodextrin results in the highest viablity of bacteria. Furthermore different protectants such as trehalose and sorbitol were added. Using sorbitol is more suited for the protection of the bacteria cells during fluidized bed drying than trehalose. For shelf life determination the granulated microorganisms are stored at -20 °C, 4 °C and 20 °C. The storage at room temperature without adding a cell protectant resulted in none viable *Lactobacillus plantarum* cells after three months. In contrast adding the protectants trehalose or sorbitol resulted in viable cells. High recovery of viable cells was achieved at 4 °C and -20 °C regardless of using trehalose or sorbitol.
The project is part of the WIGRATEC and was founded by the BMBF (FKZ 03WKBQ04B).

FMV007

Generation of new flavours in wheat doughs supplemented with by-products and fermented with non-*Saccharomyces cerevisiae* yeasts

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Consumer studies show the demand of bread and rolls with good taste and flavour. One way to improve the flavour of wheat based products is the use of fermented doughs. However, during fermentation of doughs the formation of flavour is generally limited by the substrate availability in the wheat flour and the metabolic potential of the baker's yeast *Saccharomyces cerevisiae*. To overcome these limitations, we set up a new approach by combining the addition of new substrates and yeasts to the wheat dough fermentation. By-products of the food industry like apple, carrot or grape pomace were chosen to increase the availability of substrates involved in flavour formation. In addition, non-*S. cerevisiae* yeasts differing in their metabolic potential were applied to obtain new flavours during the dough fermentation. Fermented doughs were prepared by using strains of the species *Kluyveromyces marxianus*, *Torulasproa delbrueckii*, *Wickerhamomyces anomalus* and *Pichia jadinii* as starter culture and wheat flour supplemented with 25% of pomace and subsequently subjected to microbial and sensory analysis. Analysis of the microbiota revealed that the yeast strains were competitive and thus belonged to the dominating yeast biota (10^6 to 10^8 cfu/g) at the end of fermentation. Furthermore, the fermentation of wheat flour supplemented with by-products and the non-*S. cerevisiae* yeasts resulted in doughs with fruity, flowery and sweet flavours. A strain- and species-specific formation of the flavours and a certain influence of the accompanying lactic acid bacteria biota could be observed. The effect of varying by-product concentration, dough yield, pH and the time of fermentation was demonstrated. Baking of white bread by using 20% of the fermented doughs showed that some of flavours could be transferred to the bread. With regard to the biochemical background it is tempting to speculate that the Ehrlich pathway (fruity esters) or the conversation of terpenes to terpenoides (flowery and sweet) play a certain role. However, the metabolic pathways of non-*Saccharomyces* yeasts involved in flavour formation are currently unknown, demonstrating the further research need.

FMV008

A novel enzymatic approach for growth inhibition of undesired wine related microorganisms

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Microorganisms play an important role in winemaking. In addition to the eukaryotic ethanol producing yeasts also bacteria occur in must and wine. The most important representatives are lactic acid bacteria (LAB) and acetic acid bacteria (AAB). Most of these microorganisms are undesired in must and wine because of their metabolic activities which can negatively influence the wine quality. For example some strains of *Pediococcus damnosus* or *Leuconostoc mesenteroides* are able to produce exo-polysaccharides which lead to problems during wine filtration. Further undesired byproducts of the metabolism of LAB are biogenic amines. These compounds can cause health problems such as migraine, hypertension and digestive disorders.
To inhibit the growth of these spoilage organisms most winemakers use sulphur dioxide. Studies have shown that the addition of sulphur dioxide can lead to an inhibition of desired wine yeasts and especially malolactic bacteria.
Since 2001 the usage of lysozyme for microbiological stabilisation of wine is allowed. This enzyme is isolated from hen-egg and therefore not optimally adapted to the milieu of wine with its low pH value and high concentration of ethanol. Also examples of incomplete inhibition caused by the occurrence of resistant strains are described. Sensitive persons can show allergic reaction to lysozyme. Due to the disadvantages of the current agents for

microbiological growth inhibition the research of alternatives are of special interest.
Streptomyces sp. produce a wide range of bacteriolytic enzymes such as muramidases and peptidases. Two of them, a muramidase and a muropeptidase were isolated, purified and characterised. These enzymes showed higher activities than lysozyme under wine making conditions. Also a wider spectrum of organisms was inhibited by the *Streptomyces* enzymes. For the hydrolysis of the exo-polysaccharides of peddiococci a glucanase was isolated from *Delftia tsuruhatensis*. By the use of this glucanase an inhibition of yeast growth could be achieved as well.
These three novel enzymes are discussed as potential tools for monitoring microbial growth during winemaking.

FMP001
Influence of light on food relevant fungi

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Mycotoxins are toxic secondary metabolites of several food relevant filamentous fungi, like *Aspergillus*, *Penicillium*, *Fusarium* and *Alternaria*. The influence of light of varying wavelength on growth and mycotoxin biosynthesis on representatives of this species has been analysed. Generally the Penicillia seem to be more sensitive to light treatment than the other species. Interestingly wavelengths from both sides of the spectrum, e. g. red (long wavelength, 627 nm) and blue (short wavelength 470 - 455 nm) had the strongest inhibitory effects on growth and ochratoxin A biosynthesis. Blue light generally had a stronger effect. Light of moderate wavelength, 590 to 530 nm, (yellow to green) had more positive than negative influences on growth or ochratoxin A biosynthesis compared to the control (dark incubation). How the fungus reacts depends strongly on the light intensity. Depending on the resistance of the species a complete cessation of growth and/or inhibition of mycotoxin biosynthesis could be achieved. Interestingly some kinds of secondary metabolites seem to have some protective effect against light irradiation. The biosynthesis of two mycotoxins of *P. verrucosum*, ochratoxin A and citrinin are mutual regulated when this strain is irradiated with light. Citrinin is produced under light conditions which inhibited ochratoxin A biosynthesis. The same is true for a derivative of ochratoxin, in particular a derivative of ochratoxin β in *A. carbonarius*. *A. carbonarius* produced high amounts of the ochratoxin β derivative under blue light when the production of ochratoxin A was ceased at the most inhibiting conditions used (royal blue light, 455 nm, 1700 Lux). Moreover light has only a growth stalling effect on aerial mycelia but an inactivating effect on spores of light sensitive species. If a non-growing colony under light is shifted to the dark it immediately grows normally. After incubation of spores of *P. verrucosum* for 24 h under blue light up to 97 % of the spores were no longer able to germinate.

FMP002
Viability tests for granulated microorganisms

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This project is part of the research project WIGRATEC which concentrates on fluidized bed technology. Aim of the present study is to develop new methods for testing the viability of granulated microbial starter cultures.
To test the activity and viability of microorganisms currently methods like colony forming units (CFU) and the Live/Dead BacLight Assay (Invitrogen) are used. It would be beneficial to have a fast method for detecting bacterial viability during production and as a quality control tool for granulated starter cultures. Within the framework of this project alternative assays for biomarkers are developed by means of MALDI-TOF-MS (Matrix-assisted laser desorption/ionization time of flight mass spectrometry) and protein analysis (1D- and 2D-electrophoresis). These assays will then be used to test different batches of granulated microorganisms to determine viability. Aim is to develop an assay for viability detection that is able to fulfill the requirements of an assay used in routine process control.
This research is funded by BMBF, Innovative regionale Wachstumskerne (WIGRATEC), FKZ 03WKBQ04C.

FMP003
Detection of *Salmonella spp* contamination in liver and heart raw poultry products in Libyan market

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Salmonella species are major cause of food borne illness in humans worldwide including an estimated 1,400,000 cases of salmonellosis. The egg and poultry meat considered one of the main sources of Salmonella which lead to food borne illness in humans, handling of raw heart and liver of the poultry and consuming of the undercooked poultry meal are could be the main cause of infection.
Libyan people prefer to eat poultry meat rather than another types of meat, poultry liver and heart product is one of the common products in the Libyan market.
One hundred thirty nine samples of poultry liver and heart were collected randomly from north, middle and south regions in Tripoli from Libyan poultry markets and then pre enriched in buffered peptone water (BPW), traditional scientific method to isolate the Salmonella spp, the suspected samples were confirmed by using biochemical tests Were used and the results showed that there were no contamination with salmonella spp but another types of bacteria were isolated.

FMP004
Genus specific light sensitivity in food relevant Penicillia

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Filamentous fungi are known contaminants of several foods like fruits, vegetables, cereals and meats. The biosynthesis of ochratoxin (OTA) and citrinin (CIT), two food relevant mycotoxins produced, beside by some Aspergillus species, by *Penicillium nordicum* (OTA) and *P. verrucosum* (OTA and CIT), is influenced by extrinsic conditions like water activity, temperature, pH or light. The fungi react to external conditions by altered growth or mycotoxin biosynthesis rates. Light, especially blue light treatment differentially affects growth and mycotoxin biosynthesis of various taxonomically related *Penicillium* species. Especially sensitive are the two ochratoxin A producing Penicillia, whereas citrinin producing species are much more resitant. While the production of ochratoxin A is strongly decreased by light, the production of ochratoxin B (a precursor of ochratoxin A) in *P. nordicum* as well as the biosynthesis of citrinin in *P. verrucosum* is strongly increased, suggesting a putative light protective effect of the latter two secondary metabolites. The physiological mechanism behind blue light perception is the well known photoreceptor complex, white collar, first described in *Neurospora crassa.* White collar 1, the blue light receptor which acts as a transcriptional factor permitting a reaction to light, is involved in circadian rhythmicity and modulates either positively or negatively the expression of so called clock controlled genes. Comparative alignments of different white collar homologues identified in toxin producing Penicillia revealed sequence differences which correlate to the phylogenetic relationship of the species and perhaps more interestingly to the sensitivity of the respective *Penicillium* species to light.

FMP005
Development of *Lactobacillus S*tarter Cultures for the Malolactic Fermentation of Wine

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Lactic acid bacteria such as *Oenococcus oeni* and *Lactobacillus plantarum* strains can play an important role in the malolactic fermentation (MLF) in wine. In this study we characterised *L. plantarum* strains of oenological origin for their suitability as starter cultures for the MLF in wine making. Nineteen *L. plantarum*, 2 *L. paracasei* and 2 *L. hilgardii* strains were investigated for their ability to produce bacteriocins and their ability to grow in synthetic wine medium at 20°C (pH 3.5; 12.5% ethanol). Furthermore, the strains were also screened for the production of the biogenic amines. Two *L. plantarum* strains, i.e. B184 and B188, grew in the synthetic wine medium,

showed antimicrobial activity against wine spoilage bacteria and possessed the *pln*EF, *pln*JK and *pln*N bacteriocin genes. These strains did not produce biogenic amines and were thus selected as potential starters. They were tested for malate catabolism in synthetic wine medium. The malate gene expression was measured by quantitative PCR, and the gene was shown to be active in the presence of malate. Both starter strains also quickly degraded malate in the medium, with strain B188 appearing to be faster in it's malate catabolic activity then strain B184. Glucose and fructose consumption was also measured and *L. plantarum* B 188 preferentially metabolised glucose, while *L. plantaru*m B184 metabolised both these sugars at approx. equal concentrations. Malate gene induction studies, done in synthetic wine medium to which malate was added during bacterial growth, clearly showed that in both strains the malate gene was strongly induced. The effect of the starter strains on the population structure of synthetic wine medium inoculated with the either of the starter strains in the presence of spoilage bacteria *Pediococcus pentosaceus*, *Oenococcus oeni* and *L. fermentum* was determined by co-culture experiments and rep-PCR fingerprinting. These studies showed that the starter strains had an inhibitory effect on the presence of the contaminating microorganisms.

FMP006
Characterization of potential adhesion genes in the probiotic strain *Lactobacillus plantarum* BFE 5092

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Two potential adhesion genes of the probiotic strain *Lactobacillus plantarum* BFE 5092, the gene with similarity to an aggregation promoting factor gene *apf*5092 and a mucin binding protein gene *mbp*5092, were investigated in this study at the genetic level. The genes were tested for expression under different conditions, and transcriptional studies on *apf* showed that the expression could be influenced by temperature and pH within 30 minutes. The aggregation behavior of the cells was also changed by the varying conditions and aggregation noticeably increased under conditions prevalent in fermented foods (low pH and low temperature of 37°C) indicating that *apf*5092 does probably not play a role for probiotic activity. To further investigate the role of *apf*5092 in aggregation, it was cloned and expressed in *E. coli.* The transformed strain showed higher coaggregation ability with gram-positive bacteria. Transcription studies on *mub*5092 revealed that it could be induced by mucin when added to the growth medium within30 min. The data suggested that *L. plantarum* BFE 5092 can adapt its adhesion factors to changing environmental conditions. Tthe gene encoding *apf*5092 was identified to play a role in the aggregation of *L. plantarum* BFE 5092.

FMP007
Investigation of biofilm-mediated *Pseudomonas aeruginosa* contamination of a drinking water distribution system, using a combination of cultural and molecular methods

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P. aeruginosa is an opportunistic pathogen which can be involved in sporadic or persistent contamination events in drinking water systems. The basis of the present study was the recurring cultural detection of *P. aeruginosa* in water samples from a German drinking water distribution system during warmer months of the year. In order to track the source of this contamination, both water and biofilms were investigated for the presence of *P. aeruginosa*, using a combination of culture-based and culture-independent molecular methods. First, genotyping of 18 *P. aeruginosa* water isolates from throughout the distribution system and the waterworks was performed, using pulsed-field gel electrophoresis. Independent of sampling site and date, a single clone of *P. aeruginosa* was detected, indicating that a systemic contamination of the water distribution system was highly probable, which seemed to originate from the waterworks. In a second step, 22 biofilm samples from the distribution system and waterworks were analyzed for *P. aeruginosa*. The bacteria were not detected culturally in any of the biofilms. However, by means of culture-independent fluorescence *in situ* hybridization, *P. aeruginosa* was identified in 17 out of the 22 biofilms. In conclusion, *P. aeruginosa* occurred in biofilms of pipes and/or other technical components of the distribution network and the waterworks in a viable state which could not be recognized by routine culture analysis. Thus, the biofilms were a reservoir of *P. aerguinosa* and presented a continuous contamination potential for the water phase. A possible explanation for cultural detection of *P. aeruginosa* in drinking water during certain time periods may be the transition from the non-culturable to culturable state under favorable environmental conditions which have yet to be defined (e.g. warmer water temperatures). In addition, the results showed that complimentary to conventional culture, molecular methods may be helpful in a target-aimed approach for the elucidation of biofilm-medated contamination events in drinking water systems.

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FMP008
Genus and Reliable Species Identification of *Cronobacter* spp. by MALDI-TOF MS

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Genus *Cronobacter* (formerly *Enterobacter sakazakii*) consists of 6 species, *C. sakazakii*, *C. dublinensis*, *C. malonaticus*, *C. muytjensii*, *C. turicensis* and *C.* genomospecies 1. These Gram-negative opportunistic food-borne pathogens have to be fast and reliably identified. Enrichment cultures of food samples are streaked on selective media for their detection. The potential of MALDI-TOF MS for confirmation of potential *Cronobacter* spp. colonies was evaluated.
19 *Cronobacter* sp. strains were investigated, 9 were culture collection strains and 10 were field isolates. All strains were identified as *Cronobacter* spp. using the MALDI Biotyper system. Even the correct species was detected for most of the 19 strains. However, peak pattern of mass spectra of *Cronobacter* spp. were similar so the distance - expressed in log(score) values - to another species was low and two *Cronobacter* species were assigned to another *Cronobacter* species by using the MALDI Biotyper standard algorithm. Therefore, a comparison of raw spectra was evaluated for the potential of a secure identification of *Cronobacter* species. Indeed, at least one specific peak was determined for every species. An identification scheme was designed by using these peaks. All 19 samples were analysed and identified for a second time as blinded sent samples and successfully identified as the correct *Cronobacter* species by means of this identification scheme.
The reliable identification of genus *Cronobacter* is important in food industry and is guaranteed by using the MALDI Biotyper system. A secure species identification of a *Cronobacter* spp. strain can be achieved by applying an adapted identification scheme.

FMP009
Biochemical characteristics of *Listeria monocytogenes* isolated from goat milk in Iran

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Background: Reports of outbreaks due to *Listeria spp*. occurring in both man and animals have been steadily increasing over the past two decades, and listeriosis as a disease entity has emerged as a serious threat to animals and public health.Till now different methods with a variety of microbiological media have been used and proposed. Recently some commercial kits such as Lister API (BioMerieux, Lyon, France) and Micro-ID Listeria (Remel, Durham, USA) have been used for identification of *Listeria spp*. In this research work *Listeria monocytogenes* identified by use of one of this kits.
Methods: About 210 samples of raw milk collected from private goat farms in Shahrekord area (Iran) were processed by use of two enrichment method. Listeria enrichment broth (LEB) and Fraser secondary enrichment broth were used as primary and secondary enrichments. The suspected isolates after purification, Gram,s staining, motility test at 20-25 degree centigrade and CAMP test identified by Micro- ID Listeria kit.

Results: Out of 210 samples of raw goat milk 9 samples showed the presence of *Listeria monocytogenes* which confirmed by Micro- ID Listeria kit. All the isolates of *Listeria monocytogenes* were beta haemolytic and positive for CAMP reaction. All the isolates were negative for phenyl alanine deaminase, ornithine decarboxylase, lysine decarboxylase, malonate utilization and beta galactosidase tests. These were also negative for acid production from arabinose, D-xylose, mannitol, soluble starch and sucrose but acid was produced in rhamnose, salicin, and trehalose. Hydrogen sulfide production was recorded in tripticase soy broth with lead acetate paper strips but negative with triple sugar iron agar. Out of 9 isolates of *L. monocytogenes* only one produced acid from lactose. In serotyping all the isolates were serotype 4b.
Conclusion: The Results showed the presence of Listeria in goat milk which may act as source of infection in man. The use of Micro- ID Listeria kits for isolation of *Listeria monocytogenes* not only decrease the isolation time but also its sensitivity is high. The problem of these kits is that before using them for haemolytic activity of the organism CAMP test should be done.

FMP010
Micro-Raman spectroscopy - A promising technique for identification of milk-extracted pathogens

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In matters of quality assurance and hygienic standards, separation, concentration and detection of small numbers of microbial pathogens directly from milk without culture enrichment bear a big challenge for the food industry. Additionally the current tense political situation induces a heightened risk of bioterroristic attacks in this manufacturing branch. For example, a deliberate attack with *Brucella melitensis*, causing the highly infective zoonotic disease brucellosis, on the milk-producing industry could cause considerable damage on economy and public health. As a potential bio-weapon this agent gained notoriety for being hardly detectable.
This implies a necessity for a fast and reliable identification technique, but microbiological approaches are demanding and time-consuming so far. Also other more sophisticated and sensitive molecular genetic or immunological methods are cost-intensive and pre-cultivation is still necessary. Here vibrational spectroscopy offers a promising opportunity, since it is non-destructive and achieves high specificities [1]. Especially Raman spectroscopy enables a striking reduction of preparation and analysis times and has already verified its feasibility in this matter [2]. Since micro-Raman spectra provide a fingerprint of the total molecular composition of single cells, they inherently contain all information needed to accurately identify microorganisms down to subspecies level [3].
In this contribution *Brucella spp.* and closely related species isolated from spiked milk samples were identified successfully. For this purpose appropriate inactivation and extraction steps were developed with respect to the compatibility towards Raman spectroscopic measurements. A beforehand established database with Raman spectra of a various number of Gram-negative microorganisms together with chemometrical calculations like linear discriminant analysis provide the basis for an identification within hours.
Acknowledgement: Funding of the research projects 13N9547 and 13N9549 from the Federal Ministry of Education and Research, Germany (BMBF), is gratefully acknowledged.

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FMP011
Specific enumeration of *Lactobacillus plantarum* starter culture in the malolactic fermentation of Grauburgunder white wine using quantitative PCR

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The *Lactobacillus plantarum* B188 strain isolated from wine was investigated for use as a starter culture in the malolactic fermentation of Grauburgunder white wine. In order to identify this strain among autochthonous wine lactic acid bacteria, a method was developed which was based on quantitative PCR. For this, the qPCR primers needed to target a strain-specific sequence, which would allow discrimination between this strain and other *Lactobacillus plantarum* strains that might be present in the fermentation. *Lactobacillus plantarum* B188 was determined to contain a plasmid that could serve as a specific target sequence for qPCR. A randomly amplified polymorphic DNA (RAPD) PCR was performed using primer LB2 and the plasmid from strain B188 as a template. The randomly amplified fragments were cloned into a TA cloning vector and sequenced. A DNA fragment which did not reveal homology to other DNA sequences in the GenBank databank was used as a target for strain-specific qPCR. A 50 L Grauburgunder white wine fermentation was started in the autumn of 2010 with approx 10^8cfu/ml three days following the start of the alcoholic fermentation. The lactic acid bacteria counts were performed in regular intervals using plate counting on de Man, Rogosa and Sharpe (MRS) agar. The microbial counts were assessed in paralell using quantitative PCR. The qPCR results were correlated to microbial counts with the aid of a previously established standard curve that was generated from DNA of known amounts of live *Lactobacillus plantarum* B188 bacterial cells. As the background lactic micropopulation in the wine fermentation was negligible, as determined by a control fermentation without starter culture, we could show that the viable plate counts correlated well with quantitative PCR enumeration of the L. plantarum B188 strain.

FMP012
A novel Muroendopeptidase from *Steptomyces albidoflavus* DSM 40233 and its Application in Growth Inhibition of Wine-relevant Bacteria

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In Germany, winemaking has long tradition. The wine-related microbiota such as yeasts, lactic acid bacteria and acetic acid bacteria can largely influence the wine quality. Some are well known for their off-flavour or biogenic amine production, which can lead to wine spoilage. Therefore it is necessary to inhibit the growth of undesired microbes. This is done so far by sulphuring or the addition of lysozyme. However, these measures are limited in their efficiency. Sulphuring can cause problems with the alcoholic fermentation because of the partial or total growth inhibition of the wine yeasts. Lysozyme does not attack many wild type strains of lactic acid bacteria due to modifications of their cell wall composition. Furthermore these treatments may interfere with the health of the consumer. Sulfur may lead to incompatibility ans lysozyme may cause allergic reactions. In this study an alternative method will be described. Bacteria of the genus *Streptomyces* are able to produce hydrolytic exoenzymes. A novel muroendopeptidase from the supernatant of *Streptomyces albidoflavus* was isolated by cation exchange chromatography and gel filtration. The biochemical characteristics of the enzyme were determined. Under the conditions of winemaking such as low pH and low temperature values it is relatively stable and it exhibits high lytic activity against wine-related organisms. N-terminal sequencing showed a similarity with a metalloprotease of the family M23 from *Streptomyces albus* J1074. The exact cleavage site of this protease in the cell wall of *Micrococcus luteus* was determined. In terms of winemaking, the use of this enzyme is much more effective than the use of lysozyme due to the higher lytic activity. It is much more stable and lysis a broader spectrum of target microbes.

FMP013
Differentiation of Microorganisms Associated with Wine by DNA-fingerprinting analysis

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The conversion of must into wine is a complex microbiological process in which various yeasts and bacteria could be involved. Due to the negative influences of some metabolic compounds of several of these organisms, methods for rapid identification of these species are required. In the early stages of wine making, if the must is exposed to oxygen, acetic acid bacteria are able to generate high amounts of acetic acid, which can lead to spoilage of the wine. Mainly, lactic acid bacteria are responsible for the formation of diacetyl, biogenic amines, mousy off-flavour and exopolysaccharide slimes. In this case some molecular methods for the identification of bacteria like 16S rDNA sequencing are often less specific because of the high conservation of this gene within these genera. For further classification several time consuming physiological tests would be needed. With regard to the determination of yeasts the ITS region is often insufficient for the identification of wine related yeast species. Especially, the differentiation of the *Saccharomyces* sensu stricto group, including the wine-relevant species *Saccharomyces cerevisiae*, *Saccharomyces bayanus* and *Saccharomyces paradoxus* is not possible.
For a better monitoring of the vinification process identification of wine related microorganisms is essential. Therefore, we applied a DNA-fingerprinting method based on the specific amplification of certain genomic regions which are flanked by the *Not*I recognition site (Nested Specifically Amplified Polymorphic DNA-PCR, nSAPD-PCR) for the classification of wine-related lactic acid bacteria, acetic acid bacteria, *Sclerotinia/Botrytis, Dekkera/Brettanomyces*, and *Saccharomyces* species.

FMP014
Cleaning and disinfection of work shoes from the field of PPE

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In many industrial branches, including food, pharmaceutical and cosmetic industry, hygiene demands are increasing. Although personal protective equipment (PPE) includes shoes as well as other clothing that need to be changed, cleaned or disinfected frequently, hygienic demands for shoes were often neglected in the past. Classical, water-based cleaning and disinfection methods damage the footwear, protective properties or do not yield in satisfactory disinfection.
wfk and PFI have developed together a cleaning and disinfection procedure for work shoes from the field of personal protective equipment based on a liquid CO_2-cleaning method, which allowsgentle but disinfecting cleaning and preserves required safety and protective functions of this footwear after several reprocessing cycles.
Specifications have been worked out for safety and protective shoes suitable for a disinfecting CO_2-cleaning procedure.
In a liquid CO_2 treatment shoes are not moistened - no drying is required. Inactivation of pathogens in liquid CO_2 depends on the species, the incubation time, water content and the presence of additives.

FMP015
Raman spectroscopic detection of meat spoilage by *Pseudomonas putida*

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Infrared and Raman spectroscopy have been proven useful tools for rapid and non-invasive detection of meat spoilage. The Raman method can be applied even to samples wrapped with standard packaging materials if visible or near-infrared excitation lasers are applied [1].
We have demonstrated recently that the spoilage status of meat can be appraised by means of multivariate statistical analysis of the Raman spectra [2]. For the further development of such analyses it is essential to distinguish between bacterial and non-bacterial induced alterations of the meat surface and of the spectra.
Here, we report results of a study on pork meat which has been sterilized on the surface to remove the natural flora and which was then artificially spoiled with *Pseudomonas putida*, a prevalent psychrophilic spoilage bacterium. Simultaneous measurements of bacterial growth and corresponding Raman spectra were performed with three sets of meat samples over a period of two weeks of storage at 4°C. These comprised: (i) uncontaminated meat as a control for sterility, (ii) meat which has been contaminated on its surface with *P. putida*, and (iii) meat covered by a membrane with a pore size of 0.2 mm on which P. putida was spreaded.
When the inoculated bacteria proliferating on the surface of the samples entered the late exponential growth phase, they caused strong laser-induced fluorescence (LIF) which was absent in the uncontaminated samples. Our results show how the Raman technology can be used to differentiate between the three model conditions. We also demonstrate that the microbial status of cold stored meat can be classified correctly and conveniently by the corresponding Raman spectra.

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Applied Spectroscopy, 64 (2010) 888-894.
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FMP016
A tool for rapid detection of old and new types of *Streptococcus thermophilus* bacteriophages in dairies

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Many strains of Streptococcus thermophilus starter cultures are susceptible to infection by lytic bacteriophages in dairies, e.g., during production of yoghurt, mozzarella and Swiss-type cheeses. Phages of these thermophilic lactic acid bacteria have been isolated and characterized world-wide and are currently grouped into two distinct subgroups on basis of their phage structural proteins and their mode of DNA packaging. The two groups comprise either cos-type phages containing DNA with cohesive terminal ends or pac-type phages revealing a „head-full" mechanism of DNA packaging. Representatives of both phage groups cannot be differentiated morphologically (Siphoviridae phages). For the simultaneous detection and differentiation of cos- und pac-type S. thermophilus phages, a PCR system has been established on basis of conserved DNA regions of the non-related genes coding for the major head proteins (mhp) of both phage types. This multiplex PCR system can be used both for the detection of lytic phages in whey and product samples and for the detection of prophages in lysogenic S. thermophilus cultures as well.
When the multiplex PCR tool was tested with a broad set of lytic S. thermophilus phages, one phage failed to generate a PCR amplicon. By electron microscopy it was shown that this new phage differed morphologically from all other well-known S. thermophilus phages. It is notable that this new phage also exhibited a number of physiological characteristics unrelated to those of all other cos- or pac-type S. thermophilus phages.
DNA sequence analysis of the major head gene region of the new phage indicated high DNA homology to the corresponding DNA region of Streptococcus pyogenes phages. The standard multiplex-PCR for comprehensive and reliable detection of S. thermophilus-phages was updated with a DNA primer pair specific for the mhp gene of the new phage.

FMP017
Prevalence and pathogenicity of mycobacteria on a farm in Upper Franconia (Bavaria, Germany)

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The increase of allergic and asthma diseases in the industrialized world has often been explained using the „Hygiene Hypothesis" which assumes a decline of human contact with microorganisms [1]. Farm environment in childhood reduces the development of allergies and asthma [2]. Mycobacteria, which are most efficient in the stimulation of the immune system, were assumed to be responsible for this so called „Farm-Effect" [3]. Farm animals and thereby also the quality of food, produced on farms, benefit from this effect as well. According to their growth speed and pigmentation, mycobacteria can be classified into four Runyon-Groups. The members of these groups exhibit either weak pathogenic (Group I & II), increased pathogenic (Group III) or non pathogenic (Group IV) mycobacteria [4].
In this study we have located the reservoirs of mycobacteria and their respective Runyon-Group on a farm in Upper Franconia (Bavaria, Germany). Mycobacteria were isolated from several farm sites and assigned according to the Runyon-classification.
We were able to identify mycobacteria of all four Runyon-Groups in open land habitats (soil, manure, and dunghill). Their number was between 5,760 and 18,200 cfu per gram dry weight. Mycobacteria of Runyon-Group II were characteristic of old cattle shed (80 to 2,130 cfu per gram dry weight). The corn silage and the new cattle shed revealed no mycobacteria.
The data show that various locations on farms are indeed populated by mycobacteria and that weakly pathogenic mycobacteria of the Runyon-Group II in combination with high levels of mycobacteria from all Runyon-groups may account for the stimulation of the immune system of humans and livestock on farms.

FMP018
The use of UV-C light to inactivate microorganisms in fruit juices

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UV-C light (200-280 nm) can be used for the inactivation of microorganisms, as the absorption maxima of DNA components fall in this range (260 nm). This technique has been successfully applied for the disinfection of drinking water in Germany for many years. UV-C treatment of more coloured and turbid liquids, such as milk and wine, is also applied in foreign countries. One limiting factor, however, is the low penetration depth of UV-C into liquids such as fruit juices, which are coloured and may contain particles. To overcome this problem, UV-C technologies which are currently used are based on thin films or turbulence flows. In this study, a new technology using Dean vortices was used to compensate the problem of lack of penetration depth. Four different microorganisms, i.e. *Lactobacillus plantarum* BFE 5092, *Escherichia coli* DH5α, *Alicyclobacillus acidoterrestris* DSM 2498 and *S. cerevisiae* DSM 70478 were investigated. Parameters like optical density, turbidity and viscosity, which influence the inactivation of microorganisms, were evaluated using Ringer`s buffer solution coloured with a dye, or fruit juices such as elderberry nectar, cloudy apple juice or blood orange juice, which differ considerably in optical density and turbidity. The optical density appeared to be the most important factor which influenced the bacterial inactivation. Cell counts of *L. plantarum* BFE 5092 could be reduced in Ringer`s solution adjusted with dye from an initial level of ca. $1x10^8$ cfu/ml to $1x10^1$ cfu/ml at an optical density of 20 with a dosage of 9.6 kJ/L. However, only a log 1.5 reduction could be achieved at an optical density of 140 using the same dosage. Furthermore, no noticeable effect on inactivation was determined by varying the turbidity or the viscosity. However, an increasing flow rate, and the corresponding higher Dean Number improved the efficacy of UV-C treatment.

FMP019
Detection and inactivation of *Cronobacter* species in infant formula

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The novel genus *Cronobacter* combines five bacterial species formerly classified as *Enterobacter sakazakii* [1]. In the last decades, these opportunistic pathogens have been implicated in several incidents as the cause of meningitis and enterocolitis with high mortality rates in premature infants resulting from feeding with contaminated powdered infant formula (PIF) [2].
PIF therefore is strictly recommended to be „*sakazakii*-free" which is defined as the absence of any colony forming unit in 30 samples of 10g of PIF [3]. As *Cronobacter* is ubiquitous in the environment and can survive for long periods in dried products and even has been shown to survive spray drying, the problem of this bacterium in PIF continues to be a major challenge to the industry [4].
In cooperation with a large scale producer of PIF we are developing a modified production process assuring the inactivation of *Cronobacter*. Detection of viable cells is accomplished by quantitative RealTime PCR as well as selective growth on chromogenic media following enrichment culture.

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FMP020
Effect of different protectants on viability of thermotolerant acetic acid bacterium *Acetobacter sengalenisis Acetobacter sengalenisis*

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Production of vinegar at high temperature (>37° C) needs special processes and equipments; one of the key elements in the process, is the accessibility of active and stable starters. In this study the influences of different cryo-protective agents on some steps (freezing, drying and storage) of starter production were investigated. To achieve this goal, *Acetobacter senegalensis,* was used as a thermotolerant acetic acid bacterium.
Glucose was used as carbon source in fermentor to produce biomass. Different cryo-protectants (manitol (20%), glycerol (3%), sucrose (10%), trehalose (5%), glutamate (3%), maltodextrin (10%), skimmed milk (10%) and spent growth medium) were added to washed and unwashed biomass. The lyophilized cells (92-93% water content) were stored in darkness under different temperatures (-20° C, +4° C and 35° C). The viability of cells after rehydration, activity of glucose dehydrogenase, gluconate dehydrogenase and soluble protein contents were determined up to 6 months.
According to the results, washing of cells by tap water has no effect on viability of cells during freezing and more than 97% of cells are alive in all treatments. After lyophilization, unwashed cells showed higher viability in all treatments in comparison to washed cells. On the basis of residual viable cells, manitol, maltodextrin, and spent growth medium showed the highest protective effects (92.3%, 88.2% and 82.1% survival, respectively) on cells during drying process whereas glycerol had the lowest protective effect on viability (15.4% survival).
During storage of lyophilized cells at 35° C, 100% of cells are dead in all treatments after 15 days. Unwashed cells treated with manitol, maltodextrin and spent growth medium showed 79.2%, 68.3% and 62.7% viability, respectively after keeping at 4°C for 6 months.
There is direct relationship between the soluble protein contents of cells and storage temperature. Cells stored at -20° C showed highest soluble protein contents after 6 months of storage while the lowest amount of soluble protein contents was detected in cells stored at 35° C. On the other hand, glucose dehydrogenase and gluconate dehydrogenase activities decreased during storage of cells at 4°C, whereas more than 90% of the enzymes activity remained during storage of different cells at -20° C, so it can be assumed that higher temperature can inactivate cell proteins.

In conclusion, lyophilization of *Acetobacter senegalensis* by the mentioned methods can provide a promising and economic tool for production of stable and active vinegar starters.

GWV001
Paralogues aspartokinases from *Pseudomonas stutzeri* A1501: synthesis of the precursor for the compatible solutes ectoine and hydroxyectoine

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The compatible solutes ectoine and hydroxyectoine are widely synthesized by bacteria as osmostress protectants. Their synthesis is catalyzed by the EctABCD enzymes from L-aspartate-beta-semialdehyde, a central hub in amino acid biosynthesis. Aspartate-beta-semialdehyde is produced from aspartate via the sequential reactions of the aspartokinase (Ask) and the aspartate-semialdehyde-dehydrogenase (Asd). Ask is typically highly regulated by allosteric control in order to avoid gratuitous synthesis of the precursor aspartylphosphate. Most organisms have evolved multiple forms of this enzyme and feedback regulation of these specialized Ask´s is adapted to the respective biosynthetic pathways. In a number of microorganisms, the gene clusters (*ectABCD*) for ectoine/hydroxyectoine biosynthesis is followed by an *ask* gene, suggesting that the Ask_Ect is a specialized enzyme for this biosynthetic pathway. To study the enzymatic and regulatory characteristics of Ask_Ect and its influence on ectoine and hydroxyectoine synthesis, we focused on the non-halophilic bacterium *Pseudomonas stutzeri* A1501 who possesses paralogues Ask enzymes: Ask_Lys and Ask_Ect. We found that the *ectABCD_ask* gene cluster is organized as an osmotically inducible operon. Accordingly, *P. stutzeri* A1501 synthesized ectoine and hydroxyectoine in an osmotically controlled fashion with hydroxyectoine being the dominant solute. We cloned the *ectABCD_ask* genes and expressed them functionally in an *E. coli* strain under the control of the natural osmotically inducible *ect* promoter. A strain carrying a plasmid with the entire *ectABCD_ask* gene cluster produced significantly more ectoine/hydroxyectoine in comparison to a strain with a plasmid carrying only the *ectABCD* genes. We purified both the Ask_LysC and Ask_Ect enzymes and found significant differences with regard of their allosteric control: Ask_LysC was inhibited in a concerted fashion by threonine and lysine, whereas Ask_Ect showed only inhibition by threonine. Our data provide novel insight into the enzymatic properties of Ask_Ect and clues for the recombinant production of two commercially interesting compatible solutes that have already found wide applications.

This study was funded by the LOEWE program of the State of Hessen (Synthetic Microbiology; SynMicro, Marburg).

GWV002
A remarkable stable and active styrene oxide isomerase from *Rhodococcus opacus* 1CP with high biotechnological potential

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Microorganisms from various phyla utilize styrene as a sole source of carbon and energy. The most commonly found degradation pathway designated as *side-chain oxygenation route* has been described in detail for several pseudomonads and is responsible for the conversion of styrene into phenylacetic acid as a central metabolite. In a first step styrene monooxygenase oxidizes styrene to (*S*)-styrene oxide which is afterwards converted by a styrene oxide isomerase (SOI) into phenylacetaldehyde. A dehydrogenase then oxidizes the aldehyde into the acid. SOI-genes (*styC*) can be found in all hitherto published styrene-catabolic gene clusters *styABCD* from pseudomonads. So far only two SOIs were roughly characterized on protein level, one from a *Xanthobacter* strain and another one from a *Corynebacterium* strain. Together with the described enzyme from *Pseudomonas putida* S12 evidence was provided for a membrane-bound location of this type of isomerase.
Here we report on the identification, enrichment, and biochemical characterization of a further representative of SOIs from the actinobacterium *Rhodococcus opacus* 1CP. The enzyme is strongly induced during growth on styrene as well as weakly in the presence of styrene oxide or phenylacetaldehyde. The localization of the enzyme was shown to be membrane-integrated and a procedure was developed to highly enrich the protein in active form. A final specific activity of 600 U mg^{-1} meets the highest intramolecular oxidoreductase activity reported for this enzyme class, so far. The wide pH- and temperature stability range, as well as a considerable long-time stability favors this enzyme for the biotechnological preparation of pure phenylacetaldehydes.
In order to assess the suitability of purification procedure as a general strategy to obtain highly enriched SOIs the corresponding isomerase of *Pseudomonas fluorescens* ST was investigated, too. The purified enzyme (313 U mg^{-1}) was compared in respect to biochemical properties with the SOI of strain 1CP.

GWV003
The enzyme laccase as biocatalyst for the synthesis of various novel organic compounds with potent bioactive properties

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Laccases [E.C. 1.10.3.2] are copper-containing oxidoreductases produced by insects and plants as well as by several microorganisms. Fungal laccases are generally extraordinaryly stable extracellular enzymes with ligninolytic activity [1]. They are stable to high temperatures and function at up to 70 °C. Furthermore, they require no co-substrates, using only atmospheric oxygen as non-toxic oxidant and their low specificity results in large substrate spectrum (>100 aromatic compounds). One of these numerous substrate classes is that of the diphenols, which are oxidized by laccase to reactive quinonoid radicals. These radicals undergo non-enzymatic coupling reactions with various types of compounds e.g. antibiotics or amino acids. This enables the laccases to derivatize a broad range of compounds, including many which are not directly substrates the enzyme itself. This opens up a variety of applications in „green chemistry".
The application of laccase for biobleaching of pulp or for textile dye degradation is well-known. In addition, we have developed a new technology using laccase from the ligninolytic fungi *Pycnoporus cinnabarinus* and *Myceliophthora thermophila* as catalysts for the synthesis of new organic compounds as potent antifungals and biomaterials. The laccase-catalyzed reactions of diphenols with amines like azoles or amino acids resulted either in C-N coupled dimers and oligomers or gave rise to novel types of ring closure products [2]. The process starts with the formation of quinones from the *para*-dihydroxylated compounds followed by a nucleophilic attack of the amine, resulting in C-N-coupled heteromolecular products. Depending on the substituents mono- or oligoaminated products were formed. The synthesis of cyclic products e.g. cycloheptenes and cyclooctenes can be described as regioselective domino reaction. Yields of up to 71% showed the high efficiency of the reaction. The MS and NMR analyses confirmed the structures of the novel compounds.
The introduced enzymatic process demonstrates that laccase can be used for the environmentally friendly synthesis of various types of substances. In particular, the formation of cyclic products which are not accessible by standard procedures. This considerably broadens the application properties of laccase and makes this enzyme interesting for „white biotechnology" [2].

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GWV004
The first hyperthermophilic D-arabitol dehydrogenase catalyzes the regiospecific oxidation of D-arabitol to D-ribulose

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The hyperthermophilic bacterium *Thermotoga maritima* is an abundant source of thermophilic and thermostable enzymes. The genome sequence is known and it encodes the largest number of sugar/polyol transporters, oxidoreductases and hydrolases of any prokaryotic genome sequenced to date. Here we present the first detailed characterization of a

hyperthermophilic D-arabitol dehydrogenase from *Thermotoga maritima,* which was heterologously purified from both *Escherichia coli* and *Gluconobacter oxydans*. The protein was first purified by Strep-Tactin affinity chromatography and exclusively catalyzed NAD-dependent oxidation of D-arabitol or D-xylitol and the NADH-dependent reduction of D-ribulose and D-xylulose. Increased catalytic rates were observed upon Mg^{2+} or K^+ addition. However, the tagged protein was not thermostable and was rapidly inactivated at 85 °C. In contrast to the tagged protein, the D-arabitol dehydrogenase was thermostable when purified by heat precipitation and ion exchange chromatography without an affinity tag. The tag-less form of D-arabitol dehydrogenase had similar kinetic parameters compared to the tagged enzyme, demonstrating that the Strep-tag was not deleterious to protein function but decreased protein stability. A single band at 27.6 kDa was observed on SDS-PAGE for the tag-less D-arabitol dehydrogenase and native PAGE revealed that the protein formed homohexamer and homododecamer. The enzyme catalyzed the oxidation of D-arabitol to D-ribulose and therefore belongs to the class of D-arabitol-2-dehydrogenases, which are typically observed in yeast not bacteria. The product D-ribulose is a rare ketopentose that has numerous industrial applications. Given its thermostability and specificity, D-arabitol 2-dehydrogenase is a desirable biocatalyst for the production of rare sugar precursors.

GWV005

Cofactor regeneration: understanding the catalytic properties of the NAD^+-reducing [NiFe]-hydrogenase from *Ralstonia eutropha* by investigating its sub-complexes

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Several industrially relevant enzyme-driven syntheses, such as the production of L-*tert* leucine, require the reductant NADH, which needs to be continuously regenerated during catalysis. Formate dehydrogenase is currently the only enzymatic system used for NADH regeneration at industrial scale. However, its activity is limited, and formate leads to a significant decrease in pH [1]. The NAD^+-reducing soluble hydrogenase (SH) from *Ralstonia eutropha* H16 represents a promising alternative as the SH reduces NAD^+ to NADH at the expense of H_2 in the presence of O_2 [2-3].

The SH is composed of the six subunits HoxHYFUI$_2$ which accommodate the H_2-cycling catalytic Ni-Fe center, two flavin mononucleotides (FMN) and an electron relay made of iron-sulfur clusters [2]. The functional role and interplay of these cofactors in the context of O_2 tolerance is so far unknown [4], and we have therefore investigated separately the hydrogenase module HoxHY and the diaphorase module HoxFU.

In vitro assays and direct electrochemical studies show that as-isolated HoxHY is catalytically inactive, but, after reductive activation at low potentials, exhibits both H_2 oxidation and H^+ reduction activities. Optical spectroscopy revealed the presence of FMN at substoichiometric levels, which is consistent with an increase of H_2-oxidizing activity in the presence of supplemental FMN [5].

Quantification of the metal and FMN content of the HoxFU module in combination with UV/Vis spectroscopy revealed one [2Fe2S] cluster, one FMN and a series of [4Fe4S] clusters per HoxFU, which is consistent with its close relationship to Complex I. The Michaelis constants for the substrates NADH, NADPH and NAD^+ were 56 µM, 6.78 mM and 197µM, respectively. Protein film electrochemistry revealed that NADH oxidation is product-inhibited by NAD^+ with $K_I = 0.3 \pm 0.2$ mM [6].

For HoxHY and HoxFU the overpotential relative to $E(2H^+/H_2)$ and $E(NAD^+/NADH)$ is minimal consistent with the role of the SH in bidirectional catalysis [5-6]. The results are discussed in terms of possible control mechanisms for the direction of catalysis and implications for aerobic cofactor regeneration.

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GWV006

Design-based construction of a lysine hyper-producing strain by *Systems Metabolic Engineering*

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Since its discovery in the 1950´s *Corynebacterium glutamicum* has been intensely exploited for amino acid production [1]. The annual production volume of L-lysine nowadays exceeds 1 million tons thus forming a large sector in biotechnological food and feed industry. From early on, the industrial demand for this amino acid strongly stimulated the creation of efficient production strains, including development of progressive techniques that allow strain optimization. Still, the production properties achieved today are significantly below the theoretical capacity predicted [2]. With the advent of recombinant DNA technology, a targeted genetic optimization of *C. glutamicum* became possible which, in combination with systems-level omics platforms, allows a precise and targeted optimization - systems metabolic engineering.

Here we describe the stepwise construction of a genetically defined lysine hyper-producing strain. Characteristic metabolic flux patterns observed experimentally and predicted by in silico simulation, revealed that concerted, systems-wide pathway engineering is required towards optimal performance. The desired flux re-direction was achieved by only 12 genome-based modifications located in the central metabolism and the lysine biosynthetic route. The finally obtained production strain achieved a remarkable carbon conversion yield of 55 %, a lysine HCl titre of 120 g L^{-1}, and a productivity of 4 g L^{-1} h^{-1}. With this production performance the lysine hyper-producer created in this work is the best wild type based production strain so far described and lies at the maximum performance of classically derived strains.

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GWV007

The gene rosA encoding N,N-8-amino-8-demethyl-D-riboflavin dimethyltransferase is located within a gene cluster possibly involved in biosynthesis of roseoflavin in *Streptomyces davawensis*

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Streptomyces davawensis was first isolated from a Philippine soil sample in a screening program for antibiotic-producing organisms. In the stationary growth phase this strain produces the antibiotic roseoflavin (RoF) (8-dimethylamino-8-demethyl-D-riboflavin) one of the few known natural riboflavin-analogs [1,2]. It was postulated that RoF is synthesized from riboflavin *via* 8-amino- (AF) and 8-methylamino-8-demethyl-D-riboflavin (MAF) [3,4]. Using a cell-free extract derived from stationary-phase *S. davawensis* cells, an *S*-adenosyl methionine dependent conversion of AF into MAF and RoF was observed. The corresponding *N*,*N*-8-amino-8-demethyl-D-riboflavin dimethyltransferase activity was enriched by column chromatography (3 steps). The final most active fraction still contained at least 5 different proteins. With the major protein present in the final active fraction an enzymatic digest was performed and peptides were used for de novo sequencing by MS/MS. The obtained peptide sequence was compared to a protein database derived from the genomic sequence of *S. davawensis*. The sequence matched a hypothetical protein derived from a yet uncharacterized open reading frame (ORF7678) located in the middle of a (putative) gene cluster within the *S. davawensis* genome. Overexpression of ORF7678 in *Escherichia coli* revealed that the corresponding gene product had *N*,*N*-8-amino-8-demethyl-D-riboflavin dimethyltransferase activity. The ORF was named *rosA*, being the first gene of the RoF biosynthetic pathway to be identified. RosA was purified from a recombinant *E. coli* strain and was kinetically characterized (apparent K_m for AF 69,46 µM; V_{max} = 0,63 µmol/min mg protein; k_{cat} = 24,38 min^{-1}). The putative *S. davawensis* gene cluster containing *rosA* was overexpressed in *Streptomyces lividans* and *Streptomyces albus*, which naturally do not synthesize roseoflavin. The resulting recombinant strains were found to not produce RoF in the

stationary phase, indicating that the gene cluster did not contain all relevant genes for RoF biosynthesis. The *rosA* transcript was detected by reverse transcription PCR in *S. davawensis* cells in the stationary growth phase but not in the exponential phase.

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GWV008
Enzyme Engineering of an Enoate Reductase from *Zymomonas mobilis* Affecting the Enzyme Activity and Enantioselectivity

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Recently, the stereoselective bioreduction of activated alkenes has emerged as a valuable tool for the synthesis of various enantiopure compounds.
In this light, flavin-dependent enoate reductases are interesting enzymes for the industrial production of such chiral compounds, because they are able to reduce activated alkenes exclusively in a *trans*-specific fashion, which goes in hand with the creation of up to two new chiral centers [1].
In this project we used a site directed mutagenesis approach and the exchange of several loops between two enoate reductases, OYE1 from *Saccharomyces carlsbergenisis* and NCR from *Zymomonas mobilis* [2] to identify new enzyme variants that are able to reduce various α, β - unsaturated aldehydes and ketones [3,4].
Three variants possessed increased activity towards all substrates tested compared to wild type NCR. Furthermore one variant was obtained that showed a significant influence on the enantioselectivity of the enzyme.

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GWV009
Corynebacterium glutamicum engineered for efficient isobutanol production

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We recently engineered *Corynebacterium glutamicum* for aerobic production of 2-ketoisovalerate by inactivation of the pyruvate dehydrogenase complex, pyruvate:quinone oxidoreductase, transaminase B, and additional overexpression of the *ilvBNCD* genes, encoding acetohydroxyacid synthase, acetohydroxyacid isomeroreductase, and dihydroxyacid dehydratase (1). Based on this strain, we engineered *C. glutamicum* for the production of isobutanol from glucose under oxygen deprivation conditions by inactivation of L-lactate and malate dehydrogenases, implementation of ketoacid decarboxylase from *Lactococcus lactis*, alcohol dehydrogenase 2 (ADH2) from *Saccharomyces cerevisiae*, and expression of the transhydrogenase genes *pntAB* from *Escherichia coli*. The resulting strain produced isobutanol with a substrate specific yield ($Y_{P/S}$) of 0.60 ± 0.02 mol per mol of glucose. Interestingly, a chromosomally encoded alcohol dehydrogenase rather than the plasmid-encoded ADH2 from *S. cerevisiae* was involved in isobutanol formation with *C. glutamicum* and overexpression of the corresponding *adhA* gene instead of the ADH2 gene increased the $Y_{P/S}$ to 0.77 ± 0.01 mol isobutanol per mol of glucose. Inactivation of the malic enzyme significantly reduced the $Y_{P/S}$, indicating that the metabolic cycle consisting of pyruvate and/or phosphoenolpyruvate carboxylase, malate dehydrogenase and malic enzyme is responsible for the conversion of $NADH+H^+$ to $NADPH+H^+$. In fed-batch fermentations with an aerobic growth phase and an oxygen-depleted production phase, the most promising strain *C. glutamicum* Δ*aceE* Δ*pqo* Δ*ilvE* Δ*ldhA* Δ*mdh* (pJC4*ilvBNCD-pntAB*) (pBB1*kivd-adhA*) produced about 175 mM isobutanol with a volumetric productivity of 4.4 mmol $l^{-1}h^{-1}$, and showed an overall $Y_{P/S}$ of about 0.48 mol per mol of glucose in the production phase.

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GWV010
Phosphotransferase system (PTS) independent glucose utilization in *Corynebacterium glutamicum* by inositol permeases and glucokinases and application for improved L-lysine production

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Corynebacterium glutamicum is used for the annual production of 1.3 million tons of L-lysine from starch hydrolysates and molasses. The predominant carbon sources in these feedstocks, glucose, sucrose, and fructose, are substrates of the phosphoenolpyruvate dependent phosphotransferase system (PTS), which is the major path of glucose uptake and which is essential for sucrose and fructose utilization by *C. glutamicum.* Some growth from glucose is retained in the absence of the PTS. The growth defect of a deletion mutant lacking the general PTS component Hpr in glucose medium could be overcome by suppressor mutations leading to high expression of inositol utilization genes or by addition of inositol to the growth medium if a glucokinase is overproduced simultaneously. PTS-independent glucose uptake was shown to require at least one of the inositol transporters IolT1 or IolT2 as a mutant lacking IolT1, IolT2 and the PTS component Hpr could not grow with glucose as sole carbon source. Efficient glucose utilization in the absence of the PTS necessitated overexpression of a glucokinase gene in addition to either *iolT1* or *iolT2*. IolT1 and IolT2 are low affinity glucose permeases with K_S-values of 2.8 mM and 1.9 mM, respectively. As glucose uptake and phosphorylation via the PTS differs from glucose uptake via IolT1 or IolT2 and phosphorylation via glucokinase by the requirement for phosphoenolpyruvate, the roles of the two pathways for L-lysine production were tested. The L-lysine yield by *C. glutamicum* DM1729 was lower than by its PTS-deficient derivate DM1729Δ*hpr*, which, however, showed low production rates. Combined overexpression of *iolT1* or *iolT2* with *ppgK*, the gene for PolyP/ATP-dependent glucokinase, in DM1729Δ*hpr* enabled L-lysine production as fast as by the parent strain DM1729, but with 10 to 20 % higher L-lysine yield.

GWV011
Biotechnological conversion of glycerol to 2-amino-1,3-propanediol (serinol) in recombinant *Escherichia coli*

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The biodiesel industry is very much interested to convert the huge surplus of glycerol, which is obtained during transesterification of the fatty acids from vegetable oils or fats with methanol, into higher value products. One promising molecule is 2-amino-1,3-propanediol better known as serinol. It has become an important intermediate for several chemical applications in the last years. Amino alcohols like serinol are widely used as precursers for non-ionic contrast agents like 1-*N*,3-*N*-*bis*(1,3-dihydroxypropan-2-yl)-5-[(2*S*)-2-hydroxypropanamido]-2,4,6-triiodobenzene-1,3-dicarboxamide (iopamidol). Iopamidol is used as contrast agent for angiography throughout the cardiovascular system. Serinol is also an intermediate for drugs dealing with pain treatment, and chiral (1R,2R) phenylserinols have been used as precursors in chloramphenicol synthesis since 1947. Until now serinol is normally produced chemically from 2-nitro-1,3-propanediol, dihydroxyacetone and ammonia, dihydroxyacetone oxime or 5-amino-1,3-dioxane. A biological approach to synthesize serinol was designed using amino alcohol dehydrogenases like the AMDH from *Streptomyces virginiae* IFO 12827 *in vitro*. We constructed an artificial pathway and established for the first time an *in vivo* serinol production. Therefore, we expressed the bifunctional dihydroxyacetonephosphate aminotransferase/ dihydrorhizobitoxine synthase RtxA from *Bradyrhizobium elkanii* USD94 in recombinant *Escherichia coli* strains. In flask experiments these strains were able to accumulate serinol up to 3 g/l in the supernatant. 2-amino-1,3-propanediol was isolated by converting it into the corresponding hydrochloride. Further purification was achieved by cation exchange chromatography employing a Dowex® fine mesh resin and elution with ammonium hydroxide. With this method 60 % of the product was recovered.

GWV012
Autotrophic Production of Stable Isotope-labelled Amino Acids

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Stable isotope (SI)-labelled biomolecules are increasingly in demand as standards for quantitative mass spectrometry and multidimensional NMR. The production of ^{13}C-labelled substances by autotrophic bacteria is a popular method since it uses the competitively priced ^{13}C-carbon source, $^{13}CO_2$. The β-proteobacterium *Ralstonia eutropha* H16 is able to grow lithoautotrophically with H_2 and CO_2 as sole sources of energy and carbon, respectively [1]. The industrial production of SI-biomaterial with *R. eutropha* is already established [2] and *R. eutropha*-based SI-enriched diet has been used successfully in quantitative proteomic analyses of mouse models [3]. Of particular interest for quantitative proteomics are SI-labelled amino acids, e.g. SI-arginine, which are required for amino acid specific labelling of proteins in cell free assays as well as for standards in quantitative proteomics. In the current study arginine enrichment in autotrophically grown *R. eutropha* cells was accomplished by accumulation of the arginine-containing polymer cyanophycin [multi-L-arginyl-poly-(L-aspartic acid)]. Overproduction of cyanophycin was achieved by heterologous expression of the cyanophycin synthase gene (*cphA*) of *Synechocystis* sp. strain PCC6308 under control of the ribulose-1,5-bisphosphate carboxylase (*cbb*) promoter. The constructed strain accumulates cyanophycin under lithoautotrophic growth conditions as cytoplasmic inclusions. The cyanophycin content of the cells reached up to 5.5% of cellular dry weight (CDW). The plasmid-based overexpression strain showed a decrease of cyanophycin yield when grown without antibiotic, most likely due to the loss of the corresponding plasmid during cultivation. Plasmid stability is crucial for enhanced cyanophycin synthesis [4]. To overcome plasmid curing, we integrated the *cphA* gene together with the appropriate *cbb* promoter sequence into chromosome 2 of *R. eutropha* to ensure stable production of cyanophycin, resulting in a strain offering possibilities for cost-effective production of SI-arginine, a particularly marketable product.

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GWV013
Systems Metabolic Engineering of *Basfia succiniciproducens* for Biobased Production of Succinic Acid

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Succinic acid, a key building block for important bulk chemicals, is currently derived from petrochemical origin. With regard to the shortage and increasing prices for fossil resources, the biotechnological production of succinic acid becomes an attractive alternative to the traditional route. Hereby, the bio-based production process, using renewable resources and fixing carbon dioxide, a prominent greenhouse gas, appears to be more sustainable.
Towards, an economically competitive bio-based production process for succinic acid we focus on the recently isolated microorganism *Basfia succiniciproducens*. It belongs to the *Pasteurellaceae* family having the ability to naturally overproduce succinic acid. It grows on a variety of different carbon sources (e.g. glucose, glycerol) [1]. Volumetric productivity for succinic acid of up to 1.3 g L^{-1} h^{-1} and a yield of 0.6 g g^{-1} are reached on glucose as sole carbon source [2]. By using glycerol as sole carbon source, remarkably increased succinic acid yields of up to 1.2 g g^{-1} can be obtained [1]. To determine genetic and metabolic targets for the elevation of yield and production efficiency these studies focus on the metabolic network of *B. succiniciproducens*.
Taking the development of a minimal medium as a starting point, several genetic modifications were introduced into the organism to improve the production efficiency. The systems biotechnological approach of comprehensive ^{13}C metabolic flux analysis program led to a detailed insight into the metabolic network of *B. succiniciproducens*.

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GWV014
Chemoenzymatic synthesis and microbial degradation of enantiopure aromatic beta-amino acids

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Chiral beta-amino acids are valuable building blocks for the production of fine chemicals and pharmaceuticals.
As their chemical synthesis is still inefficient and costly our approach is a modification of the well studied hydantoinase/carbamoylase system. Aryl-substituted dihydropyrimidines were synthesized as substrates for whole cell biotransformation experiments with different wild type bacteria and recombinant *E. coli* strains expressing hydantoinases with known activity for aryl-substituted hydantoins. Most strains tested were able to hydrolyze the substrates to the corresponding *N*-carbamoyl beta-amino acids [1]. Two isolates showed enantioselective conversion of the model substrate phenyldihydrouracil and also were able to hydrolyze *p*-chlorophenyldihydrouracil. The gene sequences of two novel hydantoinases and one carbamoylase were elucidated.
Furthermore we investigated the microbial degradation of beta-Phenylalanine. In all bacteria tested so far, the initial reaction is a (S)-selective transamination to the corresponding beta-keto acid by inducible PLP dependent transaminases. We established a chiral HPLC analysis suitable for the enantioseparation of several aromatic beta-amino acids to study the substrate spectrum of these enzymes [2].

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GWV015
Natural Product Synthesis by Squalene-Hopene Cyclases (SHCs)

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Considering the membrane fraction of cells, one difference between bacteria and eukaryotes is the absence of sterols as membrane constituents. In contrast to eukaryotes, it is considered that some eubacteria produce pentacyclic triterpenes of the hopanoid class as structural and functional equivalents of sterols [1, 2]. Hopanoids are synthesized by squalene-hopene cyclases (SHC; EC 5.4.99.17), which catalyze the cyclization of triterpenes via cationic intermediates in one of the most complex and powerful one-step reactions known in biochemistry. Most of our understanding about the biochemical and molecular mechanism of this reaction has been obtained by the characterization of a SHC from *Alicyclobacillus acidocaldarius* (AaSHC; GI: 1435434).
In our study, we characterized a novel SHC from the gram-negative, alcohol producing bacterium *Zymomonas mobilis* (ZmSHC1; GI: 56552444) and compared its activity and substrate spectrum with another, previously described squalene-hopene cyclase (ZmSHC2; GI: 6466213) from the same organism [1, 3]. In order to do this, we optimized the expression system for these enzymes in *Escherichia coli* and the conditions for the enzymatic reactions. Subsequently, we determined the enzymatic activity of ZmSHC1 with a variety of substrates including citronellal, homofarnesol and squalene. Despite the differences in chain length (C_{10}-C_{30}) and the presence of C=C double bounds or functional groups like aldehydes at the position where protonation needs to occur for the initiation of the reaction, conversion could be found for all of these substrates. Beside the conversion of squalene to hopene, the cyclization of homofarnesol to ambroxan and citronellal to isopulegol is of particular interest, as these compounds are commonly used in the manufacturing of fragrance and flavour concentrates or could provide a bio-catalytic access for the production of menthol, respectively. Furthermore, our results revealed significantly higher rates of substrate conversion of ZmSHC1 in comparison to those, previously described for the squalene-hopene cyclase AaSHC from *A. acidocaldarius* [4].

From these results, we conclude that the squalene-hopene cyclase ZmSHC1 from *Z. mobilis* has a high bio-catalytic potential for a large variety of industrial applications.

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GWV016
Rhamnolipids- Green Surfactants Based on Renewables

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Biosurfactants are green alternatives to conventional chemically-synthesized surfactants. Rhamnolipids are glycolipids with good physico-chemical properties. However, the main obstacles towards an economic production are low productivity, high raw-material costs, relatively expensive downstream processing, excessive foaming problems and a lack of understanding the rhamnolipid regulation in bioreactor systems.
A recent study shows that the sequenced *Pseudomonas aeruginosa* strain PAO1 is able to produce high quantities of up to 40 g/L of rhamnolipid during 30 L batch bioreactor cultivations with sunflower oil as sole carbon source [1]. The rhamnolipid production capacity of PAO1 was evaluated in comparison with the over-producer strains DSM7108 and DSM2874 [2]. PAO1 not only seems to be an appropriate model, but surprisingly has the potential as a strain of choice for actual biotechnological rhamnolipid production.
The induction of rhamnolipid biosynthesis is known to be quorum sensing regulated. Nevertheless little is understood about the up- and down-regulation of rhamnolipid production. In 30L bioreactor cultivations of *P.aeruginosa* PAO1 the specific rhamnolipid productivity shows a distinct maximum correlated with the transition of the logistic growth to a stationary phase. In contrast to well-established production strains, PAO1 allows knowledge-based „systems biotechnological" process development combined with the frequently used heuristic bioengineering approach. Thus PAO1 could be an appropriate model for rhamnolipid production in pilot plant bioreactor systems. In a current project different fed-batch cultivation strategies and an optimized down-stream procedure are evaluated. The aim is to develop an economic cultivation process where the cells are kept at high levels of specific rhamnolipid productivity.

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GWV017
Regioselective hydroxylation of medium-chain *n*-alkanes and primary alcohols by CYP153 enzymes

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Cytochrome P450 monooxygenases are a very large and diverse superfamily of heme-containing proteins found in all domains of life. The enzymes catalyze a variety of reactions including hydroxylation of C-H bonds, heteroatom oxygenation, heteroatom release (dealkylation), oxidative deaminations, dehalogenations, desaturations and epoxide formation [1]. They have long been the focus of biochemists because of their interesting ability to introduce a single oxygen atom from O_2 into an organic substrate, that results in an one-step synthesis of complex molecules. Most of these enzymes only work as part of a multiprotein complex with redox partners providing electrons from NADH cofactors to the heme domain.
Selectively hydroxylated hydrocarbons are of great interest in the chemical industry, due to their role as intermediates for the synthesis of bulk and fine chemicals. The selective terminal hydroxylation of alkanes is still problematic and there is to date no efficient chemical strategy to direct the introduction of hydroxyl groups on primary non-activated C-H bonds. CYP153 enzymes are such enzymes catalyzing the terminal hydroxylation of aliphatic, alicyclic and alkyl-substituted compounds with high regio- and stereoselectivity under mild reaction conditions [2]. Two CYP153A enzymes were cloned and expressed in *Escherichia coli*. The activity of each P450 was reconstituted with artificial electron transfer partners [3]. The CYP153A enzymes were assayed *in vitro* with purified proteins using C_5-C_{12} *n*-alkanes and C_6-C_{12} primary alcohols as substrates.
This work was performed within the "Systembiologie in Pseudomonas für die industrielle Biokatalyse" and financial support by the BMBF is gratefully acknowledged.

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GWV018
Whole-cell biotransformation for the stereospecific hydroxylation of the incompatible solute guanidino-ectoine

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The Fe(II)/α-ketoglutarate-dependent ectoine hydroxylase (EctD) from the halotolerant *Halomonas elongata* uses, besides ectoine, a broad spectrum of unusual cyclic substrates, including synthetic ectoine derivatives [1]. In this study, we investigated whether the enzyme is able to hydroxylate the so called incompatible solute guanidino-ectoine (2-amino-3,4,5,6-tetrahydro-4-pyrimidine-carboxylic acid). During salt stress *E. coli* accumulates this chemically synthesized substrate via the osmotically induced proP and/or proU transport systems. But in contrast to the compatible version ectoine, guanidino-ectoine displayed a negative effect on the growth rate of *E. coli* [2]. In order to supply and investigate the hydroxylated form of guanidino-ectoine, we employed a whole-cell biotransformation system in *E. coli* BL21, which is based on heterologous expression of the *ectD*-gene from *H. elongata* [3]. This biotransformation system enabled a 100% conversion of guanidino-ectoine into its 5-hydroxy derivative (2-amino-5-hydroxy-3,4,5,6-tetrahydro-4-pyrimidine-carboxylic acid). The hydroxylated version itself proved inhibitory to cellular metabolism, and therefore represents a new addition to the group of cyclic incompatible solutes.

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GWV019
Catalytic biofilms: Real time solvent tolerance analysis of *Pseudomonas* sp. strain VLB120ΔC and profiling of EPS matrix

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Biofilms are ubiquitous surface associated microbial communities, which are embedded in an extra cellular polymeric matrix (EPS) responsible for biofilm structural integrity and strength. Biofilm grown cells exhibit enhanced tolerance towards adverse environmental stress conditions (e.g. antimicrobial substances, toxic chemicals and heavy metals) and thus there has been a growing interest in the recent years to use biofilms for biotechnological applications [1]. We present a time and loci resolved, non-invasive, quantitative approach to study biofilm development and its response to the toxic solvent styrene. *Pseudomonas* sp. strain VLB120ΔC-BT-*gfp*1, was grown in modified flow cell reactors and exposed to the solvent styrene. Biofilm grown cells displayed stable catalytic activity producing (*S*)-styrene oxide continuously during the experimental period. The pillar like structure and growth velocity of the biofilm was not influenced by the presence of the solvent. However, the cells experience severe membrane damage during the styrene treatment, although they are obviously able to adapt to the solvent as the amount of permeabilized cells decreased from 80% to 40% in 48 hours. Concomitantly the fraction of ConA stainable polysaccharides increased, substantiating the assumption that those polysaccharides play a major role in structural integrity and enhanced biofilm tolerance towards toxic environments. Compared to control experiments with planktonic grown cells, the *Pseudomonas* biofilm adapted much better to toxic concentrations of styrene, as nearly 65% of biofilm cells were not permeabilized (viable) as compared to only 7% in analogous planktonic cultures [2]. Preliminary biochemical profiling of the

EPS matrix showed that it consists mainly of polysaccharides (50%), proteinaceous compounds (20%), and lipids (10%), while humic acids, uronic acids, and nucleic acids account for the rest of the matrix. Styrene stimulated substantially the production polysaccharides compared to non-solvent conditions. This study underlines the enhanced robustness of biofilms in solvent saturated environments, which is supposedly a combination of effects such as the increased EPS production, physiological changes due to adaptation, slow growth and other resistance mechanisms.

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GWV020
Asymmetric benzylic hydroxylation and epoxidation of alkylbenzenes and styrene derivatives by *Agrocybe aegerita* aromatic peroxygenase

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Optically pure C_α-hydroxy alkylbenzenes and C_α-C_β styrene epoxides are of vital interest as building blocks in the synthesis of pharmaceuticals and fine chemicals. In particular chiral epoxides offer two defined stereo centers in ring opening reactions. The agaric mushroom *Agrocybe aegerita* produces a heme-thiolate peroxygenase (*Aae*APO) belonging to a new class of aromatic peroxygenases (APOs) that shares spectral and catalytic properties both with peroxidases and cytochrome P450 monooxygenases and catalyses various oxygenation reactions. Hydroxylations and epoxidations proceed with sometimes virtually perfect stereoselectivity yielding phenyl alcohols and oxiranes, respectively. Typical substrates are non activated hydrocarbons such as alkylbenzenes and styrene derivatives as well as their cyclo alkylbenzene analogons. Enantiomeric excesses greater than 99% and total turnovers up to 110,000 cycles are achieved. Besides this, the apparent kinetic K_M- (k_{cat}-) values determined for ethyl and propyl benzene hydroxylation to 694 µM (409 s^{-1}) and 480 µM (194 s^{-1}) support implementation into technical processes. Mechanistic aspects regarding stereoselectivity and oxygen incorporation are further objects of discussion.

GWP001
Characterization of immobilized alkaline cyclodextrin glycosyltransferase from a newly isolated Bacillus agaradhaerens KSU-A11

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Alkaliphilic bacteria were isolated from soil and water samples obtained from Egyptian soda lakes (Wadi Natrun area, Egypt). Screening for cyclodextrin glycosyltransferase-producing alkaliphilic bacteria resulted in isolation of 10 positive strains. Strain KSU-A11 was selected as the best CGTase producer (2.1 U/ml). 16S rDNA sequence analysis identified the KSU-A11strain as *Bacillus agaradhaerens*. CGTase was partially purified using starch adsorption technique. The partially purified CGTase was immobilized on chitin by covalent binding tecnique using cross linking reaction with high immobilization yield (85%). The properties of the free and immobilized CGTase were determined. The optimum pH of the immobilized enzyme was slightly higher than that of the free enzyme, pH 10 and 10.5 respectively. In addition, both free and immobilized CGTase retained 94 to 100 % of its initial activity over a wide pH range (pH 6.0 to 11.0). The enzymatic activity of both free and immobilized CGTase was highest at temperature 50 ºC; however, the relative activities of the immobilized enzyme were slightly higher than those of the free enzyme. Furthermore, investigation of thermostability of the enzyme indicated that the immobilization process of CGTase on chitin significantly protected the enzyme against thermo-inactivation. Kinetic parameters Km and Vmax values for free and immobilized enzymes were estimated and while there was no change in the Vmax value for both free and immobilized CGTase (83.3 µmol/min. mg), the Km of the enzyme increased from 14.28 to 20 mg/ml upon immobilization. The immobilization of the enzyme showed high operational stability by retaining about 50% of the initial activity after nine uses.

GWP002
Investigations of microalgal growth kinetics and the flashing light effect

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The construction of economic pilot plant photobioreactors exhibits still major problems due to insufficient scale-up of technical parameters from lab to larger scale. The layout of large scale photobioreactors requires the knowledge of the physiological reactions of the specific strain(s) moreover the resulting kinetics of growth and product formation under different illumination conditions (light intensity, frequency of light/dark cycles) are of fundamental interest and importance. Therefore a scale-down approach was successfully applied for identification and determination of these relevant and critic parameters. The conditions in one volume element of a large reactor are mimicked in a small scale model reactor. The configuration and successful application of this model reactor for investigation of Chlamydomonas' growth under constant and flashing light conditions will be presented in this contribution.
The model reactor comprises a special designed illumination device developed using light emitting diodes (LEDs) and collimating lenses resulting in an entirely homogenous illuminated volume. The focusing effect (lens effect) of the light source to the center of the reactor compensates the mutual shading of the algae cells. Measurements of light intensity distribution inside the reactor containing media of different optical densities will be shown which could verify the homogenous illumination in ranges relevant for determination of growth kinetics.
Besides the model reactor was applied for kinetic investigations of different algae in various operating modes (Batch, Fed-Batch and Continuous Culture). For instance the effect of reduced antenna size on growth rates of Chlamydomonas reinhardtii mutants at different light intensities compared to the non-mutated strain will be depicted. Experiments have been performed under constant illumination up to very high, saturating and already inhibiting light conditions. Furthermore the positive effect of fast light/dark cycles on growth rate (flashing light effect) was examined and relevant rates were identified.

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GWP003
Modified Galactitol-Dehydrogenase from *Rhodobacter sphaeroides* D for Electrochemical Applications

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Dehydrogenases represent an important class of enzymes in biotechnology. By enantioselective reduction or oxidation they provide access to rare sugars and alcohols which may serve as optically pure compounds and chiral building blocks in the pharmaceutical and chemical industry.
The 765 bp sequence of the galactitol dehydrogenase [1] (GatDH) gene coding for a subunit of the homotetrameric enzyme with 254 amino acids and a molecular mass of 26.4 kDa was functionally expressed in *Escherichia coli* BL21Gold(DE3). The heterologously expressed GatDH was elongated by the attachment of a His(6)-tag to the N-terminus of the protein which facilitated the purification and did not affect the catalytic activity. The active conformation is strictly dependent on the presence of bivalent cations like Mg2+. The crystal structure revealed that the Mg-ions are coordinated by the last three amino acids of the C-termini from two dimers, which let the tetramer appear as a dimer of dimmers [4]. To illustrate the mechanisms of the active site, the deduced oxidation of pentanediol is depicted.

The recombinant enzyme was modified by addition of two cysteins, arranged in front of the His-tag, to make use of the enzyme for electroenzymatic applications. The enzyme variant exhibited unaffected activity in solution and the cystein-residues enabled a directed immobilization of the enzyme to gold electrodes without a further linker-molecule. The electrochemical reoxidation of the co-substrate NAD+, hence the successful biofunctionalization of the electrode was monitored by cyclic voltammetry (CV) [2,3]. Determination of CVs with increasing substrate concentrations enabled Km-value determinations of 0.06 mM, exhibiting a slightly increased affinity to the biochemical value in solution of 0.2 mM.
By use of bioinformatic methods several amino acid exchanges have been suggested for developing enzymes with increased stability and the mutants have been constructed.

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GWP004
Uptake and utilization of glucosamine in *Corynebacterium glutamicum*

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Corynebacterium glutamicum is a Gram-positive biotin-auxotrophic actinobacterium primarily applied for the industrial production of amino acids like L-glutamate and L-lysine. Since feedstock costs hold a significant part of overall fermentation costs it is desirable to use low-cost, renewable resources like hydrolysed lignocellulose and/or chitin-rich polysaccharides. Such feedstocks contain a variety of different carbohydrates including glucose, glucosamine or N-acetylglucosamine. Interestingly, utilization of amino sugars was not investigated for *C. glutamicum* yet. We addressed uptake of glucosamine to explore the metabolic set-up for its utilization.
C. glutamicum is able to grow on glucosamine as sole carbon and nitrogen source, but only with half the growth rate than on glucose as carbon source was observed. In contrast to many other substrates glucosamine and glucose are not simultaneously metabolized by *C. glutamicum*. Since a hpr mutant of *C. glutamicum* is not capable to utilize glucosamine participation of a PTS-type carrier (phosphostransferase systems) was indicated. Growth assays with mutants lacking PtsG (glucose), PtsF (fructose) or PtsS (sucrose) revealed that PtsG is the sole uptake system for glucosamine in *C. glutamicum*. Transport assays with radiolabeled substrates were performed to characterize the kinetics of glucoseamine uptake indicating that PtsG mediates glucoamine uptake as side activity. The impact of the PtsG dependent glucosamine uptake on the efficiency of its utilization will be discussed.

GWP005
Class IIa bacteriocins from *Pediococci*: Production in fermentation systems

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Class IIa bacteriocins of lactic acid bacteria are structurally related proteins with a molecular weight ranging between 2-6 kDa. Their N-terminus contains a highly conserved region known as the pediocin box, with the characteristic amino acid sequence of -YGNGV-. These bacteriocins are known as the pediocin-like and Listeria-active bacteriocins. Since the first isolated pediocin PA-1 from *Pediococcus acidilactici*, a number of other pediocins from more or less related species have been isolated and partly characterized. These bacteriocins, apart from their broad inhibition spectra towards Gram-positive food spoilage and pathogenic bacteria, exhibit technologically important properties -e.g. maintenance of activity at sterilization or freezing temperatures and at pHs ranging from 2 to 10, which make them important potential biopreservatives.
In this work, we report on the common characteristics of production of the newly identified pediocins from *P. acidilactici*, *P. pentosaceus* and *P. damnosus* in batch and fed-batch fermentation systems. Kinetic studies on fermentations carried out under different dissolved oxygen levels in a 3-L stirred tank bioreactor, run at 150 rpm and 30°C, revealed that in all cases DO levels of 50-60% supported bacteriocin production with growth-associated and primary metabolite kinetic patterns and higher specific productivities compared to anaerobic or fully aerobic conditions under which mixed fermentation kinetics were observed. Bacteriocin production commenced only when the broth pH dropped to levels of ~3.5 due to acid production at approximately 15 hours.
These observations suggest that an oxidative metabolic pathway is involved in biosynthesis and that conversion of prepeptides to active peptides takes place upon acidification of the broth. Irrespective of the microorganism used, a feeding strategy for maintaining the pH at 3.5 and glucose at 20 g/L from 10 hours, extended the production period and almost doubled the yield of secreted proteins in all cases.

GWP006
Pediocin production from *Weissella paramesenteroides* in fermentation systems: Glucose feeding strategies and yields.

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An atypical meat isolated *Weissella paramesenteroides* strain was found to produce a class II a bacteriocin of approximately 5 kDa M.W. The bacteriocin was partly characterized and studied with respect of various physicochemical and biochemical properties of its molecule. Following the bacteriocin molecule studies, attempts were made to optimize production in fermentations carried out in a 3-L stirred tank bioreactor.
The effect of the concentration of glucose in the substrate was studied in batch cultures in which initial concentrations varied between 10 and 30 g/L. The glucose effect was also studied in fed-batch cultures operated as glucostats and pH-stats. In these runs feeding started at 10 hours while glucose and pH were maintained at stable levels: 10, 15, 20, 25, and 30 g/L glucose and pH 3.5. Another feeding strategy was also applied in which a glucose solution was fed in single shots in batch runs with initial glucose concentration of 10, 15, and 20 g/L. Maximum bacteriocin production levels in batch cultures reached 850 AU/ml under semiaerobic conditions and with 25 g/L initial glucose concentration.
Maintaining stable levels of glucose and pH extended the production period in all runs however, an optimum was observed for glucose concentration levels at 25 g/L, at which production levels were the highest (1500 AU/ml). Fermentation process kinetic studies showed growth-associated production for glucose levels ≥20 g/L. Substituting the production medium with glucose at time-points at which specific production rates started to drop resulted in higher specific productivities however, the optimum concentration of 25 g/L glucose was again observed in this type of feeding.
Therefore, the concentration of the carbon source plays an important role in bacteriocin production from *W. paramesenteroides* and manipulating its levels by choosing the appropriate mode of fermentation can lead to significant increases in process productivities.

GWP007
Characterization of the 1,3-PD dehydrogenase of *Clostridium* sp. IBUN 13A and establishment of an enzyme assay for measuring 1,3-PD

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The non-pathogen strains *Clostridium* sp. IBUN 158B and IBUN 13A were isolated from the soil of Colombian tomato and potato fields. These strains belong to a new species, closely related to *C. butyricum*, and are able to transform residual glycerol from biodiesel production into 1,3-propanediol (1,3-PD). In order to establish a competitive industry based on these strains, their 1,3-PD yield should be increased by means of genetic engineering. For measurement of 1,3-PD, an enzymatic assay based on the enzyme 1,3-PD dehydrogenase of strain IBUN 13A (encoded by the *dhaT* gene) was established. This NAD^+-dependent enzyme converts 1,3-PD into 3-hydroxypropionaldehyde, and the proportional amount of NADH produced can be detected spectrophotometrically at 340 nm. In order to overproduce the enzyme, the *dhaT* gene of IBUN 13A was cloned into the vector pET-28a(+) of Novagen® (which adds an N-terminal his-tag to the enzyme), and the resulting plasmid was transformed into *E. coli* BL21(DE3). As the enzyme is sensitive to oxygen, the whole purification process was performed under anaerobic conditions. After cell disruption using a French Press, the

enzyme was purified via metal ion affinity chromatography. Additionally, imidazole and excess NaCl were removed from the concentrated protein fraction by using PD-10 desalting columns (GE Healthcare), because of their disturbing effects for the measurement of enzyme concentration and activity. The enzymatic assay was performed anaerobically, using a photometer adjusted to 37 °C. The pH was kept at 9.2 with a $KHCO_3$ buffer, and the reaction was started with the 1,3-PD samples. The overproduced 1,3-PD dehydrogenase was characterized concerning its stability, substrate specificity, and the optimal pH and temperature for its activity. Furthermore, a calibration curve between 0 and 60 mM 1,3-PD with a correlation coefficient of 0.992 could be obtained for the overproduced enzyme, which allows for the determination of 1,3-PD concentrations in culture samples of clostridial strains grown on glycerol as substrate.

GWP008
Effective biocatalytic synthesis of D-Rhamnose, a major building block of carbohydrate-based vaccines

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D-Rhamnose (6-deoxy-D-mannose) is rare in nature but it is the major constituent of immunogenic oligosaccharides (*O*-antigens) of various human and plant pathogenic gram-negative bacteria. Therefore, the availability of D-Rhamnose is of concern to syntheses of carbohydrate-based vaccines against human pathogens [1]. As chemical synthesis of D-Rhamnose and its extraction from bacterial lipopolysaccharides are both tedious and low-yielding, we have developed a concise biocatalytic route to D-Rhamnose with yields >40%.
The route started from 6-deoxy-D-glucose **1** which was quantitatively converted in water to 6-deoxy-D-glucosone **2** (30 $g \cdot l^{-1} \cdot h^{-1}$) using a catalytically improved pyranose 2-oxidase variant [2]. Downstream processing of **2** was facile and comprised ultrafiltration and lyophilisation (>90%). Solid **2** was dissolved in deionized water and quantitatively reduced (9 $g \cdot l^{-1} \cdot h^{-1}$) to a mixture of D-Rhamnose **3** (42%) and 6-deoxy-D-glucose **1** (58%) with 1,5-Anhydro-D-fructose-Reductase (AFR) [3] and cosubstrate regeneration. When the conversion was complete, residual D-glucose from cosubstrate regeneration and 6-deoxy-D-glucose **1** were oxidized to the corresponding gluconic acids with glucose oxidase. Downstream processing of D-Rhamnose **3** comprised ultrafiltration, removal of charged compounds by ion-exchange chromatography and lyophilisation to give solid **3**.

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GWP009
L-Sorbitol-Dehydrogenase (LSDH) from *Bradyrhizobium japonicum* USDA110: Cloning and Characterisation of an Interesting Enzyme for Rare Sugar Synthesis

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The rare sugar D-sorbose is an interesting synthon for pharmaceutical applications and can be produced from easily prepared L-sorbitol by LSDH [1]. BLAST search with the N-terminal amino acid sequence of the *Stenotrophomonas maltophilia* enzyme [1] listed putative enzymes with best similarities for an assumed ribitol-DH of *Bradyrhizobium japonicum*. The gene was amplified, tagged with histidines and heterologously expressed. The enzyme was biochemically characterized collecting data on temperature and pH-optimum, isoelectric point, substrate spectrum and subunit composition. First structural data suggest temperature stability and crystallisation experiments are in progress. The enzyme exhibited high activity for D-sorbitol transformation to D-fructose but also reasonable activity with L-sorbitol resulting in D-sorbose as the single product. The reaction products were analysed via HPLC, the cofactor is regenerated with lactate-dehydrogenase. A cost effective co-factor regeneration system for these procedures can be achieved with electrochemical methods as has been shown for DSDH from *Rhodobacter sphaeroides* [2].

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GWP010
Development of genetic tools aiming at strain improvement in *Bacillus pumilus*

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Members of the Gram positive endospore forming genus *Bacillus* are intensively used for the industrial production of secreted enzymes such as proteases, amylases, and chitinases. Recently, the only sparsely investigated species *Bacillus pumilus* got into focus due to its high secretion capacity for extracellular enzymes serving as an alternative producer strain for industrial enzyme production. However, the scientific knowledge concerning *B. pumilus* is currently rather poor. Thus, genetic tools have to be developed and applied for strain improvement of *B. pumilus*. Here, we focus on the development, improvement, and application of basic genetic tools for *Bacillus pumilus* such as transformation techniques for plasmid-transfer (PEG-mediated protoplast transformation, electroporation, conjugation, natural competence), procedures for gene replacement and direct knock outs (induced competence with pMMcomK[1], *upp* counter selection system[2]), generation of stable and safety strains (*spoIV*, *uvrBA* and *recA* deletion mutants), establishment of random mutagenesis systems (*mariner-Himar1* transposon system for *Bacilli*[3]) or construction of reporter gene systems.
This work is supported by the Bundesministerium für Bildung und Forschung (BMBF, grant no. 0315594C).

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GWP011
Characterisation of Friulimicin Production during Cultivation of *Actinoplanes friuliensis* in a bioreactor

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Friulimicin is a lipopeptide antibiotic which is active against a broad range of multiresistant gram-positive bacteria such as methicillin-resistant *Enterococcus spec.* and *Staphylococcus aureus* (MRE, MRSA) strains. The producer strain of Friulimicin, *Actinoplanes friuliensis*, is a Gram positive soil-inhabiting bacterium which belongs to the group of rare actinomycetes. *A. friuliensis* is a filamentous growing bacterium having a complex life cycle, which includes morphological differentiation.
For the characterization of Friulimicin biosynthesis, *A. friuliensis* was cultivated in a bioreactor under defined and controlled conditions. A chemically defined production medium, especially developed for *A. friuliensis,* was used. This defined medium is a prerequisite for the quantitative analysis of cell metabolism during the cultivations. A new developed middle infrared spectroscopy method (AquaSpec Technology, micro-biolytics GmbH) was applied to analyse substrates and metabolites.
In order to improve the understanding of the complex regulatory network of the friulimicin biosynthesis in *A. friuliensis*, a genome-scale network model will be developed and characterized. To validate the model and to perform metabolic flux analysis, data from the cultivations are integrated into this model. This model should give hints for directed genetically modifications and development of process control strategies with the objective to redirect metabolic fluxes towards Friulimicin production.

GWP012
In vitro characterization of *Escherichia coli* phage K1E RNA polymerase and its *in vivo* application for protein production in *Bacillus megaterium*

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Gene „7" of *Escherichia coli* K1E phage, predicted to encode a DNA-dependent RNA polymerase (RNAP), was cloned and heterologously

expressed in *E. coli*. The corresponding protein was purified by metal-affinity chromatography to >90 % purity and concentrated to 12 mg ml^{-1}. Subsequently, the protein was subjected to an enzyme activity assay demonstrating its functionality as RNAP. Putative promoters for this RNAP within the bacteriophage K1E genome were predicted computationally and summerized in a sequence logo. In *in vitro* transcription experiments the K1E RNAP revealed optima of pH 8, 37°C to 40°C, with a strong dependency on Mg^{2+} ions and a stimulation by spermidine. Further, even low salt concentrations (>30 mM NaCl) inhibited enzyme activity. Based on these results, a system for high-yield *in vitro* RNA synthesis using K1E RNAP was established.
Additionally, new protein production systems for *Bacillus megaterium* were developed based on the K1E RNAP. It was shown, that a system combining the K1E RNAP with a SP6 RNAP promoter produced highest amounts of the intracellular model proteins Gfp (61.4 mg g_{CDW}^{-1}) and the extracellular Tfh (2971 U l^{-1}; 3.2 mg l^{-1}) *in vivo*.
Now, with the help of its just published genome sequence it is possible to characterize bottle necks in the protein production, especially secretion, process of *B. megaterium* by systems biotechnology approaches utilizing microarrays, proteome, metabolome and fluxome data. The bioinformatical platform (MEGABAC, http://www.megabac.tu-bs.de) integrates obtained theoretical and experimental data.

GWP013
Isolation and characterization of methanogenic Archaea from on-farm biogas plants

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Electricity and heat generation from methane-rich biogas often provides the advantages of utilizing renewable energy sources and heat thus reducing the emission of climate-relevant greenhouse gases. Various efforts in raising the efficiency of biogas production were focused on improving the technical aspects. The microbial biocoenosis in general as well as the specific microbial interactions leading to methane formation in biogas plants remains largely a black box. Methanogenic Archaea were isolated from on-farm continuously stirred tank reactor (CSTR) biogas plants. In this study the obtained isolates were compared with biodiversity predictions of culture-independent methods and morphological and physiological characterizations were performed.
Reactor samples from five biogas plants fed with corn and cattle manure were used as an inoculum for enrichment of methanogenic Archaea. To achieve pure cultures, anaerobic variants of the serial dilution- or solid media plating- techniques were applied. Selective growth of hydrogenotrophic, methylotrophic and acetoclastic methanogens was achieved by application of modified DSMZ culture media. Morphological examinations were accomplished by fluorescence microscopy. Culture purity and biodiversity analysis were performed by denaturating gradient gel electrophoresis (DGGE), as well as 16S- rDNA cloning experiments in connection with RFLP. With *Methanobacterium formicicum*, *Methanosarcina mazei*, *Methanosarcina barkeri*, *Methanosaeta concilii* and *Methanoculleus bourgensis* species from four different families could be isolated. Their impact in biogas formation is discussed.

GWP014
Biochemical and genetic characterization of ethylene glycol metabolism in *Pseudomonas putida* KT2440 and JM37

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The large versatility in biosynthetic pathways together with its broad substrate spectra renders *P. putida* a model organism for various industrial applications, such as bioremediation or biocatalysis processes.
We used the *P. putida* strains KT2440 and JM37 for the characterization of ethylene glycol (EG) metabolism with the overall goal to develop a biocatalytic route for the synthesis of glyoxylic acid (GXA), a proposed intermediate in the metabolism of EG. Being an important building block for flavors and polymers, GXA is a valuable product for many industrial processes. Since production of GXA is currently limited to chemical synthesis, a biotechnical production route is of great economical interest.
In contrast to strain KT2440, we could demonstrate that *P. putida* JM37 was able to use EG as well as GXA as sole source of carbon and energy. Despite this difference, dense cell suspension experiments revealed complete conversion of 50 mM EG and GXA for both strains within 50 h. During conversion of EG, accumulation of 4.1 mM glycolic acid (GCA), 12.8 mM GXA, and 13.7 mM oxalate (OXA) was detected in supernatants of strain KT2440. To identify enzymes involved in the metabolism of EG in KT2440 and JM37, a differential proteomic approach was used.
Increased expression of tartronate semialdehyde synthase (Gcl), malate synthase (GlcB) and isocitrate lyase (AceA) in JM37 as well as AceA in strain KT2440 was found during incubations with EG or GXA. These proteins represent key enzymes of known pathways involved in the metabolism of GXA. The corresponding triple mutant strain harboring an additional deletion of the gene *prpB,* encoding for methyl isocitrate lyase, was constructed in strain KT2440 and characterized for GXA accumulation. This mutant strain possessed a significant reduction in its EG conversion rate compared to the wildtype strain and was found to accumulate up to 15 mM GCS, 11.2 mM GXS and 8.6 mM of OXA. Further analysis uncovered the induction of two PQQ-dependant ethanol dehydrogenases [pp_2674, pp_2679], indicating an important role for these proteins within the oxidative metabolism of EG. A double deletion mutant of the two isoenzymes in KT2440 resulted in a dramatic decrease in EG metabolism. The absence of GCS and GXS accumulation in this mutant further highlights the importance of these enzymes for EG metabolism.

GWP015
Production of lignin-modifying enzymes *via* co-cultivation of white-rot fungi

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Lignin, the third most abundant polymer present in nature, is expected to play an important role as raw material for the world's biobased economy in the post-oil era. White-rot fungi are efficient lignin degraders, which makes them ideally suited for industrial applications where phenolic compounds such as lignin must be altered or removed. Most biodegradation processes in nature take place by division of work of different microorganisms in complex ecosystems. However, most of what known about lignin biodegradation is from pure culture studies with basidiomycete fungi. Co-cultivation approaches for production of lignin modifying enzymes with white-rot fungi have been paid little attention.
With this work we investigated the effects of co-culturing of white-rot fungi on lignin-modifying enzyme production. In a prescreening concerning paired growth characterisation and ligninolytic ability *Bjerkandera adusta, Dichomitus squalens, Hypoxylon fragiforme, Phlebia radiate, Pleurotus eryngii* and *Pleurotus ostreatus* were cultured in pairs on PDA agar plates or rather Remazol Brilliant Blue R dye containing agar plates. The degree of decolourization was clearly stimulated due to mycelia interactions.
Combinations of species with good prospects were studied under submerged co-cultivation concerning the production of the three main lignin-modifying enzymes, laccases (EC 1.10.3.2), lignin peroxidases (EC 1.11.1.14) and manganese peroxidases (EC 1.11.1.13). Compared with the monocultures, co-cultures of *P. radiate* with *D. squalens* and *P. ostreatus* with *P. radiate* showed positive effects on production of lignin modifying enzymes.
Current work is using secretomic approaches in connection with activity overlay detection and MALDI-TOF-MS peptide mapping to characterize molecular differences of lignin modifying enzyme expression between the co-cultures and the monocultures.

GWP016
O-demethylenation catalyzed by fungal aromatic peroxygenases

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The (methylenedioxy)phenyl group (Phe^1-O-C-O-Phe^1) is found in pharmacologically active compounds, insecticides and diverse products of the secondary plant metabolism. In principle this group protects a reactive catechol (OH-Phe^1-OH) and is therefore suggested to increase the half life (action, toxicity etc.) of the respective substance. In human, this moiety is predominately oxidized by cytochrome P450s (P450s) to yield a reactive catechol that can be further metabolized. Here we report that fungal aromatic peroxygenases (APOs) secreted by *Agrocybe aegerita*, C*oprinellus radians* and *Coprinopsis verticillata* are able to oxidize the methylenedioxy group to give the corresponding catechols. The peroxygenases oxidized various 1,3-benzodioxoles including natural compounds such as pisatin, safrole and myristicin as well as the entactogenic drug methylenedioxymethamphetamine (ecstasy). Moreover, we could show that a colorless aqueous solution of 1,2-(methylenedioxy)-4-nitrobenzene turned immediately into a bright yellow at pH 7 caused by the formation of 4-nitrocatechol. This reaction was exploited to develop a peroxygenase assay for the rapid spectrophotometric and colorimetric characterization of this reaction. Moreover, steady-state kinetics results with 1,2-(methylenedioxy)-4-nitrobenzene, gave parallel double reciprocal plots suggestive of a ping-pong mechanism and the presence of $H_2{}^{18}O_2$ gave no incorporation of ^{18}O into the catechol group of the resulting 4-nitrocatechol, which points to an mechanism similar to that observed in P450s. Our results show that fungal peroxygenases are able to oxidize the methylenedioxy group, which is a physiological interesting reaction and can be a useful tool in the screening of fungi and other microorganisms for extracellular peroxygenase activities.

GWP017
Production of human drug metabolites with fungal peroxygenases

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The synthesis of oxygenated/hydroxylated human drug metabolites via selective monooxygenation is still a challenging task for the synthetic chemist. Here we report that aromatic peroxygenases (APOs) secreted by agaric fungi such as *Agrocybe aegerita* (*Aae*APO) and *Coprinellus radians* (*Cra*APO) catalyze the H_2O_2-dependent selective monooxygenation of diverse drugs. The results showed that the reactions proceeded regioselectively, giving isomeric purities of 99% and yields up to 90% of the desired metabolites. Mass spectral analysis of the metabolites formed during the *Aae*APO-catalyzed oxidation of tolbutamide in the presence of $H_2{}^{18}O_2$ in place of H_2O_2 showed a shift of the principal $[M+H]^+$ ion from m/z 287 to m/z 289 in case of 4-hydroxytolbutamide, which points to a true peroxygenase mechanism. Moreover, the intramolecular deuterium isotope effect $[(k_H/k_D)obs]$ of the peroxygenase-catalyzed *O*-dealkylation of *N*-(4-[1-^{2}H]ethoxyphenyl)-acetamide was 3.1 ± 0.2, which indicate a P450-like reaction mechanism. Interestingly, both enzymes oxidized the estrogen receptor antagonist tamoxifen to 4-hydroxytamoxifen, endoxifen and *N*-desmethyltamoxifen, whereas oseltamivir (Tamiflu®) was only oxidized by *Cra*APO (80% conversion into the ester cleavage product oseltamivir carboxylate). Our results clearly indicate that fungal peroxygenases may be a useful biocatalytic tool to prepare diverse pharmacologically relevant metabolites.

GWP018
Analysis on biotinylation and biotin metabolism in *Corynebacterium glutamicum*

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The biotin auxotrophic bacterium *Corynebacterium glutamicum* is used for large-scale production of amino acids. In the genome of this organism the genes genes *bioA, bioD* and *bioB* encoding for a fragmentary biotin synthesis pathway are annotated, as well as a gene cluster showing high identity on protein level to a biotin transport system (*bioYMN*) from *R. etli* [1]. *C. glutamicum* also possesses a gene that is annotated as a putative biotin protein ligase gene (*birA*). Here we describe the functional analysis of *bioYMN* and *birA*.

Since glutamate production of *C. glutamicum* is triggered by biotin limitation the influence of biotin was analysed with a *bioYMN* overexpressing strain. It could be shown that overexpression of the gene cluster led to a two fold decrease in yield of glutamate production per biomass ($Y_{p/x}$) under biotin limiting growth conditions. This corroborates the assumption that *bioYMN* encodes a biotin transport system in *C. glutamicum*.

In *E. coli* the biotin genes are regulated by the bifunctional BirA protein, which is active as biotin-protein ligase and as transcriptional repressor of the *bio*-genes [2]. BirA from *C. glutamicum* lacks an N-terminal DNA-binding domain and is not regulating biotin metabolism, as it was shown by transcriptome analysis. In order to characterize the function of BirA from *C. glutamicum*, an enzyme assay was developed. A short (105 aa) His-tagged biotin-carrier-protein (BCCP) was constructed from the AccBC subunit (591 aa) of acetyl-CoA carboxylase from *C. glutamicum* and isolated via Ni-NTA affinity chromatography. This BCCP protein was used as substrate for BirA in a discontinuous assay, and it could be shown that BCCP was biotinylated dependent on the over expression of the *birA* gene. Therefore it can be concluded, that *birA* encodes biotin protein ligase in *C. glutamicum*.

Moreover, *birA* expression was analysed in regard to growth and lysine production, and it could be shown, that overexpression of *birA* resulted in a significant growth advantage, both, on glucose and lactate as sole carbon source and, compared to the control strain, resulted in a higher lysine yield on glucose.

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GWP019
Metabolic Engineering of *E. coli* HB101 for acetone production

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In the ABE fermentation by *Clostridium acetobutylicum* a mixture of solvents is produced (acetone, butanol, ethanol). The heterologous expression of the corresponding genes in an industrial production strain such as *Escherichia coli* is one possibility to yield acetone as the only product.

We established a metabolic pathway for an acetate independent acetone formation in *E. coli*. The production is based on plasmid-mediated expression of thiolase A (ThlA) and acetoacetate decarboxylase (Adc) from *Clostridium acetobutylicum* in combination with YbgC from *Haemophilus influenzae*. YbgC showed thioesterase activity *in vitro* with acetoacetyl-CoA as substrate. The corresponding gene *ybgc* was cloned together with *thlA* and *adc* from *C. acetobutylicum* as an operon under control of the *lac* promoter. Among several strains, production of acetone up to 66 mM (3.8 g/l) could be demonstrated in strain *E. coli* HB101.

To increase the production of acetone, several specific genes on the chromosome of strain HB101 were knocked-out. As a first step, we chose such genes that code for proteins that use acetyl-CoA itself or earlier intermediates of the glycolysis as substrate. The aim was to increase the availability of acetyl-CoA as the substrate of acetone way, so that a possibly excess could lead to a higher acetone production.

The genes were replaced by a FRT-flanked resistance cassette, which was removed by a FLP-recombinase step. Resulting clones were tested for acetone production. Indeed, the *ppc* mutant (PEP carboxylase), the *gltA* mutant (citrate synthase) and the double mutant *gltA*/*ppc* (citrate synthase/PEP carboxylase) showed higher amounts of acetone. In comparison to the HB101 strain, which produced 6.4 g/l acetone in 100 ml cultures, these strains produced 7.0 g/l (Δ*ppc*), 9.8 g/l (Δ*gltA*) and 9.5 g/l (Δ*gltA*/Δ*ppc*) acetone respectively.

GWP020

Corynebacterium glutamicum engineered as a designer bug for the production of pyruvate

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Corynebacterium glutamicum is a non-pathogenic, Gram-positive organism that grows on a variety of substrates and is used for the production of amino acids, e.g. L-glutamate, L-lysine and L-valine, as well as organic acids, e.g. lactic and succinic acid. The aim of the present work was to engineer *C. glutamicum* to produce pyruvate. The resulting strain is supposed to be used as a platform for production strains of amino acids and other organic acids derived from pyruvate, e.g. dicarboxylic acids of the citric acid cycle. These might be used as precursors for a variety of bulk chemicals and commercially important polymers, which are these days produced primarily from petrochemicals via chemical synthetic processes.
In our study we modified *C. glutamicum* for the production of pyruvate and decreased formation of byproducts (e.g. amino acids). By stepwise inactivation of the pyruvate dehydrogenase complex, the pyruvate:quinone oxidoreductase, the L-lactate dehydrogenase and attenuation of the acetohydroxyacid synthase [AHAS] by deleting the C-terminal domain of its regulatory subunit, efficient pyruvate production was achieved. The deletion of the genes encoding alanine aminotransferase and pyruvate:valine aminotransferase led to a strong reduction of the side product L-alanine and together with the attenuation of the AHAS to decreased L-valine formation (below 5 mM). Above all, we observed efficient pyruvate formation up to nearly 200 mM in shake flask experiments, with a yield of ~0.7 g pyruvate per g of glucose. The most critical step for pyruvate formation is the attenuation of the AHAS. In fed-batch fermentations with the newly constructed *C. glutamicum* strain, final pyruvate concentrations of more than 500 mM have been observed. Thus, the strain represents an efficient pyruvate producer and an ideal platform for pyruvate-derived metabolites.

GWP021

Extension of the substrate utilization range of *Ralstonia eutropha* strain H16 for mannose and glucose by metabolic engineering

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The Gram-negative facultative chemolithoautotrophic bacterium *Ralstonia eutropha* strain H16 is known for its narrow carbohydrate utilization range which limits its use for biotechnological production of PHAs and possibly other products from renewable resources. To broaden its substrate utilization range, which is for carbohydrates and related compounds limited to fructose, N-acetylglucosamine and gluconate, strain H16 was engineered to use mannose and glucose as sole carbon sources for growth. The genes for a facilitated diffusion protein (*glf*) from *Zymomonas mobilis* and for a glucokinase (*glk*), mannofructokinase (*mak*) and phosphomannose isomerase (*pmi*) from *Escherichia coli* were alone or in combination constitutively expressed in *R. eutropha* strain H16 under control of the *neokanamycin*- or *lac*-promoter, respectively, using an episomal broad host range vector. Recombinant strains harboring pBBR1MCS-3*::glf::mak::pmi* or pBBR1MCS-3*::glf::pmi* grew on mannose, whereas pBBR1MCS-3*::glf::mak* and pBBR1MCS-3*::glf* did not confer the ability to utilize mannose as carbon source to *R. eutropha*. The recombinant strain harboring pBBR1MCS-3*::glf::pmi* exhibited slower growth on mannose than the recombinant strain harboring pBBR1MCS-3*::glf::mak::pmi*. These data indicated that phosphomannose isomerase is required to convert mannose-6-phosphate into fructose-6-phosphate for subsequent catabolism via the Entner-Doudoroff pathway. In addition, all plasmids conferred to *R. eutropha* also the ability to grow in presence of glucose. Best growth was observed with a recombinant *R. eutropha* strain harboring plasmid pBBR1MCS-2::P_{nk}*::glk::glf*. In addition, expression of the respective enzymes was demonstrated at the transcriptional and protein level and by measuring the activities of mannofructokinase (0.622 U mg^{-1} ± 0.063 U), phosphomannose isomerase (0.251 U mg^{-1} ± 0.017 U), and glucokinase (0.518 U mg^{-1} ± 0.040 U). Cells of recombinant strains of *R. eutropha* synthesized poly(3-hydroxybutyrate) to about 65% - 67% (wt/wt) of cell dry mass in presence of 1% (wt/vol) glucose or mannose as sole carbon sources.

GWP022

Conversion of 3-sulfinopropinonyl-CoA, a structural analogue of succinyl-CoA, to propionyl-CoA in *Advenella mimigardefordensis* strain DPN7^T

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Degradation of 3,3-dithiodipropionate (DTDP), a sulfur containing precursor substrate for polythioester production, was investigated in *Advenella mimigardefordensis* strain DPN7^T. This bacterium was isolated due to its ability to utilize DTDP as a sole source of carbon and energy [1]. DTDP is initially cleaved by a disulfide-reductase (LpdA) into two molecules of 3-mercaptopropionic acid [2]. In the next step a thiol dioxygenase (Mdo) catalyzes the oxidation to 3-sulfinopropionate (3-SP), which is thereafter activated to the corresponding 3-SP-CoA thioester by a succinyl-CoA synthetase (SCS) [3, 4]. A Tn*5*::*mob*-induced mutant, defective in growth on DTDP and 3-SP, was genotypically characterized. The transposon insertion was mapped in an open reading frame with highest homologies to an acyl-CoA dehydrogenase (CaiA) from *Verminephrobacter eiseniae* strain EF01-2 (63 % identical amino acids). A defined Δ*caiA* mutant verified the observed effects in the Tn*5*::*mob* induced mutant. For enzymatic studies CaiA was heterologously expressed in *E. coli* using pET23a::*caiA*. The purified enzyme catalyzed the conversion of 3-SP-CoA to propionyl-CoA. It is therefore a novel reaction for the abstraction of sulfur. FAD, as a putative cofactor of CaiA, has been isolated from the purified protein and its identity was confirmed via HPLC-ESI-MS.

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GWP023

Enhancing the Biodesulfurization of Dibenzothiophene with *Rhodococcus erythropolis* IGTS8 using Synthetic Surfactants

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Background: Combustion of fossil fuels releases hazardous emissions like SO_2 into the environment. This is due to the presence of high amounts of organosulfur compounds such as dibenzothiophene (DBT) and its alkylated derivatives. Strict environmental regulations imply that the amount of sulfur in transportation fuels be drastically reduced. The petroleum industry relies on hydrodesulfurization to remove sulfur from petroleum -derived fuels. This technique is costly, not completely efficient, and even environmentally polluting. Biodesulfurization with dedicated microorganisms has been proposed as an environmentally friendly and cost effective alternative or complement. However, large scale application of microbial desulfurization is limited by the low biocatalytic efficacy.
Aim of the work: In this work, *Rhodococcus erythropolis* IGTS8 was adopted to study the influence of some synthetic surfactants on the biodesulfurization activity using DBT as a model substrate.
Methods: All experiments were conducted in mineral salts medium containing glucose and dibenzothiophene as a sole sulfur source in the presence or absence of surfactants. Cell-free culture supernatants were analyzed by HPLC to monitor the consumption of DPT and the formation of the dead end product 2-hydroxybiphenyl.
Results: Among the tested surfactants, SDS was chosen to complete the studies because it did not inhibit the growth of the IGTS8 strain. The IGTS8 strain grew on glucose in the presence of SDS as a sole sulfur source after a lag period of 3 days. However, The IGTS8 strain was not able to utilize SDS as a sole carbon source even after 2 weeks of incubation. The data also showed that the biodesulfurization enzymes of the 4S pathway are functional in the presence of different concentrations of SDS. Cultures growing on DBT in the presence of 1000 ppm of SDS transformed DBT faster than control cultures lacking SDS.
Conclusion: The surfactant SDS improved the biodesulfurization activity of the IGTS8 strain. The potential role of SDS in the biodesulfurization process appears to be promoting the solubility of the hydrophobic substrate, DBT, in water. This would improve the mass transfer and, consequently, lead to enhanced biodesulfurization rates.

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GWP024
Identification and characterization of a 1,3-propanediol oxidoreductase from *Pectobacterium atrosepticum*

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The compound 1,3-propanediole (1,3-PD) is a valuable chemical for polyester production used in textile fiber, film and plastic industry. It is routinely converted from acrolein or ethylene oxide via 3-hydroxypropionaldehyde (3-HPA) using chemical approaches. Since the chemical synthesis of 1,3-PD produces toxic intermediates and is highly expensive, much effort has been spent on its microbiological production. In nature, anaerobic microbial metabolism of glycerol involves a reductive pathway, enabling the NADH-dependent formation of 1,3-PD and a coupled oxidative pathway, which generates the reducing power for the reductive branch. In a first reaction step, the conversion of glycerol to 3-HPA is catalyzed by the enzyme glyceroldehydratase. Finally, 1,3-propanediol oxidoreductase (PDOR) reduces 3-HPA to 1,3-PD.
To identify biocatalysts with novel properties for the production of 1,3-PD, we performed BLAST searches using the sequences of PDOR from bacteria of the genera *Citrobacter*, *Clostridium* and *Klebsiella*, which are known to convert glycerol to 1,3-PD. In addition, the sequence of the gene *yqhD* from *Escherichia coli* was used as query. This open reading frame encodes a NADPH-dependent aldehyde reductase capable of catalyzing the formation of 1,3-PD from 3-HPA. Homologues of PDOR and YqhD were identified in the genome of the facultative anaerobic bacterium *Pectobacterium atrosepticum*. Both genes were cloned into pQE30 and pQE80 expression vectors and were purified after heterologous production in *E. coli*. Results on the characterization of the enzyme, including physicochemical and kinetic properties, will be presented.

GWP025
Expression of metagenomic membrane-bound dehydrogenases from acetic acid bacteria: The design of new oxidative catalysts.

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Acetic acid bacteria are used in biotechnology due to their ability to incompletely oxidize a great variety of carbohydrates, alcohols and related compounds in a regio- and stereo-selective manner. Most of these reactions are catalyzed by membrane-bound dehydrogenases with a broad substrate range.
Acetic acid bacteria contain a multitude of such dehydrogenases and many of them cannot be grown as pure cultures. Therefore we expect habitats rich in acetic acid bacteria, such as a mother of vinegar to be good sources of uncharacterized metagenomic dehydrogenases of potential value for biotechnology. We investigated the diversity of several mothers of vinegar by 16S rDNA sequencing as a preparation to construct metagenomic libraries.
The metagenomic membrane bound dehydrogenases will be screened by sequence similarity and functionally expressed in tailor made *Gluconobacter oxydans* strains devoid of their own dehydrogenases to avoid overlapping specificities. Expression in an acetic acid bacterium should facilitate functional integration in the membrane physiology of these organisms. To achieve this goal we developed a clean deletion system for *Gluconobacter* strains based on 5' fluorouracil counter selection. This system was used to delete various genes. Furthermore we developed the shuttle vector (*E. coli-G. oxydans*) system pKOS4 that is needed for the expression of metagenomic dehydrogenases controlled by natural constitutive and inducible promoters of such enzymes.
As currently very little is known about the promoters of membrane-bound dehydrogenases we investigated the regulation and transcription start point of different dehydrogenases in *G. oxydans* 621H.

GWP026
Modification of the fatty acid composition of the bacterial membrane of *Rhodobacter capsulatus*

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Unsaturated or functionalized fatty acids are used for a multitude of biotechnological applications. As these compounds can only be found in trace amounts in their natural source and chemical synthesis is not efficiently feasible, they are usually produced via biocatalytic processes. To produce fatty acids with potentially high biological activity, fatty acid-modifying enzymes are widely used for their functionalization. However, enzymatic functionalization of fatty acids is mostly limited by the availability of substrates as many fatty acid modifying enzymes only specifically convert defined acyl chains of membrane phospholipids.
Recently we developed a novel expression system which is based on the photosynthetic bacterium *R. capsulatus* [1-3]. In contrast to standard expression hosts, *R. capsulatus* is particularly suited for the heterologous expression of membrane proteins because it forms an extensive system of intracytoplasmic membranes (ICM) during phototrophic growth. Since ICM formation basically allows accommodation of heterologous membrane proteins as well as efficient storage of phospholipids, we now tested if the fatty acid composition of the bacterial membrane can be modified in order to biotechnologically produce functionalized fatty acids in high amounts. Here we demonstrate that fatty acids of different chain length and degree of unsaturation that have been supplemented to the growth medium were efficiently integrated into the *R. capsulatus* membrane. Furthermore, the incorporation efficiency of foreign fatty acid could be significantly increased by specific inhibition of the biosynthesis of endogenous unsaturated fatty acids.

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GWP027
A pathway transfer system that facilitates the heterologous expression of large gene clusters in a broad range of bacterial hosts

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To access valuable natural substances synthesized by microorganisms it is necessary to establish complex biosynthetic pathways in heterologous bacterial hosts. However, several limitations associated with cloning, transfer, stable maintenance and functional expression of all pathway genes retain this process challenging. In order to overcome these limitations we developed a novel biosynthetic pathway transfer and expression system, which facilitates the expression of unmodified large gene clusters in different heterologous hosts.
The novel *in vivo* auto cloning and expression system (IVAC) consists of two different cassettes, named L- and R-IVAC. The two cassettes comprise structural elements allowing (i) the conjugational transfer of large DNA fragments encompassing all genes of interest into the expression host, (ii) the integration of the IVAC-labelled gene cluster into the host chromosome via transposition, and (iii) the expression of all target genes irrespective of their orientation and natural DNA elements that might affect their coordinated expression in the respective host strain.
Using a carotenoid biosynthetic gene cluster we could demonstrate that the IVAC-system is a powerful tool that allows the concerted functional expression of clustered genes in different bacterial hosts.

GWP028

Application of the soluble NAD^+-reducing hydrogenase (SH) of *Ralstonia eutropha* H16 for solar-driven H_2-production in cyanobacteria

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Hydrogenases catalyze the reversible formation of $2e^-$ and $2H^+$ from H_2 [1]. Hydrogen is discussed as a promising renewable fuel replacing fossil energy carriers in the future. Therefore, the H_2-production capability of these enzymes is of significance for biotechnological applications.
Theoretically cyanobacteria are ideally suited to produce H_2 from sunlight and water since they generate „high-potential" electrons during photosynthesis, which could be used directly by hydrogenases for hydrogen production.
Indeed many cyanobacteria such as *Synechocystis* sp. PCC 6803 and *Synechococcus* PCC 7002, possess so called bidirectional [NiFe]-hydrogenases of the H_2:$NAD(P)^+$-oxidoreductase type. In nature, these enzymes evolve relatively small amounts of H_2 from accumulating reductant in form of NADH and NADPH under O_2-limiting conditions [2].
In order to increase the amount of H_2 and to enable continuous production of H_2 also in the presence of O_2, we are currently investigating the heterologous synthesis of the soluble NAD^+-reducing hydrogenase (SH) of the soil bacterium *Ralstonia eutropha* H16 in cyanobacteria. This bidirectional [NiFe]-hydrogenase is known to maintain H_2-conversion at high concentrations of O_2 and has already been characterized intensively by various biochemical and spectroscopic methods [3,4].
Conditions for heterologous production of functional *R. eutropha* SH in *Synechocystis* PCC 6803 are currently being explored. Very recently, SH production in the cyanobacterium *Synechococcus* PCC 7002 has been demonstrated with a new heterologous expression strategy [5,6] and is subject of comprehensive characterisation and further optimisation in our group.
Furthermore, we aim to uncover the molecular basis for the exceptional O_2-tolerance of the *R. eutropha* SH. Ongoing analysis focuses on the amino acid coordination of the Fe-S-cluster in the small e^--transferring hydrogenase subunit of the SH. The mechanism of O_2-tolerance provides essential knowledge to convert an O_2-sensitive cyanobacterial hydrogenase into an enzyme that produces H_2 directly from sunlight and water in the presence of oxygen.

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GWP029

Engineered salt-induced ectoine promoter for use in *H. elongata* as halophilic expression system

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Heterologous protein expression is commonly carried out in *E. coli*, but often limited by formation of inclusion bodies or unsatisfactory protein stability. Heterologous expression systems in the presence of salt and compatible solutes have previously been applied to demonstrate the potential of including stabilizing/protecting solutes in the process of functional recombinant protein expression [1]. As *E. coli´s* capacity to tolerate salt-stress is limited, *Halomonas elongata*, a moderate halophilic gamma proteobacterium of broad salt tolerance, has been proposed as an alternative [2].
Halomonas elongata can grow over a salinity range of 1-20% NaCl and is able to adjust osmotic pressure by the intracellular accumulation of the protein-protecting osmolytes ectoine and S,S-beta hydroxyectoine. The biosynthesis of these solutes is under the control of a salt-inducible promoter region, promA. We present here the construction of a pBBR1-derived vector containing an engineered version of the promA promoter. By modifying the ribosomal binding site, we obtained a suitable vector (pWUB1) for salinity-controlled protein expression in *H. elongata*.
Using GFP_{UV} as a reporter gene, we monitored the expression pattern controlled by the modified promoter region and also investigated the influence of osmotic stress and the presence of compatible solutes for fine-tuning of promoter regulation.

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GWP030

Synthesis of citrulline-rich cyanophycin by use of *Pseudomonas putida* ATCC 4359

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The provision of cyanophycin (multi-l-arginyl-poly-l-aspartic acid, CGP) as a putative precursor for biodegradable technically employed chemicals makes it important to synthesise CGP in recombinant organisms. Furthermore derivates of CGP, harbouring other constituents, are of special interests for further research. As shown previously, cyanophycin synthetases with wide substrate ranges are able to incorporate other amino acids than arginine like citrulline and ornithine, but are still dependent on additional supplementation of these amino acids in order to achieve sufficient incorporation rates [2]. Therefore, using an organism which produces the needed supplement by itself, was the next logical step. *Pseudomonas putida* strain ATCC 4359 is such an organism because it was previously shown that it produces large amounts of L-citrulline from L-arginine [1]. Synthesis of CGP in this *P. putida* strain was achieved by expressing the cyanophycin synthetase of *Synechocystis* sp. PCC 6308. Using an optimised media for cultivation, the strain was able to synthesise insoluble CGP amounting up to 14.7 ± 0.7 % (w/w) and soluble CGP amounting up to 28.7 ± 0.8 % (w/w) of the cell dry matter, leading to an overall CGP-content of 43.5 %. The occurrence of soluble CGP was dependent on the temperature during cultivation. HPLC-analysis of the soluble CGP showed that it was composed of 50.4 ± 1.3 mol % aspartic acid, 32.7 ± 2.8 mol % arginine, 8.7 ± 1.6 mol % citrulline and 8.3 ± 0.4 mol % lysine, while the insoluble CGP contained amounts of less than 1 mol % of citrulline. Using mineral salt media with 1.25 or 2 % (w/v) Na-succinate, respectively, and 23.7 mM L-arginine, this strain synthesised amounts of 25 to 29 % of the CDM insoluble CGP showing only a very low citrulline content of less than 1 mol %.

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GWP031

Functional genomics of the prophage CGP3 causing population heterogeneity in *Corynebacterium glutamicum*

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The Gram-positive soil bacterium *Corynebacterium glutamicum* ATCC13032 is one of the most important organisms in White Biotechnology as it is used for the industrial production of more than two millions tons of amino acids per year. Genome sequencing of *C. glutamicum* revealed the existence of three prophages (CGP1, CGP2 and CGP3) highly diverse in size and grade of degeneration. The largest prophage CGP3 (187 kbp) accounts for 6% of the genome and is inserted in a cluster of tRNA genes.
Recent studies demonstrated via a fluorescence microscopy approach that the CGP3 prophage exhibits spontaneous induction in a small fraction of *C. glutamicum* wild type cells. Upon induction CGP3 excises from the genome and exists as a circular double-stranded DNA molecule (Frunzke *et al.*, 2008). In several cases, phage induction was accompanied by lysis of the cells suggesting the expression of functional phage lysins. Therefore, studies were initiated in order to understand spontaneous CGP3 induction leading to population heterogeneity and cell lysis in *C. glutamicum* cultures.
A first series of experiments focused on the identification of the putative phage regulator controlling the lysogenic/lytic switch of CGP3. A potential candidate is the putative transcriptional regulator Cg2040 encoded by the CGP3 genome. Transcriptome comparisons of a strain overexpressing Cg2040 and *C. glutamicum* wild type resulted in significantly reduced mRNA levels of five phage genes, which are located next to or in close vicinity of cg2040. Subsequently, binding of purified Cg2040 to putative target promoters was verified via electrophoretic mobility shift assay (EMSA). Furthermore, first results will be provided concerning the functionality of the putative phage integrase Int2 of CGP3. These efforts

finally aim at the inactivation of CGP3 in *C. glutamicum* in order to obtain strains with enhanced genomic stability and improved performance in industrial production processes.

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GWP032
Production of the lantibiotic mersacidin by *Bacillus amyloliquefaciens* FZB42

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Mersacidin belongs to a group of ribosomally synthesized peptide antibiotics called lantibiotics. Lantibiotics are characterized by the presence of the unusual amino acids lanthionine and methyllanthionine that are introduced via extensive posttranslational modifications. Genes coding for the prepeptide and for proteins involved in modification, processing, transport, regulation and immunity are organized in gene clusters.
Mersacidin (MW: 1824 Da) consists of 20 amino acids and is produced by *Bacillus spec*. HIL Y-85,54728. Its antimicrobial activity is due to complex formation with the peptidoglycan precursor lipid II, thereby inhibiting cell wall biosynthesis. It exerts interesting antimicrobial activities against human pathogens, including MRSA strains.
Since the mersacidin producer strain is not easily transformable, the aim of this study was the production of mersacidin in the naturally competent *B. amyloliquefaciens* FZB42. A blast analysis in the NCBI database identified the 5'part of the *mrs* gene cluster including the genes for immunity and regulation (*mrsEFG; mrsKR2*) within the genome sequence of this strain. These genes are located in the same region as in the wildtype producer. MIC determinations showed that *B. amyloliquefaciens* FZB42 exhibits immunity to mersacidin at the same level as the wild type producer. The completion of the gene cluster was performed using genomic DNA of a wild type producer mutant (*B. spec*. rec1) that is characterized by an exchange of the structural gene (*mrsA*) for *ermB* and *mrsA* was reconstituted *in trans*. Production of active mersacidin was confirmed by agar well diffusion assays, HPLC and MALDI-TOF analysis. Genomic sequences obtained from the wild type producer showed a high identity (98.5%) to corresponding genes of *B. amyloliquefacien*s FZB42. Furthermore, metabolic profiling and 16sRNA sequencing also indicate that *B. spec*. HIL Y-85,54728 might be a member of the species *B. amyloliquefaciens*.

GWP033
Biotechnical production of homopolythioester applying 3,3´-dithiodipropionic acid as precursor substrate and a recombinant strain of *Advenella mimigardefordensis*

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In the past, homopolymers of polythioester (homo-PTE) could only be produced using recombinant strains of *Escherichia coli* and more or less growth inhibitory 3-mercaptoalkanoates [1]. Nontoxic and more stable PTE precursors would be valuable for high-scale biotechnical applications. By adapting the knowledge unraveled for the 3,3´-dithiodipropionic acid (DTDP) catabolism and pathway in *A. mimigardefordensis* strain DPN7^T, an alternative homo-PTE production strain was successfully constructed. Cells of *A. mimigardefordensis* cleave DTDP symmetrically into two molecules of 3-mercaptopropionic acid (3MP) [2, 3]. 3MP is the building block of the basic homo-PTE poly(3MP). However, in *A. mimigardefordensis* wild type cells, a 3MP dioxygenase (Mdo) sulfoxygenates emerging 3MP into 3-sulfinopropionic acid, which is afterwards metabolized and used as a carbon source for growth. Deletion of *mdo* and introduction of the artificial BPEC-pathway (genes encoding the butyrate kinase (Buk) and the phosphotransbutyrylase (Ptb) from *Clostridium acetobutylicum* as well as the PHA synthase (PhaEC) from *Thiococcus pfennigii* [4]) resulted in the potent poly(3MP) producing strain *A. mimigardefordensis* Δ*mdo buk_ptb*::*recA* pBBR1MCS5::*phaEC*. This strain synthesized poly(3MP) and accumulated it to more than 15% (wt/wt CDW) of poly(3MP), if cultivated in mineral salts media containing propionate and/or glycerol as utilizable carbon sources in addition to DTDP as the sulfur harboring PTE precursor.

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GWP034
Microbial Production of Single Cell Oil (SCO) from Low-Cost Carbon Sources with the Yeast C*ryptococcus curvatus*

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Oleaginous microorganisms are species of yeasts, molds, bacteria and microalgae which contain more than 20 % lipids in their cellular dry weight, so called single cell oils (SCOs). These SCOs are produced intracellularly in the stationary growth phase under nitrogen limitation with simultaneous excess of a carbon source. The amount of such storage lipids can reach up to 70 % of the cellular dry weight. Some oleaginous microorganisms are known to produce high proportions of polyunsaturated fatty acids (PUFAs) which are important for the food and cosmetic industry. SCOs can be produced from renewable resources without competition with food or feed.
The aim of this study is the setup of a process for the economical production of SCO with the model yeast strain *Cryptococcus curvatus*. For this purpose different low-cost carbon sources, including waste material containing carbohydrates, e.g. raw glycerol from biodiesel production or lignocelluloses waste material, are to be tested regarding to quantity and quality of the produced SCO. Additionally, the influence of process parameters (temperature, pH value, aeration, C/N-ratio) and the influence of amount and manner of nitrogen addition on the quality and quantity of the produced SCO should be examined.

GWP035
Conversion of „alpeorujo" by agaricomycetes and their oxidoreductases

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Mediterranean countries generate high amounts of a solid by-product of the olive oil production, the so-called „alpeorujo" (approximately 800 kg t^{-1} of processed olives). The water soluble fraction of alpeorujo contains polyphenols and monoaromatic hydrocarbons, which are structurally heterogeneous and inhibit microorganisms and plant growth, such as the main monomeric phenols tyrosol and hydroxytyrosol. Proper handling and detoxification practices are, therefore, required if „alpeorujo" is to be revalorized as a potential fertilizer or amendment. Research is needed to understand the transformation mechanisms of the residue and the implications of its use for agricultural purposes.
Wood and leaf-litter colonizing Basidiomycotina (mostly Agaricomycetes) are the most efficient degraders of persistent natural polymers. They secrete extracellular oxidoreductases like class II peroxidases (e.g. manganese peroxidase - MnP, lignin peroxidase - LiP, versatile peroxidase - VP) and laccases (Lac). These biocatalysts are thought to be the key enzymes of lignin degradation and due to their broad substrate spectrum and enzymatic stability they are of particular interest as potential catalysts for bioremediation purposes.
In the present work we demonstrate the effects of *in-vivo* and *in-vitro*-conversion of „alpeorujo" by wood-degrading fungi (e.g. *Bjerkandera adusta* and *Auricularia auricula-judae*). Besides stimulating effects on the enzyme secretion (e.g. up to 55 U ml^{-1} MnP of *B. adusta* and 2000 U g^{-1} MnP of *A. auricula*) by this agricultural waste material, we found a significant decrease in total phenolic content (~90%) during liquid and solid fermentation of „alpeorujo". These findings are correlated by a distinct shift of water-soluble aromatics (detected at 260 nm) from low mass (3 kDa) to higher molecular mass (30 kDa) fractions, measured by HPLC-SEC, which implicates a polymerization process. *In-vitro*-conversion of the phenol-rich „alpeorujo" with purified MnP and Lac resulted in a decrease of the total

phenol content (23% and 55% respectively) in combination with a characteristic shift of the fragmentation pattern of water-soluble aromatics. Our results show that the phenol-rich biopolymer stimulates the secretion of extracellular fungal biocatalysts (e.g. MnP), which finally leads to a decomposition effect of agricultural waste material suitable for agricultural purposes.

GWP036

Heterologous production of the non-proteinogenic amino acid L-pipecolic acid in *Corynebacterium glutamicum*

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The non-proteinogenic amino acid L-pipecolic acid is found as a building block in the structure of many microbial secondary metabolites such as the immunosuppressants rapamycin and FK506 or the antibiotics pristinamycin and friulimicin [1]. Due to its ability to introduce reverse turns in peptides, pipecolic acid increases the stability and potency of such compounds. The non-racemic production of the amino acid is catalyzed by lysine cyclodeaminases such as the Pip protein in the friulimicin biosynthesis (2). The *pip* gene of the friulimicin biosynthetic gene cluster was heterologously expressed both in *Escherichia coli* and different strains of the industrial lysine producer *Corynebacterium glutamicum* (wildtype, DlysE mutant, DM1730). The functionality of corresponding His- and MalE-tagged recombinant enzymes was shown by the detection and quantification of L-pipecolic acid production by thin layer chromatography. The best result was found in *C. glutamicum* DM1730 carrying the MalE-tagged Pip protein. Due to its positive characteristics, L-pipecolic acid represents a useful building unit for production of bioactive natural or synthetic peptides. According to our results, *C. glutamicum* DM1730 seems to be a suitable heterologous host for a prospective biotechnological production of this unusual amino acid.

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GWP037

Enzymatic and chemical modification of biosurfactants

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There has been an increasing interest in biologically produced surfactants such as Sophorolipids and Rhamnolipids. Rhamnolipids are produced by *Pseudomonas aeruginosa* when grown on glycerol, triglycerides or n-alkanes. Sophorolipids are produced by *Candida bombicola* in high yields [1]. These substances are able, e.g., to enhance the biodegradation of hydrocarbons in soil [2].
In this study we are interested in modifying microbial glycolipids in order to get additional interesting properties such as improved surface/ interfacial activity or bioactivity.
Starting with these biosurfactants we try to achieve the sophorose, di-rhamnose and mainly the uncommen fatty acids by hydrolysis. Herefore, we want to use chemical hydrolysis to get the β-hydroxydecanoic (RL) acid and 17-hydroxyoctadecenoic (SL). Enzymatic hydrolysis will be used for the 3-(3-hydroxydecanoyloxy) decanoic acid. These first products shall be used as building blocks for the syntheses of new glycolipids using various glycosidases and/or lipases to show if the special surface/interface activity and bioactivity is founded in the fatty acids or in the unusual sugars.
The new glycolipids will be purified and afterwards characterized concerning their molecular structures (NMR, mass spectrometry, elemental analysis). Additionally, we plan to determine their antimicrobial and other bioactive properties, e.g. anti-tumor promoting activity (in cooperation with H. Tokuda, Kanazawa University, Japan).

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GWP038

Three Novel Thermostable Lipases from Different Metagenomes Ranging from Soil Enrichments to Hydrothermal Vents

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Metagenomics reveal culture-independent insights into microbes´ diversity and the enzymes they feature [1, 3]. Lipolytic enzymes, namely carboxylesterases (EC 3.1.1.1) and triacylglycerol lipases (EC 3.1.1.3), catalyze both hydrolysis and synthesis reactions on a broad spectrum of substrates at various conditions rendering them especially suitable for biotechnological applications. Most lipases used today originate from mesophilic organisms and are susceptible to thermal denaturation (Levisson et al. 2009). Here we report on the identification of novel thermostable archaeal and bacterial lipases from three different microbial communities. Our metagenomic libraries were constructed from an enrichment using heating water as inoculum, a long term soil enrichment culture and a deep-sea hydrothermal vent-derived enrichment. Cultures were maintained at 65° to 70°C and microbial communities characterized on a phylogenetic level based on 16S rRNA genes. Mainly thermophilic Firmicutes were identified in the soil enrichment after several months of incubation, while the heating water culture contained mostly novel *Thermales*. The hydrothermal vent culture consisted predominantly of archaeal species that are closely related to *Thermococcales*. The metagenomic libraries constructed from the designated enrichments comprised 800 to 8,500 clones. Screening of the libraries on *p*NP-substrates (C_4 and C_{12}) at temperatures between 50°C and 70°C resulted in the identification of 15 lipolytically active clones. Until now, three enzymes, LipS, LipT and LipZ have been expressed recombinantly in *E. coli* and in *P. antarctica* and have been characterized biochemically. Current studies show a half life time of up to 48 h at 70°C (LipS) and 50 min at 90°C (LipZ). The temperature optima ranged between 70°C (LipS) and 100°C (LipZ). All three enzymes are able to catalyze the hydrolysis of long-chain fatty acid esters like *p*NP-palmitate (C_{16}), -stearate (C_{18}) and -oleate ($C_{18:1}$; LipT), indicating lipase activity. Current work focuses on further biochemical characterization with unusual substrates and synthesis reactions in organic solvents as well as crystallographic analyses.

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GWP039

Obtaining and selection haploids of distillery yeasts

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Haploidization is a crucial step during obtaining yeast strains with improved technological properties by means of yeast sexual hybridization. This natural method improving of industrial strains of yeast is used for over 60 years.
Aim: The aim of this study was to obtain and isolate haploid cultures of different Saccharomyces cerevisiae strains, also evaluate them as a possible strains for conjugation and hybrids selection to obtain a new distillery yeasts for fermentation of concentrated broths prepared from sugar beet juices.
Methods: 9 strains of S.cerevisiae from our Pure Cultures Collection were used in experiments: PA1, PA2, PA3, PS2, PS3, M2, M3, OH2, and BC16. 2 strains: S.cerevisiae Ma and S.cerevisiae Mα - stable haploid markers with known mating type were also used for mating type assay. Presporulation medium containing sodium pyruvate, glucose, yeast extract, bacto-peptone was used. Modified McClary medium: potassium acetate 10g/L, yeast extract 2,5g/L, glucose 0,2g/L, agar 25g/l, was used for sporulation. Haploid clones were obtained according to the procedure Johnston and Mortimer using enzymes from Helix pomatia to asci walls digestion. Single spores were isolated from tetrads by using micromanipulator with glass needle. Yeast colonies grown out of individual spores were transferred to YPG slants.The criteria in selecting parent strains and their haploid clones obtained from the spores were: morphological features, ability to ferment and assimilate selected sugars, ability to assimilate glycerol as well as fermentation of 20 and 25°Blg broths prepared from concentrated sugar beet juice.

Results: 4 of 9 parent strains were not able to form ascospores in the experiment conditions. 198 spores were isolated after asci dissection. Spore survival ranged between 10 and 85% depending on the strain. After multi-step selection, 16 haploid strains were assessed during fermentation of 1L (25°Blg) medium prepared from concentrated sugar beet juice and the results were compared with those obtained for initial diploid strains. Ethanol yield ranged between 60-77% - for haploids and 72-90% for parent strains.
Conclusions: Selected haploids will be used during obtaining yeast hybrids designed for dynamic and efficient fermentation of sugar beet juices for ethanol.
This work was financed from funds for Polish Science in 2009-2010 years as a part of projects: R&D N R12 0062 06 and R&D N312 301037.

GWP040
Chitin deacetylases - towards patterns of acetylation

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Chitosan, the linear heteropolysaccharide of glucosamine and N-acetylglucosamine residues, is the only naturally occurring polycationic polymer. As such, it has a number of highly attractive physico-chemical properties and, through interaction with the mostly anionic components of living cells, interesting biological activities.
Today's commercially available chitosans are produced chemically from chitin isolated from shrimp shell wastes. They can be well defined concerning their degree of polymerization and degree of acetylation, but they are invariably characterized by a random pattern of acetylation (PA). The biological activities of chitosan, such as antimicrobial, plant strengthening or immuno-stimulatory activities, may be greatly influenced by their PA. However, no methods are available today for the production of chitosans with defined non-random PA.
A chitin deacetylase from the phytopathogen fungus *Puccinia graminis* f. sp. *triticum* was heterologously synthesized in the fission yeast *Schizosaccheromyces pombe*. The hexahistidin tagged protein was purified to electrophoretic purity and used for kinetics with chitin trimers, tetramers, pentamers and hexamers. The resulting products were analyzed by mass spectrometry and high performance thin layer chromatography (HP-TLC). After hydrolysis the resulting products of the substrates ranging from tetramer to hexamer retained two acetylated units (A) at the non-reducing ends, whereas the following units were completely deacetylated (D). These products (AADD, AADDD, AADDDD) were identified by electrospray ionization mass spectrometry, whereas the PA of the hydrolysis product of the trimer was ambiguous. Polymeric substrates treated with this chitin deacetylase may also contain a non-random blockwise PA as NMR data suggests.
A putative peptidoglycan deacetylase from *Bacillus licheniformis* DSM13 was heterologously synthesized as a strepII-tagged protein in *Escherichia coli* Rosetta 2 (DE3) [pLysSRARE2] with a pET-22b(+) vector construct and enriched to electrophoretic purity. This new enzyme shows strong deacetylating activity towards chitin and may also generate a PA. In an analogous way to the *P. graminis* enzyme this enzyme should also be analyzed towards the potentially generated PA.

GWP041
Systems metabolic engineering of biotechnological diaminopentane production by *Corynebacterium glutamicum* for bio-based polyamides

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Polyamides, nowadays produced mainly from petrochemically derived monomers, display an important fraction in the field of polymers. Due to the very durable material properties they are used e. g. in the automotive industry or in high value consumer products. The growing shortage of fossil resources, connected to rising oil price, escalating CO_2 production and global warming, has led to a search for suitable monomers derived from renewable resources. A very promising candidate in this context is the biotechnologically produced C5 diamine diaminopentane (DAP), a key building block for novel bio-based polyamides like PA 5.4 or PA 5.10.

Here, we describe the biotechnological production of diaminopentane through systems-wide metabolic engineering of *Corynebacterium glutamicum*. Lysine-overproducing strains of *C. glutamicum* were used as promising starting point, as diaminopentane is directly available from lysine by decarboxylation. This was realized by expression of lysine decarboxylase (*ldcC*) from *E. coli*. Subsequent studies revealed this reaction as key target for efficient production, since *in vitro* activity and *in vivo* flux towards diaminopentane closely correlated in mutants expressing different variants of *E. coli ldcC*. High-level expression from a single genomic copy was achieved by combining the use of the strong constitutive promoter EFTU and a codon usage optimized for the translation system of *C. glutamicum*. The corresponding strain, *C. glutamicum* $P_{eftu}ldcC^{opt}$, produced diaminopentane on glucose with a yield of 0.2 mol mol^{-1} (1). Using then iterative rounds of systems-wide pathway analysis and engineering supporting pathways were successfully engineered. Metabolome analysis, however, revealed substantial formation of a so far unknown by-product, which was identified as an acetylated variant, N-acetyl-diaminopentane. The undesired by-product reached levels of more than 20% of that of diaminopentane. By identification and elimination of the competing pathway towards N-acetyl-diaminopentane in $P_{eftu}ldcC^{opt}$, the yield for DAP was further increased (2).

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GWP042
Effect of yeast strains and nitrogen content on efficiency of alcoholic fermentation of raw juice based worts

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Introduction: Production of bioethanol from by-products of sucrose factories such as raw and thick juices or molasses is a promising alternative for Polish sugar industry. In distilleries these raw materials can be directly fermented after pH adjustment and inoculation with yeast (raw and thin juices) or easily converted to fermentation worts (thick juice, molasses) only through dilution, supplementation with some nutrients and inoculation with yeast cells.
Aim: The aim of this study was to evaluate the effect of various strains of yeast *S. cerevisiae* on dynamics and efficiency of alcoholic fermentation of raw sugar beet juice based worts.
Materials and methods: Four yeast strains were used: M1, M2, M3 (derived from Pure Culture Collection of the Institute of Fermentation Technology and Microbiology of the Technical University of Lodz) and As-4 (commercial yeast preparation). As the raw material, the raw sugar beet juice was used. Raw juice based worts were prepare with and without addition mineral nitrogen source $(NH_4)_2HPO_4$.
Results: It was found that all used yeast strains fermented raw juice based worts dynamically and efficiently. Total time of fermentations not exceeded 48 hours. Addition of mineral nitrogen nutrient has not affected on fermentations capacity and their dynamic.
Analysis of the chemical composition of distillates obtained, showed correlation between wort supplementation with mineral nutrient and concentration of fermentation by-products, especially higher alcohols. Supplementation of raw juice based wort with mineral nitrogen contributed to a significant decrease of over 30% content of 2-methyl-1-butanol, 3-methyl-1-butanol and 2-methyl-1-propanol, and more than 13% of n-propanol and n-butanol in distillates.
Application of molasses yeast strains (M1, M2, M3) for raw juice worts fermentation had significant impact on by-products concentration in distillates. The content of higher alcohols (in particular isoamyl ones) in the majority of distillates from the raw juice (worts with mineral nitrogen) obtained by using yeast strains: M1, M2 and M3 were much lower than in distillates from the worts fermented with yeast As-4. Contents of 2-methyl-1-butanol and 3-methyl-1-butanol in these distillates ranged between 91.3±6.5 - 348.4±23.6 mg/l spirit 100% v/v and 692.5±37.5 - 1191.9±83.7 mg/l spirit 100% v/v respectively. In the relation to trials without supplementation, contents of higher alcohols obtained from worts with additional mineral nitrogen were reduced in distillates in ranges from 7% (M2) to 55% (M3).
The study was financed by the Polish Ministry of Science and Higher Education under R&D Grant No N R12 0062 06.

GWP043
Hemicellulose for Production of the Biopolyamide Monomer 1,5-Diaminopentane by *Corynebacterium glutamicum*

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The production of bio-based polymers from renewable resources represents an attractive alternative to classical petrochemical routes in respect of ecological problems and increasing raw material prices. Recent interest focused on biotechnological production of polyamides, whose material properties are improved compared to biologically derived polylactic acid and polyhydroxyalkanoates. Recent studies realized production of 1,5-diaminopentane as monomer building block of polyamides by *Corynebacterium glutamicum* via heterologous expression of lysine decarboxylase [1]. The raw materials such as glucose or starch for the production of 1,5-diaminopentane however compete with food industry. The non-food lignocellulose derived hemicellulose seems therefore attractive as alternative since it ensures abundant and cost-effective supply. In our research project the bio-based production of 1,5-diaminopentane by *C. glutamicum* was extended to hemicellulose utilization [2]. For this purpose, the metabolism of 1,5-diaminopentane producing C. *glutamicum* was engineered to the use of the C_5 sugar xylose. This was realized by heterologous expression of the *xylA* and *xylB* genes from *Escherichia coli* mediating the conversion of xylose into xylulose 5-phosphate, an intermediate of the pentose phosphate pathway. The resulting *C. glutamicum* mutant exhibited efficient production of 1,5-diaminopentane from xylose and from mixtures of xylose and glucose. The novel strain was tested on industrially relevant hemicellulose fractions, mainly containing xylose and glucose as carbon sources. For this purpose, hemicellulose from dried oat spelts was first hydrolyzed enzymatically and subsequently utilized for biotechnological production of 1,5-diaminopentane. Obtained yields are promising towards bio-based 1,5-diaminopentane and bio-polyamides from non-food raw materials.

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GWP044
Cytochrome P450 monooxygenase-catalysed conversion of aromatic compounds

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In general, monooxygenases catalyse the incorporation of a single oxygen atom from molecular oxygen into organic substrates, while the other oxygen atom is reduced to water. An important member of this monooxygenase family are the cytochrome P450 monooxygenases (CYPs). CYPs are heme-thiolate proteins which catalyse the introduction of oxygen into generally non-polar, aromatic or aliphatic molecules, thereby leading to hydroxylation, aromatisation, epoxidation or cleavage of carbon-carbon bonds. Because of their unique and versatile catalytic properties, they are of great interest as potential biological catalysts for industrial applications [1].
The direct and selective introduction of the hydroxyl group into aromatic rings is one of the most challenging fields in organic chemistry. The number of direct hydroxylations, as well as their selectivity, is still limited. In this project, cytochrome P450 monooxygenase CYP116B3 from *Rhodococcus ruber* is applied for the enzyme-catalysed conversion of various phenylalkanes and derivatives thereof to be used in the synthesis of natural products, natural product analogues and precursors of pharmaceuticals. Recently, CYP116B3, a natural self-sufficient fusion protein consisting of a P450 monooxygenase, ferredoxin and a flavin-containing reductase, was reported to convert various aromatic compounds [2]. The purified recombinant CYP116B3 monooxygenase enzyme will be employed to examine and expand its substrate spectrum in addition with an increase of activity, regio- and chemoselectivity of the enzyme.

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GWP045
The intermediate products of sugar beet processing as raw material for bioethanol production

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Introduction: From an economic point of view and in comparison with cereals, sugar beet and intermediates from beet processing are very good raw materials for ethanol production due to their content of fermentable sugars.
Aim: The aims of this study were to investigate the bioethanol production of raw, thin and thick juices as intermediates of sugar beet processing and the effect of type of mineral supplement of worts, its sterilization and extract of thick juice based worts on fermentation dynamics and ethanol yield.
Materials and Methods: Raw, thin and thick juices were obtained from the sugar factory in Dobrzelin (Poland). Fermentations were carried out by using dried distillery yeast (2 g/L), strain As-4, purchased from the yeast factory in Maszewo Lęborskie (Poland). Worts were supplemented with $(NH_4)_2HPO_4$ or $(NH_4)_2SO_4$ (0.3 g/L) as mineral nitrogen sources. Raw materials were analyzed by methods recommended in sugar industry.Worts were analyzed before and after fermentation by methods recommended in distilleries.
Results: Fermentation trials revealed that yeast strain As-4, ensured the complete fermentation of the raw juice as well as of the thin juice within 48-52 hours and high ethanol yield (88.00-90.74% theoretical yield). Supplementation of raw juice-based worts with $(NH_4)_2HPO_4$ or $(NH_4)_2SO_4$, and autoclaving of raw juice had no significant impact on fermentation dynamics and ethanol yield. The degree of ethanol biosynthesis yield from the thin juice compared to the raw juice was lower when it was supplemented with $(NH_4)_2SO_4$ (85.64±1.1% theoretical yield) compared to supplementing with $(NH_4)_2HPO_4$.
The shortest (130 h) time of process was observed for the 18°Blg wort prepared from thick juice. The application of worts with higher extract, between 20 and 23°Blg, resulted in lengthening of overall process time. The highest ethanol yields (89.85±1.5 - 92.08±1.7% of theoretical) in thick juice fermentation trials were achieved when it was diluted to the extract of 18 and 20°Blg. Initial extract of thick juice based wort about 20°Blg can be considered as optimal, enabling maximal ethanol yield. An increase in sugar concentration in a fermentation medium had a significant effect on a decrease of ethanol yield. The yield of 100% alcohol from 100 kg of raw or thin juice ranged between 7.6 and 8.3 l and between 33.8 - 38.1 l spirit 100% v/v. from 100 kg of thick juice. Results of our study prove that the intermediates of sugar beet processing are attractive raw materials for bioethanol production.
The study was financed by the Polish Ministry of Science and Higher Education under R&D Grant No N R12 0062 06.

GWP046
Chemoenzymatical synthesis of acylated oligopeptides

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Acylpeptides are representing a group of biosurfactants which is of increasing interest for many laundry and cosmetic applications. Because of its similarity to the skin's surface structure, these molecules can be used as additives to increase the skin-compatibility of strongly irritating surfactants [1].
After a protease-catalysed synthesis of oligopeptides via a kinetically controlled approach, the following step will be the acylation of the peptide chain. This can be achieved either by chemical or by enzymatic means [2].
Initially we chose the Schotten-Baumann reaction, in which an amine is connected with a fatty acid chloride under alkaline conditions. Since during the peptide synthesis an alkaline hydrolysis step is involved, we consider a coupling of both, the synthesis and the acylation to a chemoenzymatic one-pot reaction.
For the future, biocatalysts should be used to couple oligopeptide and fatty acid. Therefore we started a screening of different enzymes on their capability to carry out acylation reactions of amino acid derivatives.

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GWP047
Production of microbial biosurfactants with non-pathogenic strains

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Biosurfactants are biodegradable, have low toxicity and can be produced with biological waste materials or renewable resources. They are used as detergents or surfactants in the pharmaceutical, cosmetic and food industry. Rhamnolipids produced by *Pseudomonas aeruginosa* belong to the very limited group of commercially available biosurfactants. However, the opportunistic human pathogen *Pseudomonas aeruginosa* is up to now the conventional organism used for the production of rhamnolipids.
The aim of this work is to increase the productivity of a non-pathogenic rhamnolipid production strain by optimizing culture conditions. This strain produces a rhamnolipid which is composed of two rhamnose molecules and two hydroxy fatty acids with a chain length of 14 carbon atoms. Fermentations of the production strain are carried out with a sixfold parallel bioreactor system. By now, an average volumetric productivity of about 1.83 mg/hL in rich medium was found.
The rhamnolipid was purified with a gradient flash system using a chloroform/methanol and a chloroform/methanol/acetic acid solvent mixture. Successful purification was proven by nuclear magnetic resonance spectroscopy. The purified rhamnolipid had a more than 95% purity.
A minimal medium on the basis of an elemental composition analysis of the non-pathogenic production strain was designed.
The next step will be further development of the minimal medium to optimize the rhamnolipid yield.

GWP048
Establishment of an alternative carbohydrate metabolism pathway in *R. eutropha.*

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The β-proteobacterium *Ralstonia eutropha* H16 utilizes fructose and gluconate as carbon sources for heterotrophic growth exclusively via the Entner-Doudoroff-pathway with its key enzyme 2-keto-3-desoxy-6-phosphogluconate (KDPG) aldolase. By deletion of the *eda* gene, which encodes this enzyme, we constructed a KDPG aldolase-negative strain, which is disabled to supply pyruvate for energy metabolism originated from fructose or gluconate as sole carbon source. To restore the fructose catabolism, an alternative pathway, similar to the fructose-6-phosphate shunt of heterofermentative bifidobacteria, was established. For this, the gene *xfp* from *Bifidobacterium animalis*, coding for a bifunctional xylulose-5-phosphate/fructose-6-phosphate-phosphoketolase (Xfp; [2]) was expressed in *R. eutropha* H16 PHB^{-}4 Δ*eda*. Xfp catalyzes the phosphorolytic cleavage of fructose-6-phosphate to erythrose-4-phosphate and acetylphosphate as well as of xylulose-5-phosphate to glyceralaldehyde-3-phosphate and acetylphosphate. The recombinant strain exhibited phosphoketolase (PKT) activity on both substrates and was able to use fructose as sole carbon source for growth, due to the fact, that the PKT is the only enzyme that is missing in *R. eutroph*a H16 to establish the artificial fructose-6-phosphate shunt. *R. eutropha* H16 PHB^{-}4 Δ*eda* pBBR1MCS-3::*xfp* should be applicable for a novel variant of a plasmid addiction system to maintain episomal encoded genetic information during fermentative production processes. Plasmid addiction systems are often used to ensure plasmid stability in biotechnological relevant microorganisms and processes without the need to apply external selection pressure like antibiotics [1]. By episomal expression of Xfp in a *R. eutropha* H16 mutant lacking KDPG-aldolase-activity and cultivation in minimal media with fructose as sole carbon source, the growth of the cells will be addicted to the use of the *xfp*-containing plasmid. This novel selection principle extends the range of further biotechnological processes using *R. eutropha* H16 as production platform.

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GWP049
Novel Lipases: Tools for biochemical synthesis and fat/oil processing

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Lipases are versatile tools in biotechnology, catalyzing a broad range of hydrolytic and/or (trans-)esterification reactions. Due to these characteristics, their industrial use is steadily expanding. For opening novel application solutions, developing lipases with new properties becomes more crucial than ever. EUCODIS Bioscience therefore attaches great importance to diversity and application-related characterization of their lipase portfolio, serving customers in the chemical, pharmaceutical, food, feed and other industries.
Here, we investigated the characteristics of a number of novel lipases with regard to potential use in the synthesis of (bio-)chemical or pharmaceutical products, and in the oil and fat processing industry. Candidate genes were selected, expressed, the enzymes processed and supplied to interested parties for application testing. The enzymes were characterised with respect to substrate specificity (chain length, preference for saturated or (poly-)unsaturated fatty acids), regio- and enantioselectivity and optimal conditions for hydrolysis or (trans-)esterification reactions. Lipases with long chain fatty acid acceptance as well as enzymes useful for the removal of short chain fatty acids were identified. Together with their preferences towards saturated or unsaturated fatty acids, these lipases are valuable tools for the development of novel processes in the oil and fat industry. To explore the suitability of the Eucodis lipases in the synthesis of (bio-)chemical and pharmaceutical products, lipases were in particular tested for their capability to catalyze (trans-)esterification reactions.
Here, we will introduce the Eucodis library of lipid modifying enzymes, their catalytic properties and reaction characteristics, and discuss their potential in industrial biocatalysis.

GWP050
Glycopeptide resistance in *the producer strain Amycolatopsis balhimycina*

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Glycopeptides are the drugs of last resort for treatment of severe infections caused by gram positive pathogens. They impair bacterial growth by binding to the terminal d-Ala-d-Ala residues of cell wall precursors and thus block cell wall biosynthesis. However, the number of glycopeptide resistant bacteria rose steadily over the last two decades.
The most common resistance mechanism of bacteria against glycopeptides is to reprogram the murein synthetic machinery resulting in resistant cell wall precursors ending on d-Ala-d-Lac. This modification is catalyzed by enzymes encoded by the *vanHAX* operon. Their transcription is activated in the presence of glycopeptides by the two component system VanRS.
The genome of *Amycolatopsis balhimycina,* the producer of the vancomycin-like glycopeptide balhimycin, contains genes with high homology to the enterococci *vanRS* and *vanHAX* genes.
The constructed *vanHAX$_b$*-deletion mutant in *A. balhimycina*, shows a glycopeptide sensitive phenotype. Surprisingly, the mutant strain still produces balhimycin after 42h of growth in balhimycin production medium. Therefore, *A. balhimycina* needs an additional set of genes which enable the mutant to synthesize a resistant cell wall. The synthesis of cell wall precursors ending on d-Ala-d-Lac was further confirmed by LC-MS analysis. In non-production medium the mutant strain exclusively produces cell wall precursors for a sensitive cell wall.
The occurance of resistance in *A. balhimycina* is independent of the VanRS two component system. The glycopeptide production is also not directly controlled by VanRS. Since we could recently show that the *vanRS*-system can activate the *vanHAX*-genes after their heterologous expression in *S. coelicolor* DvanRS, we intend to analyze which cellular functions are regulated by this two component system.

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GWP051
Glycerol 3-phosphatase from *Corynebacterium glutamicum*: evidence for the first bacterial glycerol 3-phosphatase

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Corynebacterium glutamicum is a well known amino acid producer which is used e.g. to produce over 1.3 million tons of L-lysine per year. During growth and amino acid production glycerol may be formed as a byproduct. The *C. glutamicum* genome encodes a putative glycerol 3-phosphatase (GPP). Here, we characterize this enzyme as the first of its kind described in prokaryotes. Among the substrates tested, GPP was solely active with glycerol 3-phosphate. The enzyme prefers conditions of neutral pH and requires Mg^{2+} or Mn^{2+} for its activity. The maximal activity of GPP was estimated to be 0.67 U/mg and GPP possesses a K_M of 2.9 mM for glycerol 3-phosphate. Phenotypically, slow growth of the recombinant *C. glutamicum* (pVWEx1-*glpFK*) in the presence of glycerol, which is due to intracellular glycerol 3-phosphate accumulation, could be alleviated by *gpp* overexpression with concomitant reduction of the intracellular glycerol 3-phosphate concentration. GPP was shown to be involved in utilization of glycerol 3-phosphate as sole source of phosphorus, since growth in glucose minimal medium with glycerol 3-phosphate as sole phosphorus source was reduced in the *gpp* deletion strain, but accelerated when *gpp* was overexpressed. Since glycerol formation during growth in fructose minimal medium could be abolished by deletion of *gpp*, but was increased as consequence of *gpp* overexpression, GPP plays a major role in formation of glycerol as by-product in *C. glutamicum*.

GWP052
Metabolic engineering of *Corynebacterium glutamicum* for the production of L-aspartate

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Corynebacterium glutamicum is used biotechnologically for the production of L-glutamate and L-lysine, which are used as food and feed additives, but strains for other products such as 1,4-diaminobutane and 1,5-diaminopentane have also been developed (1). The amino acid L-aspartate is used for the sweetener aspartame, and its derivatives are promising precursors for polymer synthesis. Therefore, we are aiming on engineering *C. glutamicum* for producing L-aspartate.
In bacteria, L-aspartate is the precursor for the amino acids threonine, isoleucine, methionine and lysine and for the cell wall precursor diaminopimelate. The first step of conversion of aspartate is catalyzed by aspartokinase (encoded by *lysC*) (2). Deletion of *lysC* is not possible as diaminopimelate is essential for growth. A downregulation of the gene via promoter exchange appears to be more promising and is thought to lead to an accumulation of L-aspartate.
Synthesis of L-aspartate from oxaloacetate depends on transamination e.g. by glutamate-aminotransferase (*aspB*). Oxaloacetate supply can be improved by enhancing the expression of the genes coding for the anaplerotic phosphoenolpyruvate carboxylase (*ppc*) and pyruvate carboxylase (*pyc*). Deletion of the aspartate-α-decarboxylase (*panD*) avoids conversion of L-aspartate, but yields a pantothenate auxotrophy. Limiting pantothenate supplementation indirectly improves oxaloacetate supply as flux via the pyruvate dehydrogenase complex is reduced and carboxylation of pyruvate or PEP may be enhanced.
L-aspartate may also be synthesized by reductive amination of fumarate. Heterologous expression of aspartate ammonia lyase genes (*aspA*) may yield L-aspartate if the level of fumarate is increased due to deletion of the gene coding for the fumarate hydratase (*fumC*) (3). Recombinant aspartase genes from *Escherichia coli* and *Bacillus subtilis* were expressed in *C. glutamicum* and high specific activities were obtained. Expression of the aspartase genes in *C. glutamicum* wild type did not lead to an accumulation of aspartate in supernatants of batch cultures unless the cells were supplied with sufficient amounts of fumarate and nitrogen.

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GWP053
Production of rhamnolipids in *Pseudomonas putida* by heterologous expression of *rhl*-genes from *Pseudomonas aeruginosa* PA01

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Rhamnolipids are biosurfactants with great potential for applications in industry. They can be used as detergents in laundry products, dish washing liquids or as emulsifiers in cosmetics and foods. Furthermore they exhibit antimicrobial properties. The production of rhamnolipids is well characterized for the opportunistic human pathogen *P. aeruginosa*. They are composed of one or two rhamnose-molecules linked to hydroxyfatty acids with different chain length between C_8 and C_{12}. The biosynthesis occurs in three steps: RhlA is responsible for the production of 3-(3-hydroxyalkanoyloxy)alkanoates (HAAs) by linking two hydroxyfatty acids together. RhlB bonds a rhamnose-molecule to HAA and creates mono-rhamnolipid. The genes *rhlAB* are arranged as an operon. RhlC is responsible for the production of di-rhamnolipid and bonds a second rhamnose to the mono-rhamnolipid. The *rhlC* gene forms an operon together with the gene *PA1131*, which function is still unknown.
However, especially for the uses of rhamnolipids in the cosmetic and food industry it is problematical when they are produced in a human pathogen. We here present the production of both mono- and di-rhamnolipids by heterologous expression using the non-pathogenic strain *P. putida* KT2440 as a host. Moreover, *P. putida* is able to grow with high concentrations of rhamnolipid (>100 g/L) in the culture media. The production in *P. putida* is possible, because precursors for rhamnolipid production are available in this bacterium. For the heterologous expression we cloned the *rhlAB* operon as well as the *rhlC* gene as single or in combination in the pVLT33 expression vector. Expression of *rhlAB* in *P. putida* resulted in the production of mono-rhamnolipid and if all three genes were expressed, *P. putida* is also able to produce di-rhamnolipids. Characterization of rhamnolipids produced by *P. putida* via HPLC-ESI-MS showed that it produced the same species like the *P. aeruginosa* wild type.

GWP054
Biological Hydrogen Production with Microalgae

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Microalgae are capable of producing a variety of products, including compounds of high energy content such as sugars, lipids and hydrogen. The latter is becoming a product of interest in the context of renewable energy production. Hydrogen may be used as energy carrier, ready for power generation in fuel cells for mobile and stationary applications.
Certain strains of microalgae are long known to produce hydrogen under anaerobic conditions [2]. The oxygen inhibited hydrogenase recombines electrons from the chloroplast electron transport chain with protons to form hydrogen directly inside the chloroplast. Melis and coworkers obtained a sustained hydrogen production under low sulfur conditions in Chlamydomonas reinhardtii [4], reducing the net oxygen evolution and thereby overcoming the inhibition of the hydrogenase. The development of specially adapted hydrogen production strains led to higher yields and optimized biological process preconditions [1, 3].

Based on these foregoing works we have established a process strategy to proceed further versus large scale production. To approach this problem a custom LED illumination system has been fitted to a glass stirred tank reactor, providing for homogeneous conditions with respect to light distribution and mixing. This idealized reactor was applied to determine growth and hydrogen production kinetics of Chlamydomonas production strains. These data are important for process scale up as well as to identify potential biological and technical limitations of the system.

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GWP055
Functional Metagenome Analysis - the Microbiome of Elephants Feces

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The intestinal metagenome of ruminants, rodents and even termites has been intensively studied. In contrast the elephant colonic microbiome was not elucidated up to now. Here we report on the construction and screening of a metagenome library derived from elephant feces. Altogether 20,000 clones were generated with a total of about 600 Mbp of genetic information. The library was constructed using pCC1FOS and inserts ranged from 12 - 40 kbp. In addition a phylogenetic analysis of the metagenomic DNA based on 16S rDNA revealed a high diversity within the bacteria, where the majority was affiliated to the Phylum of Bacteroidetes.
The metagenomic library was tested for the presence of novel biocatalyst encoding genes using various functional screening methods. About 60 positive fosmid clones with various activities were identified and their sequences established using 454 technology. Herewith we identified a range of glycosyl hydrolases e.g. cellulases, α-amylases and α-L-rhamnosidases but also phytases and esterases have been discovered. All of them are new and have a high potential for biotechnological applications.

GWP056
Intracytoplasmic membranes as platform for the production of membrane proteins from acetic acid bacteria

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Acetic acid bacteria are of high relevance for biotransformation of sugars and alcohols to oxidized products, e.g. sorbitol to sorbose, glucose to gluconic acid, 5-keto- and 2-ketogluconic acids or glycerol to dihydroxyacetone. A variety of membrane bound and cytoplasmic dehydrogenases perform these regio- and stereospecific oxidation reactions with their substrates, providing compounds for further processes, e.g. the production of pharmaceuticals or beverages. For membrane-bound dehydrogenases, of course, the cytoplasmic membrane is an essential mounting plate.
Electron microscopy of various *Gluconobacter*-strains reveals the presence of intracytoplasmic membranes under certain growth conditions, providing additional membrane surface, which may be of importance for overproduction of functional dehydrogenases and, hence, higher yield of products. Here we show the occurrence and distribution of membranes in *Gluconobacter* cells and present marker systems for monitoring of membranes and dehydrogenases in *Gluconobacter*. These tools will help to quantify and to optimize the expression of membrane-bound dehydrogenases in fermentative processes.

GWP057
Oxidoreductases from extremophilic microorganisms isolated from Spitsbergen and the deep sea

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Enzymes from extremophilic microorganisms offer a broad range of new biotechnological applications due to their ability to catalyze reactions under extreme conditions. As a result, these enzymes have a high potential not only for the optimization of already existing processes, but also for the development of new sustainable technologies [1]. Among different enzyme classes, oxidoreductases form a versatile class of biocatalysts, which are able to catalyze specific reduction and oxidation reactions [2]. Two different screening approaches were applied to identify novel oxidoreductases with improved activity and stability.
Psychrophilic bacterial strains from Spitsbergen were cultivated in micro-scale and submitted to a high-throughput screening for enzymatic hydrogen peroxide formation in microtiter plates. By applying denaturing gradient gel electrophoresis, two different positive strains were found, which were identified as *Carnobacterium* sp. and „uncultured bacterium clone". Gene libraries of both strains were constructed and screened using activity agar plates. Positive clones were sequenced and analyzed for open reading frames with similarity to known oxidoreductases. Three novel oxidoreductases were identified: alpha-glycerophosphate oxidase from *Carnobacterium* sp., aldehyde dehydrogenase and glycerol dehydrogenase from the „uncultured bacterium clone". Further oxidoreductases were detected in a novel metagenome database derived from a deep-sea consortium. In this approach, a sequence-based screening was performed yielding four oxidoreductase genes which were successfully cloned and expressed.

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GWP058
Directed Evolution - Training *Basfia succiniciproducens* for Succinic Acid Production under Stress Conditions

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Fossil fuel shortage and increasing market demand for raw materials requires innovative biotechnological processes based on renewable resources to produce valuable chemical building blocks. The production of succinic acid, such a promising platform chemical, with natural succinic acid producers like *Basfia succiniciproducens* [1] has high potential for future processes. In order to realize this potential it is necessary to adapt the organism to various process parameters, particularly to those that can emerge as stress factors and hamper bacterial growth during the cultivation.
A very useful method to adapt microorganisms specifically to various environmental conditions is the directed evolution that has been described over the past years as auspicious approach to gain superior clones for desired conditions. For *Basfia succiniciproducens* the cultivation in serum bottles yielding high succinic acid titers was modified to a sequential batch cultivation to realize an easy to handle method for directed evolution.

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MDV001
Single-cell analysis reveals unexpected phylogenetic and ultrastructural diversity of uncultivated magnetotactic bacteria

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Magnetotactic bacteria (MTB) are a diverse group of prokaryotes which orient along magnetic fields using membrane-coated magnetic nanocrystals of magnetite (Fe_3O_4) or greigite (Fe_3S_4), the magnetosomes. Previous phylogenetic analysis of MTB has been limited to few cultivated species and

most abundant members of natural populations, which were assigned to *Proteobacteria* and the *Nitrospira* phyla. Here, we used a novel approach that allowed the targeted phylogenetic and ultrastructural analysis of individual, low abundant MTB cells from environmental samples. Morphologically conspicous single cells were micromanipulated from magnetically collected multi-species MTB populations, which was followed by whole genome amplification (WGA) and electron microscopic analysis of sorted cells. Phlyogenetic identity of target cells was verified by fluorescence *in situ* hybridization (FISH) with probes derived from 16S rDNA sequences that were PCR-amplified from WGAs. Application of this approach to various freshwater and marine sediment samples revealed extensive and novel diversity of MTB, which escaped detection by parallel conventional 16S rRNA gene clone library analysis. While most of the newly identified MTB belonged to various lineages of *Proteobacteria* and the *Nitrospira* phylum, one morphotype termed SKK-01 could be assigned to the candidate division OP3, which extends the phylogenetic diversity of MTB to a new phylum. FISH demonstrated that SKK-01 represents only a marginal fraction of the MTB population (~ 10^{-5}). Besides intracellular sulfur inclusions, SKK-01 harbors ~175 bullet-shaped magnetosomes arranged in multiple chains which consist of magnetite as revealed by TEM and EDX analysis.
In conclusion, our approach represents a powerful tool for targeted single-analysis of low-abundance uncultivated prokaryotes from environmental samples.

MDV002
Complexity of the bacterial community in the sediment of the drinking water reservoir Saidenbach obtained by pyrosequencing

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The Sediment is an important compartment of freshwater ecosystems with favorable living conditions for a large number of bacterial species along changing redox conditions. Although microbes play an important role for the flux of matter and being part of the major biogeochemical cycles very little is known about their diversity in freshwater sediments. Here we present a study of the microbial diversity in sediment samples taken at three different locations and two sediment depths in the mesotrophic drinking water reservoir Saidenbach in Germany. The selected sampling sites comprise different environmental conditions. A pyrosequencing approach was used to analyse parts of the V6 and V7 region of the bacterial 16S rRNA gene. 17,751 sequences were classified into 21 phyla. The largest phylum with 50.7% of all sequences was Proteobacteria with a range of 37 to 64% in the individual samples. The rarefaction curves calculated at sequence divergence of 3% showed no saturation indicating that the full extent of genetic diversity was not covered. For all samples the Shannon index of diversity (H') was high and ranged from 7.29 to 7.53. Among the bacteria, the dominant groups were the Betaproteobacteria, Deltaproteobacteria Bacteroidetes, Verrucomicrobia, Acidobacteria, Gammaproteobacteria, Alphaproteobacteria Firmicutes, Chloroflexi, Nitrospira and Actinobacteria, representing 27.9%, 11.9%, 11.6%, 7,2%, 4.6%, 3.3%, 2.4%, 1.8%, 1.3%, 1% and 0.7% respectively, of all classified sequences. Differences in the community composition were observed between the sampling sites as well as with sediment depth. While one sampling site contained a large abundance of Fusobacteria in the upper 0.5cm sediment layer they were scarce in deeper sediment layers and at the other sampling sites. Surprisingly, the genus Nitrospira was found especially in the lower investigated sediment horizon (3-5cm sediment depth) where oxygen and nitrate were depleted and an increasing ammonium concentration was observed. The majority of Deltaproteobacteria could be classified into 3 families: Syntrophaceae, Geobacteraceae with the genus Geobacter and Desulfobacteriaceae.
The pyrosequencing approach in combination with the environmental conditions provided new information on this complex ecosystem.

MDV003
A close look at the diversity and dynamics of ultra-oligotrophic groundwater microbial communities during the restoration of a drinking water well

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Microbial monitoring of drinking water production and distribution systems is essential to assure water quality and to predict possible risks. Chemical and biological characteristics of the water pumped are checked regularly; however microbiological data is still based on outdated culturing tests. Here, we characterise intrinsic groundwater microbial communities before, during and after the mechanical restoration of a drinking water production well. High pressure jetting and hydrofracturing are procedures routinely used periodically to impede well clogging by fine sediments and also biofilms.
Microbial communities sampled from the groundwater were first screened by T-RFLP fingerprinting of intrinsic *Bacteria*, *Archaea* and *Microeukaryontes*. This revealed similar communities in water before and after well cleaning, however OTU abundances were more even afterwards. In contrast, well jetting preferentially purged only a subset of the dominating OTUs, indicating a tendency for attached growth in the well. Moreover, massively paralleled 454-pyrotag sequencing was performed. We used bidirectional sequencing of bacterial rRNA gene amplicons (~520 bp) which allowed for assembly, T-RF prediction and phylogenetic placement of dominating amplicon contigs. This data is still under analysis, but will illustrate a unique time series of drinking water microbial community details before and after well rehabilitation. Novel insights into microbial communities in groundwater wells will be provided, which improves our knowledge on the ecology of this ultra-oligotrophic habitat and also possible pools and fluxes of microbial taxa and potential pathogens.

MDV004
Recovery of methanotrophs from disturbance: population dynamics, evenness, and functioning.

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Biodiversity is claimed being essential for ecosystem functioning, but threatened by anthropogenic disturbances. Prokaryotes have been assumed to be functionally redundant and virtually inextinguishable. However, recent work indicates that microbes may well be sensitive to environmental disturbance. Focusing on methane oxidizing bacteria as model organisms, we simulated disturbance-induced mortality by mixing native with sterilized paddy soil in two ratios, 1:4 and 1:40, representing moderate and severe die-offs. Disturbed microcosms were compared to an untreated control. Recovery of activity and populations was followed over four months by methane uptake measurements, *pmoA*-qPCR, *pmoA*-based T-RFLP (terminal restriction fragment length polymorphism), and a *pmoA*-based diagnostic microarray. Diversity and evenness of methanotrophs decreased in disturbed microcosms, but functioning was not compromised. We consistently observed distinctive temporal shifts between type I and type II methanotrophs, and a rapid population growth leading to even higher cell numbers comparing disturbed microcosms to the control. Overcompensating mortality suggested that population size in the control was limited by competition with other bacteria. Overall, methanotrophs showed a remarkable ability to compensate for die-offs.

MDV005
Life in the cold, dark south - Microbial communities of marine methane seeps at Hikurangi margin (New Zealand)

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Marine methane seeps and associated chemosynthetic communities have been studied extensively in recent years. Many exciting discoveries have been made concerning the biodiversity of microbial communities, their

function, activity, influence on global geochemical cycles and their interaction with the environment. The bulk of research, however, was focused on a few areas situated in the Gulf of Mexico, the Mediterranean and Black Sea, the Northeast Atlantic and the Northeast Pacific. We here report on the first profound investigation of microbial communities of methane seeps on Hikurangi margin off the northeast coast of New Zealand; far away from all sites that have been described yet. The seep sites were not only characterized by novel seep associated fauna, namely Ampharetid and Frenulate polychaetes, but also differed strongly from other seeps by the present microbial communities. Results of 16S rRNA gene libraries and catalyzed reporter deposition fluorescence *in situ* hybridization (CARD-FISH) suggest that the associated fauna influences the underlying microbial community. High flows of methane at the Ampharetid habitat might not only be consumed by anaerobic methanotrophic archaea (ANME) mediating the anaerobic oxidation of methane (AOM) in the sediment, but also presumably by aerobic methanotrophs of the order Methylococcales at the sediment-water interface. In addition to an unusual ANME-2a aggregate structure and size distribution of AOM consortia, the findings suggest that ANME-2a is associated to more than one group of sulfate-reducing bacteria (SRB) in a single consortium. The microbial community of the Frenulate habitat is dominated by aerobic Crenarchaeota of marine group I. Although methane fluxes are present, ANME cells or AOM consortia were not detected in the shallow subsurface sediment. Furthermore, automated ribosomal intergenic spacer analysis (ARISA) of environmental DNA at nine sites on the margin and subsequent statistical evaluation showed that although species richness was comparable at all sites β diversity was very different revealing site-specific communities. Sediment depth and geographic location, most likely water depth, turned out to be major factors shaping microbial communities.

MDV006
Compatibility and Phylogeny - Plasmid Classification in the Genomics Era

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Whole genome sequences are present day bonanzas for taxonomists. Comparative genomics provides a promising perspective to reveal the evolutionary relationship between organisms, but this strategy is not applicable for extrachromosomal elements due to their high recombination frequencies. Classification of plasmids is based on their compatibility, i.e. the ability to coexist within the same cell. Classical compatibility testing is a laborious experimental discipline of pairwise replicon-comparisons. We pursue a complementary strategy essentially following the rationale that all plasmids identified from a single bacterium are *per se* compatible. We screened completely sequenced *Alphaproteobacteria* for plasmid specific replication modules, i.e. the replicase and two conserved partitioning genes, and focused our analyses on the *Roseobacter* clade (*Rhodobacterales*). Our phylogenetic analyses strongly indicate that distinct subtrees represent different compatibility groups. *Rhodobacterales* accordingly harbour at least nine different *repABC*-type plasmids that are expected to stably coexist within the same cell. This prediction is supported by the group-specificity of highly conserved palindromes representing well-known incompatibility regions (*inc*b).
Our new approach allows a reliable classification of different plasmid types and it is capable to deal with the exponentially increasing amount of sequence data. The systematic monitoring of plasmid-related sequences surprisingly revealed the presence of a novel *Rhodobacterales*-specific replication type. We designated it DnaA-like based on the homology to the chromosomal replication initiator DnaA. The functionality of *dnaA*-like replicons was proven by transformation experiments in *Phaeobacter gallaeciensis*. Its 262-kb *dnaA*-like plasmid is required for the brown pigmentation and incompatibility hence correlates with the formation of white colonies.

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[2] Pradella et al (2010): Genome organization of the marine *Roseobacter* clade member *Marinovum algicola*. Arch Microbiol 192: 115-126.
[3] Petersen et al (2010): Origin and evolution of a novel DnaA-like plasmid replication type in *Rhodobacterales*. Mol Biol Evol: advance access

MDV007
Bacterial speciation - aquatic *Alphaproteobacteria* as a model system

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The role of recombination, adaptation and selection in shaping bacterial diversity was elucidated by searching for different ecotypes within groups of closely related bacterial lineages (up to 100 % 16S rRNA gene sequence identity). Members of the family *Sphingomonadaceae* constituted an abundant fraction of the *Alphaproteobacteria* in the oligotrophic, alpine Walchensee and the mesotrophic, prealpine Starnberger See. Of these, two phylogenetically tight subgroups of *Sphingomonadaceae*, relatives of *Sandarakinorhabdus limnophila* as well as the novel lineage G1A, were identified in a clone library as the dominant *Sphingomonadaceae.* These two dominant groups were found to be physiologically active throughout the year by RT-PCR and DGGE of *rrn* transcripts. In parallel, a large number of *Sphingomonadaceae* could be isolated by a high throughput cultivation approach combined with PCR-based screening for *Sphingomonadaceae*. Among them 8 isolates were affiliated with the *S. limnophila*-cluster whereas 65 isolates formed the novel G1A cluster. Isolates of both groups were identical in their 16S rRNA gene sequence, but exhibit a considerable population substructure based on the rrn internal transcribed spacer (ITS1) sequences. In a final step the congruence between the population substructure and different ecotypes was elucidated by qPCR, physiological characterisation and *in situ* growth test.

MDV008
Singlet oxygen and hydrogen peroxide have different effects on Bacterioplankton community composition in a humic lake

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Reactive oxygen species (ROS) are generated by the photolysis of chromophoric dissolved organic matter (CDOM) in surface waters of humic lakes. With three independent *in situ* experiments we could show that artificially increased singlet oxygen ($^1O_2$) and hydrogen peroxide (H_2O_2) concentrations with the same inhibitory potential on ^{14}C-Leucine uptake affect bacterioplankton community composition (BCC) differently. BCC changes of abundant *Bacteria* were investigated with 16S rRNA gene clone libraries and BCC changes of metabolically active *Bacteria* by 16S rRNA gene based RT-PCR DGGE analysis. The relative abundance of *Polynucleobacter necessarius* and *Limnohabitans* related species (*Betaproteobacteria*) and *Novosphingobium acidiphilum* (*Alphaproteobacteria*) increased or were not affected by $^1O_2$ exposure [1] but decreased by H_2O_2 exposure. In contrast *Actinobacteria* of the freshwater AcI-B sub-cluster were not determined after $^1O_2$ exposure (Glaeser *et al*., 2010) but their relative abundance increased after H_2O_2 exposure. Because $^1O_2$ generation in humic particles is 2-3 magnitudes higher than in the surrounding water body [2] we investigated BCC shifts separately for particle-attached and free-living bacterioplankton. Furthermore changes in *Sphingomonadaceae*, *Betaproteobacteria*, and *Actinobacteria* communities were investigated in more detail with group specific 16S rRNA gene targeting RT-PCR DGGE analysis. ROS generation by DOM-photolysis is known since several years, but ROS were up to now not recognized as a selective factor for BCC. Our data show that the generation of ROS with different toxicity potential can shift BCC in a humic lake in a specific manner.

[1] Glaeser, S.P. et al (2010): Singlet oxygen, a neglected but important environmental factor: short-term and long-term effects on bacterioplankton composition in a humic lake. Environ Microbiol 12 (12): 3124-3136.
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MDP001
Microbial structure of biofilm communities in an uranium contaminated acid mine drainage environment

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Acid mine drainages (AMD) characterized by high concentrations of toxic metals and sulphate are an important environmental problem but also represent a unique habitat for microbial growth. The activity of chemoautotrophic microorganisms is the base of formation of these microbial communities, but also increases the rate of AMD formation. We investigated the microbial structure of biofilms grown in the underground AMD environment of a former uranium mine near Königstein (Saxony, Germany) characterized by high uranium concentrations (between 3×10^{-5} M to 6×10^{-5} M) and a pH in the range of 2.5 to 2.9 [1]. These acidophilic biofilms are macroscopic visible as filaments in flowing waters (so-called acid streamers) as well as stalactite-like forms hanging from the gallery ceilings (also designated as „snotites", [2]).
16S rRNA gene clone libraries constructed from both biofilm communities were dominated by a sequence affiliated with a beta-proteobacterium isolated by Hallberg et al. [2] from a macroscopic streamer from an abandoned copper mine. This novel acidophilic autotrophic iron oxidizer designated as *Ferrovum myxofaciens* strain PSTR accounted for 51% in the acid streamer community and for 76% of the „snotite" library. Less abundant sequences in the acid streamer library affiliated to the alpha and gamma subgroup of Proteobacteria (i.e. *Acidithiobacillus ferrooxidans*), respectively, as well as to the phyla of Actinobacteria, Firmicutes and Acidobacteria. Moreover, two clones similar to *Leptospirillum ferrooxidans* were originated from the stalactite forming biofilm.
The dominance of the *Ferrovum*-like bacterium was corroborated by *in situ* hybridization using the CARD-FISH technique. About 91% of the cells hybridized with the universal EUB probe were detected by means of a new designed *Ferrovum* specific probe.
Due to the extreme environmental conditions the biofilms grown in the former uranium mine Königstein are characterized by a limited species richness with a clear dominance of the novel acidophilic iron oxidizer *„Ferrovum myxofaciens"*.

[1] Arnold, T. et al (2010): Identification of the uranium speciation in an underground acid mine drainage environment analysed by laser fluorescence spectroscopy. Submitted to Geochimica et Cosmochimica Acta
[2] Hallberg, K.B. et al (2006): Macroscopic streamer growths in acidic, metal-rich mine waters in North Wales consist of novel and remarkably simple bacterial communities. Appl. Environ. Microbiol. 72, 2022-2030.

MDP002
Response of the *Roseobacter* clade to an experimentally-induced *Phaeocystis* bloom.

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We induced a *Phaeocystis* bloom in a 10 L-mesocosm of water collected in the German Wadden Sea by the addition of inorganic nutrients (nitrate, phosphate) to examine the response of the bacterioplankton growth and community composition and in particular of the *Roseobacter* clade. During the seven weeks experiment, the dynamics of the bacterial community of a light-dark (12:12 h) illumination were compared to a control mesocosm which was kept in the dark throughout the whole time. In the illuminated mesocosm a phytoplankton bloom, greatly dominated by Phaeocystis, developed, peaked after 14 days and declined to pre-bloom abundances after 25 days. Total bacterial numbers gradually increased until the end and reached $6x10^6$ cells ml^{-1}. CARD-FISH analyses showed that the *Roseobacter* clade constituted between 10 and 35% of total bacterial numbers with highest fractions after the decline of the bloom. DGGE analyses of 16S rRNA gene fragments with a *Bacteria*- and *Alphaproteobacteria*-specific primer set showed that the diversity, i.e. number of bands, was greatest during the decline of the bloom from day 14 to 25. In contrast, a *Roseobacter*-specific DGGE revealed a continuously high diversity of 22 to 32 bands throughout the experiment. Only the particle-associated fraction exhibited a distinct phase with a high diversity of 25 to 30 bands in the late break-down phase of the bloom and thereafter until day 35. Sequencing of bands excised from the *Alphaproteobacteria*-specific DGGE gels revealed mainly phylotypes of the *Roseobacter* clade and of surprisingly different subclusters. In the dark mesocosm, bacterial numbers and the number of DGGE bands continuously decreased until the end of the experiment yielding <4 bands finally and including only one *Roseobacter* phylotype.

MDP003
Nitrifying communities enriched from geothermal springs in Argentina

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Nitrification involves the sequential oxidation of ammonia to nitrite and further to nitrate. Two physiological groups of chemolithoautotrophic microorganisms, which perform this process, are referred to as ammonia-oxidizing bacteria or archaea (AOB and AOA) and nitrite-oxidizing bacteria (NOB). Nitrification plays a significant role in the global nitrogen cycle and occurs in a varity of moderate and extreme environments, including hot springs.
Sampling was performed in Las Máquinas, a thermal spring in the Argentinean province Neuquén in 2008 and 2009. This extreme habitat is dominated by the volcano Copahue and the surrounding geothermal area, which has not been investigated in detail in biological terms. The sampling sites are characterized through mud puddles with temperatures from 37°C to 74°C, pH values from acidic (2.5) to the neutral range as well as sulfur deposits.
Despite the fastidious growth and protracted cultivation of nitrifiers, we successfully enriched ammonia- and nitrite-oxidizing microorganism from Las Máquinas at moderate (28°C) and thermophilic (70°C) conditions.
Using 28°C for cultivation, members of *Nitrospira*, the dominant nitrite oxidizer at moderate thermophilic conditions [1], were detected by PCR with specific primers and typical cells of *Nitrosomonas* were found via electron microscopy. First fluorescence *in-situ* hybridization experiments showed the coexistence of bacteria and archaea in the cultures. Analyses of the 16S rRNA confirmed the FISH results in the case of the NOB culture and revealed the occurrence of archaeal und bacterial ammonia monooxygenase genes (amoA), a subunit of the key enzyme of aerobic ammonia oxidation.
Currently, both AOB and NOB enrichments at 70°C show a very slow consumption of the admitted substrate and cells are resistant against disruption. Therefore, identification and characterization of the potential novel nitrifiers still remain tricky. However, further investigations will gain insight into nitrification in such exclusive locations and the composition of the nitrifying community.

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MDP004
Influence of geographic location and land-use on the diversity of selected phyllosphere proteobacteria with special emphasis on pink-pigmented facultative methylotrophs

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Phyllosphere methylotrophic bacteria inhabit plant surfaces, mainly leaves. They play an important role in the methanol cycle by utilizing the methanol emitted by plants. On the other hand, they can produce plant growth promoting substances like auxins. One objective of this project is to analyse the diversity of selected phyllosphere bacteria, especially methylotrophs, in dependence of land-use type and geographic location of the sampling sites.
Cultivation of pink-pigmented facultative methylotrophs (PPFM) was performed after isolation from leaf samples of two different plants, *Trifolium repens* and *Cerastium holosteoides*. For each plant, samples were taken from sites with different land-use types. Isolates were grouped using amplified ribosomal DNA restriction analysis. From representative isolates the 16S rRNA gene sequences were analysed and phylogenetic analyses were performed using the neighbour-joining method. Community composition of

selected phyllosphere bacteria was investigated using a DGGE-analysis. Two primer systems for alpha and gamma proteobacteria were used.
The main groups of isolates could be found in both sampling periods, all sampling sites and all land-use types. Analysis of the 16S rRNA gene sequences confirmed that all isolates belong to the genus *Methylobacterium* with similarities between 97.3 and 100% to described species (*M. marchantiae*, *M. adhaesivum*, *M. mesophilicum*, *M. organophilicum*, *M. jeotgali*). Additionally, a new *Methylobacterium* species was found. Using DGGE, 96 leaf samples of *T. repens* from 3 sampling sites and different land-use types were analysed. First results suggest that some community members of the selected alpha proteobacteria occur on leaves of all different land-use types and both sampling periods. Using isolates from the same leaves as standards showed that the main community members seem to be *Methylobacterium* species. The composition of the selected gamma proteobacteria seems not to be influenced by land-use type.

MDP005
Influence of trans-resveratrol on the *Lactobacillus* populations of the human gut

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So far, little is known about the effect of secondary plant compounds on the composition of the human gut microbiota. Resveratrol was shown to favourably influence the *Lactobacillus* and *Bifidobacterium* populations in the gut of rats with DSS induced colitis. In this study, it was investigated whether resveratrol or resveratrol related compounds influenced the composition of bacteria in human faeces in vitro, with special reference to *Lactobacillus* populations, using culture-dependent and culture-independent techniques. The faecal microbiota of two individuals studied in vitro was noticeably different, which is in agreement with previous findings that the human microflora composition varies considerably between individuals. With the culture-independant DGGE technique, the gut microbiota appeared to be uninfluenced by resveratrol and related compounds, with only pterostilbene resulting in a small change in DGGE pattern of only one band difference. All other compounds, including resveratrol, piceid, ε-viniferin, piceatannol, hopeaphenol, amelopsin, ε-, R-, and R-2 viniferin, isoharpontin and trimethoxyresveratrol showed no clear effect on the faecal microbiota. For the experiments with resveratrol, culture-dependent techniques were used in addition to the culture-independent techniques to evaluate the influence of this compound on the microbial composition of the faecal microflora. In contrast to the results obtained with DGGE, these results showed that resveratrol led to an increase in the proportions of lactobacilli and bifidobacteria isolated on MRS+ agar, while those of enterococci and streptococci were decreased. Resveratrol could, therefore, positively affect the gut microflora by having a favourable effect on *Lactobacillus*- and *Bifidobacterium*-populations.

MDP006
Detection and Characterization of Rotavirus Genotypes (VP4) by RNA Electrophoretic Patterns and Phylogenetic analysis among children with acute gastroenteritis in Tehran-Iran

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Introduction: Acute gastroenteritis has been established as the major public health problem in worldwide children. Rotavirus is the most important etiological agent of gastroenteritis among children, It is also major cause of children malnutrition. Rotavirus,which is a member of the reoviridae family , has a genome 11 dsRNA segment that are enclosed in a triple- layered capsid. Rotaviruses are classified into G-type and P- type. Therefore, determining the prevalent and types of rotaviruses within region is essential to prepare for introducing a vaccine. Objectives: The genotype diversity of group A human Rotavirus and phylogenetic analysis of P-type detected by Multiplex RT-PCR and DNA- Sequencing. Material and Method: A total of 285 stool specimens were collected from children with diarrhea admitted to two pediatric hospitals between 2008 and 2010 in Tehran-Iran. Polyacrylamide gel electrophoresis (PAGE) was used for isolating positive rotavirus Stool samples and they were genotyped by Multiplex RT-PCR method. P-genotypes of rotavirus isolated were sequenced. Result: We found relatively high prevalence rate of rotavirus gastroenteritis in children. 29.1% stool specimens were positive .P [8] (81.92%) was the dominant of genotype, followed by P [4] (8.4%) , P [6] (7.2%) and mix type (2.4%). The peak of incidence was in the winter. A few sequence of P-genotypes strains isolated showed high level of similarity to strains from other Asian countries. Conclusion: we reported the VP4 genotype of rotaviruses - associated childhood diarrhea with high prevalence of P [8] genotype. Rotavirus strain surveillance programs are important for future vaccine formulation in Iran. Keywords: Human Rotavirus; VP4; Gastroenteritis; Genotype

MDP007
Investigating the diversity of endosymbiotic bacteria in the gills of the wood-boring bivalve *Teredo navalis*

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The shipworms (Teredinidae) are a family of wood-boring bivalves that harbor endosymbiotic bacteria in specialized cells (bacteriocytes) inside their gills. These symbionts are capable of digesting cellulose and of fixing molecular nitrogen under microaerobic conditions. They are thus supplementing the nutrition of their eukaryotic hosts and allow them to use wood as a primary food source.
In this work, the diversity of the endosymbiotic population in the gills of the wood-boring bivalve *Teredo navalis* Linnaeus, 1758, also known as the common shipworm, was analyzed. Four single-specimen 16S rDNA clone libraries with specimens from two different locations in the western Baltic Sea (Möltenort Harbor, Eckernförde Harbor) were created. Sequences were aligned considering secondary structure of the small subunit (SSU) rRNA molecule. The comparative sequence analysis was based on 1323 unambiguous sites. The phylogenetic inference using a Maximum Likelihood-based approach revealed a high diversity of closely related bacteria. However, these showed significant evolutionary distance to endosymbionts found in other host species like *Lyrodus pedicellatus* de Quatrefages, 1849 [1, 3].
Additionally, two bacterial strains (designated TN1023 and TN10130) were isolated from the gills of single specimens and partially characterized. Comparative sequence analysis of the SSU rRNA gene suggested close relationship to other strains of the culturable shipworm symbiont *Teredinibacter turnerae*, which have been described in the past [2]. Characterization by scanning electron microscopy, however, showed that at least one of these isolates (TN10130), in contrast to *Teredinibacter turnerae*, might be peritrichously flagellated.

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MDP008
Investigation into the effect of growth stages on the rhizospheric microbial community of rice plants

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Plants excrete diverse compounds into the soil, a process collectively known as rhizodeposition. Rhizodeposition forms the sole environment in the soil (rhizosphere) that is associated with a specific microbial community. Rice plants pass through several growth stages: the vegetative, reproductive and ripening stage. Microbial growth in the rhizosphere is stimulated by the continual input of readily assimilable organic substrates from the root. We hypothesize that changes in the plant growth and associated changes in root

exudation will influence the composition of the microbial community. In this study we investigated the community composition and genetic capacity of the rice rhizospheric microbial community. Therefore, rice plants (*Oryza sativa*) were grown in the greenhouse and rhizospheric soil samples were collected from replicate plants at five different time points during the different growth stages. Changes in the microbial community were monitored using Bacteria- und Archaea-specific terminal restriction fragment length polymorphism analysis (T-RFLP) of the 16S rRNA gene, followed by cloning and sequencing. The genetic potential of the soil microbial community was analyzed using the GeoChip microarray. GeoChip is a high throughput tool for studying microbial community structure linking to ecosystem processes and changes, that includes 28 000 probes covering 57 000 gene variants from 292 functional gene families involving carbon, nitrogen, phosphorus and sulfur cycles, energy metabolism, metal resistance as well as methanogenesis. Using the advantage of the functional gene array GeoChip to investigate the structure, diversity and metabolic activity of the microbial community and the ability of T-RFLP analysis to profile the microbial community, we will be able to get a precise overview of the impact of root exudation during the growth stages on the microbial community located in the rhizosphere of irrigated rice plants

MDP009
Extracellular bacterial chitinases from marine environments

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Chitin production in marine environments is enormous. Estimates for only a single genus of zooplankton (copepods) are exceeding billions of tons per year [2]. Due to these large amounts of chitin produced in marine environments, its degradation via chitinases is an extremely important step in nutrient cycling [4]. Chitinases mainly hydrolyse the beta-1,4-glycosidic bond between the chitin subunits and are members of the glycoside hydrolase (GH) superfamily [1]. Most of the bacterial chitinases belong to the GH 18 family [3].
In this work, 145 bacterial strains from the Baltic and the Mediterranean shallow and deep sea were investigated. The strains were screened for their genetic and physiological capability to degrade chitin. Within 53 strains a glycoside hydrolase family 18 A gene fragment was detected. The phylogenetic analysis revealed a broad distribution of chitinolytic microorganisms in the bacterial domain of life from different marine habitats. However, our findings show that only 17.8 % of the bacteria possessing the GH18 A gene fragment were able to degrade untreated chitin. Degradation of chitin was observed in 28 of the isolated strains. 12 isolates excreted a detectable extracellular chitinase.

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MDP010
Microbial communities on indwelling urinary tract catheters

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Catheter associated urinary tract infections (CAUTI) are diagnosed for millions of patients per years. They constitute 40 % of all nosocomial, mostly asymptomatic infections. Usually, after four weeks biofilm formation occurs on every catheter.
Multiple microbial species have been identified from catheter biofilms by using culture-dependent techniques. The most frequently found species are Enterobacteriaceae besides *Pseudomonas aeruginosa* and the Gram positive Stapylococci and Enterococci. But as known from other environmental analyses many more different species can be detected via the culture independent methodology than isolated.
Aim of this work is the identification of microorganisms from urinary tract catheter biofilms by using culture-independent methods. To determine the structure of the microbial community PCR-SSCP using universal 16S rRNA gene primers was performed.
A total number of 91 catheters were analysed. The communities consist of various Gram positive as well as Gram negative bacteria. Preliminary results showed that in catheters used for the first time on patients as well as in follow-up catheters the Lactobacillales and the Enterocacteriales were dominant. The populations did not shift significantly except for the absence of Pseudomonadales in the secondary catheters. Further obligat anaerobic bacteria could be detected (e.g. *Peptoniphilus harei*). The most frequent bacteria were *E. faecalis*, *Aerococcus urinae* and *P. mirabilis*.
Elucidation of the precise microbial community structure may enhance the opportunities for new directed antibiotic therapies or for the development of novel antimicrobial surfaces.

MDP011
Microbial diversity and changes in community composition in lab-scale biogas reactors depending on different substrates

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Production of biogas from agricultural resources involves a diverse community of different microorganisms. However, little is known about which species play key roles for the degradation of certain substrates in biogas plants. This knowledge could help to improve fermentation processes and enhance biogas formation by optimizing the conditions for these key organisms. Therefore lab-scale biogas reactors with volumes of 20 and 200 liters were set up. Reactors were started with a mixture of 70 % cow manure and 30 % pig manure to which different substrates like casein, starch and cellulose were added. First, a clone library was constructed in order to identify the most important groups of *Bacteria* in the basis feedstock for further analyses. These turned out to be *Bacteroidetes* and *Firmicutes*. Additionally, two subgroups of *Firmicutes* were investigated separately: *Lactobacillales* and *Clostridia* Cluster XIVa as well as *Bacteria* in general and methanogenic *Archaea*. Changes in the corresponding microbial communities were investigated with the help of SSCP (Single Strand Conformation Polymorphism) analyses. DNA fragments from predominant SSCP-bands were cloned and taxa identified by sequencing. Furthermore, quantifications of all microbial groups of interest were carried out by real-time PCR.
First results of SSCP analyses showed two dominant species of methanogenic *Archaea* for casein, starch and whipping cream as substrates for biogas production. DNA sequences found in these SSCP gels belonged to the genera *Methanospirillum* and *Methanobrevibacter* that are unable to degrade acetate. Corresponding SSCP band patterns did not show distinct changes with different substrates. Copy numbers of the *mcrA* gene encoding the methyl-CoM reductase calculated by real-time PCR resulted in about 10^8 per g dry matter (dm).
For *Firmicutes* and *Bacteroidetes*, six to seven and four to seven dominant species, respectively, could be observed depending on the substrate. In real-time PCR analyses, copy numbers of the corresponding 16S-rRNA gene fragments of about 10^9 per g dm for *Firmicutes* and 10^{10} per g dm for *Bacteroidetes* could be observed. In order to distinguish between presence and activity of the microorganisms, RNA-based analyses will follow.

MDP012
Population structure of aquatic *Sphingomonadaceae*

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The role of recombination, adaptation and selection in shaping bacterial diversity was assessed using aquatic members of the *Sphingomonadaceae* (*Alphaproteobacteria*) as a model group. Our multilocus sequence analysis (MLSA) targets a set of 9 housekeeping genes (atpD, dnaK, EF-G, EF-Tu, gap, groEL, gyrB, recA, rpoB) in *Sphingomonadaceae* and was used to elucidate the population structure and the significance of recombination events in this group. The new MLSA primers were designed based on all available genome sequences of 5 strains of *Sphingomonadaceae* and 2 strains of the closest phylogenetically related genus *Erythrobacter*. A total of 95 strains of *Sphingomonadaceae* were isolated from Starnberger See and Walchensee, and subjected to the novel MLSA approach. Based on their rRNA gene sequences, these strains fall into three different phylogenetic

groups. Multiple isolates were available for all major phylotypes. While 16S rRNA gene sequences were identical for the members of each 16S rRNA phylotype, the tree of the concatenated sequences of 9 housekeeping genes indicated a significant genomic divergence between the different strains. Based on the frequency of sequence divergence the isolated *Sphingomonadaceae* represent a clonal population. Based on its frequency, homologous recombination rather than mutation is the dominant force driving the divergence of the *Sphingomonadaceae*. Most significantly, MLSA revealed a distinct population substructure among individual phylotypes, suggesting different selection pressure between subclusters and the existence of distinct evolutionary units despite the identical or very similar 16S rRNA gene sequences.
Phenotypic clustering based on Biolog tests showed that the genotypic subpopulations as detected by MLSA did not exhibit distinct substrate utilization patterns. Evidently, niche separation by adapation to different growth substrates does not contribute towards genetic separation during the evolution of these freshwater *Sphingomonadaceae*.

MDP013
Spatial and temporal distribution of marine *Bacteroidetes* subgroups in contrasting water masses

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Members of the bacterial phylum *Bacteroidetes* are an important component of marine picoplankton and play a key role in organic matter degradation. In this study we analysed the spatial and temporal distribution of marine *Bacteroidetes* in samples taken during a cruise in September 2006 - from the Arctic Circle towards the Azores alongside the 30° W meridian and in samples taken during the spring diatom bloom 2009 at the long-term ecological research station Helgoland, Kabeltonne. Ten newly defined *Bacteroidetes* subgroups were analysed by fluorescence *in situ* hybridisation (FISH) with specific oligonucleotide probes. Generally, members of the subgroups affiliated to the class *Flavobacteria* were more abundant in all oceanic provinces examined than those belonging to the classes *Sphingobacteria* and *Cytophagia*. In the North Atlantic Ocean the distribution of several bacteroidetal subgroups indicated a specialisation for polar, temperate or subtropical oceanic provinces. For example, one of the subgroups affiliated to the DE2 clade occurred almost exclusively in the cold, nutrient-rich polar waters (DE2-805: 26.4 ± 1.0 x 10^2 cells ml^{-1}), whereas another closely related subgroup was found preferentially in the warm subtropical regions (DE2-873: 14.3 ± 1.7 x 10^2 cells ml^{-1}). Similar to the DE2-805 subgroup the *Sphingobacteria* A group was solely present in the nutrient-rich polar waters. There, it was observed that they can survive in the phycosphere of nanophytoplankton cells. During the spring diatom bloom at Helgoland the same bacteroidetal clades were generally present at higher abundances (0.13-5.20%), than in the North Atlantic Ocean (0.01-0.37%). For example, the DE2-805 subgroup achieved abundances up to 5% in early April 2009; whereas, the DE2-873 subgroup reached abundances of 0.19%. The *Sphingobacteria* clade SPC, which could not be detected in the North Atlantic Ocean showed abundances up to 0.13% during the Helgoland spring bloom. For most of the analysed *Bacteroidetes* clades distinct succession patters were revealed. Apparently, these subgroups possess different spatial as well as temporal niches. We found hints that closely related clades could represent different ecotypes with divergent lifestyles.

MDP014
Metalworking fluids reveal high unexpected diverse microbial communities

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Metalworking fluids (MWF) are widely used in metalworking industries for cooling and lubrication during e.g. drilling, cutting and grinding. It is estimated that worldwide about 2 x 10^{10} liters of MWF are used annually. Workers are exposed to MWF aerosols, which can cause health problems and can lead to illnesses such as hypersensitivity pneumonitis or asthma. Several microorganisms have been detected in MWF, mainly *Pseudomonas* or *Mycobacterium* species, which could be causative agents for health problems. Additionally, representatives of genera such as *Citrobacter, Ochrobactrum, Klebsiella, Neisseria* and *Salmonella* could be identified. So far, the microbial diversity of in-use MWF was described as quite low. Only few genera and species were found in each MWF sample.
In order to analyse the microbial diversity more systematically, ten samples of water based metal working fluids (MWF) were taken from five different companies, where MWF are used as coolants and lubricants in machining. Analysis of colony forming units (CFU), total cell counts (TCC), isolation of strains, cultivation-independent analysis of clones and 16S rRNA gene sequencing were carried out.
The results show that the number of CFU ranges from 0 to 1.3 x 10^8 CFU / ml MWF emulsion (R2A medium, Oxoid LTD, England), TCC were as high as 1.6 x 10^8 TCC / ml MWF emulsion. 37 strains from MWF were isolated and their 16S rRNA genes were sequenced. Additionally, the 16S rRNA genes for 380 clones were sequenced (500 to 900 bp), and from 183 clones the approximately full 16S rRNA genes were sequenced (1300 to 1450 bp). A total of 56 different genera could be detected, the number of different genera within one MWF sample varied between 5 and 21. Of these 56 genera, only 11 were detected by isolation and cultivation-independent methods, 9 genera were detected only by isolation and 36 genera were deteced only with cultivation-independent methods.
It could be shown, that MWFs harbour a high, previously unknown microbial diversity. Dominating genera were e.g. *Clostridium, Desemzia, Leucobacter, Pseudomonas, Serratia* and *Wautersiella*. The usage time of the MWF (1 week to 23 months of use before sampling) does not seem to have an impact on the microbial diversity within the MWF although mineral oil based MWF showed generally a higher diversity than MWF based on synthetic oil.

MDP015
Diversity of nitrite-oxidizing bacteria in WWTPs: Selective enrichment of a novel lineage II *Nitrospira* in co-culture with *Nitrotoga*

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In wastewater treatment plants (WWTPs) the population structure of nitrite-oxidizing bacteria (NOB) inhabiting activated sludge is complex. In most WWTPs the genus *Nitrospira* represents the dominant NOB and two lineages have been found in this habitat yet. Until now only *N. defluvii* (lineage I) has been highly enriched from activated sludge but no *Nitrospira* of lineage II. The only isolated representative of lineage II, *N. moscoviensis*, originates from a heating system and grows optimal at 39 °C. Other recent findings demonstrate the presence of close relatives of the novel nitrite oxidizer *Nitrotoga arctica* - previously found in permafrost soil - in wastewater, where it coexists with *Nitrospira*. In consideration that *Nitrotoga* prefers low temperatures and low nitrite concentrations, and *Nitrospira* has a broad temperature tolerance and favors also low nitrite concentrations, the competition between these NOB has to be elucidated in terms of changing conditions in WWTPs. For deeper insights into the physiological differences between *Nitrotoga* and *Nitrospira* we performed various enrichments of these NOB. In this study we cultivated a novel lineage II *Nitrospira* in co-culture with the previously discovered *Nitrotoga*-like bacterium HAM-1 at 10 °C. First physiological experiments suggested that the novel *Nitrospira* of lineage II has lower growth temperature (about 22 °C) and lower nitrite concentration preferences than *N. defluvii*, which grows optimal at a temperature of 32 °C. The culture was further studied by marker gene phylogenies of 16S rRNA genes and genes coding for the beta subunit of the nitrite oxidoreductase (nxrB) as well as FISH probes for lineage-specific detection. Additionally, an increasing diversity of *Nitrospira* was detected by analyzing further enrichments and it was shown that members of lineage I, which previously were found only in activated sludge, are also present in different habitats like desert soils, archaic cave systems and permafrost-affected soils. These results might help to explore physiological differences of *Nitrospira* within the same 16S rRNA lineage for a better understanding of the population dynamics in natural and engineered systems.

MDP016

Intrinsic differences in denitrifier community structure and abundance determine functional responses of denitrification in three organic soils

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Denitrification is an alternative anaerobic respiration process reducing nitrogen oxides (NO_3^- and NO_2^-) stepwise to N_2 via the intermediates NO and N_2O. This process completes the global nitrogen cycle and is of particular importance for the biogeochemical cycling of nitrogen in soils. Soils are important sources for N_2O, a potent greenhouse gas and contribute about 70% of the N_2O emitted to the atmosphere. The microorganisms capable of denitrification are polyphyletic and exhibit differences in the induction and activity of the denitrification system in individual strains which could result in ecosystem level differences in N_2O emission under different conditions [4]. Thus, community composition will affect community and ecosystem functioning.
In this study, we comparatively evaluated the structure and abundance as well as the similarity of denitrifier communities from three drained organic soils in Finland, Germany and Sweden differing in soil history and soil parameters. Structure and abundance of denitrifier communities were explored based on their NO_2^--reductase (*nirK*/*nirS*) and N_2O-reductase (*nosZ*) genes as proxies for the ability of the communities to produce and reduce N_2O. We hypothesized that the denitrifier communities harbored by these soils were composed differently since marked physiological differences in denitrification response to anoxia [2] and low temperature [1, 3] occurred. Moreover, a direct effect of pH had been observed when exposing bacterial consortia extracted from these soils to two different pH levels (pH 5.4 and 7.1). We evaluated differences in the diversity, composition and abundance of denitrification genotypes between soils and conclude that links exist between the genetic makeup and physiological responses across the three denitrifier communities. Moreover, we hypothesize that functional differences were enhanced due to differences in the composition of the active denitrifier community in response to different environmental triggers, e.g. temperature and pH.

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MDP017

A novel approach for alphaprotebacterial plasmid classification

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Members of the *Roseobacter* clade are endowed with a remarkable wealth of plasmids, e.g. up to twelve extrachromosomal replicons could be identified in *Marinovum algicola* (Pradella et al. 2009), comprising one third of the total genomic information. To investigate this diversity, a comprehensive plasmid classification scheme was established.
Plasmids are classified according to their compatibility, i.e. the ability of two or more plasmids to be stably maintained in a cell lineage. Our classification approach is based on phylogenetic analyses of the replication operons, which constitute the functional backbone of plasmids. These systems comprise the genes for replication and partitioning, revealing a common evolutionary history due to functional linkage. Operons of the same type can be found on up to four plasmids in a single cell, indicating their compatibility. The required functional divergence of compatible plasmids correlates with phylogenetic distance, i.e. their replication modules are located in different subtrees. In case of the alphaproteobacterial replication operon *repABC*, nine distinct groups were identified in the *Roseobacter* clade (Petersen et al. 2009). To validate our phylogeny based *in silico* predictions regarding plasmid compatibility, we developed a test system: selected *repABC* - modules were cloned into suited vectors and introduced into *Phaeobacter gallaeciensis* DSM 17395 . Successful transformation can be traced through specific antibiotic resistances provided by the respective construct. Accordingly, double transformands can be detected by the expressed double resistance resulting from maintenance of compatible constructs.
We observed that plasmids with phylogenetically closely related *repABC* operons, outcompete each other, and are therefore incompatible. In contrast, plasmids with distant *repABC* operons, stably coexist in the cell and are compatible. The results verify the predictions deduced from the *in silico* analyses.
Hence, our phylogenetic classification framework for plasmid replication systems allows the rapid allocation of new plasmids from incoming gene sequences. Furthermore it allows the development of genetic tools for entire plasmid knockouts and the comparison of plasmid knockout mutants and wild type strains will reveal the significance of alphaproteobacterial plasmids.

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MDP018

Flavobacteria of the North Sea: Diversity of Culturability

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Flavobacteria account according to cultivation-independent in situ hybridisation experiments for up to 30% of the *Bacteria* in the North Sea. They are considered as ecologically important microorganisms involved in the degradation of polymers. So far, only a few isolates have been described from the North Sea, mainly *Maribacter* (Barbeyon *et al.*, 2008) and *Dokdonia* (Riedel *et al.*, 2010). But 16S rRNA gene clone libraries have suggested that other species are the ecologically significant *Flavobacteria*.
We attempted a cultivation of *Flavobacteria* from different North Sea habitats (Harlesiel, Helgoland, Janssand, Sylt) originating from different marine sample materials (sediment, seawater and surfaces of plants, animals and stones) on agar plates with a variety of carbon sources (malate, glucose, arabinaose, cellobiose, galactose, xylose, peptone, casaminoacids, yeast extract) and sometimes the antibiotic kanamycin. Candidate colonies were identified by their yellow to orange colour and rod-shaped morphology under the microscope. Subsequently, 483 isolates were screened by PCR with a *Flavobacteria* specific primer designed for this purpose and the partial 16S rRNA gene was sequenced, revealing 307 *Flavobacteria,* 2 *Sphingobacteria* and 11 *Cytophagia*. The strains affiliated with 24 genera. Furthermore, representative isolates were analysed for flexirubin type pigments. Comparable to the literature, isolates affiliated with the genera *Zobellia*, *Grigella*, and *Aquamarina* were flexirubin positive. But some isolates of the genera *Arenibacter* and *Lacinutrix* were flexirubin negative, in contrast to the literature. In this study we were able to isolate strains of novel species of the *Flavobacteria* originating from the North Sea. A first analysis revealed a distinction between pelagic and costal isolates, as well as between isolates from sediment and sea water.

MDP019

Comparative phenomics of the wild type *Phaeobacter gallaeciensis* and its 65 kb plasmid knock-out mutant

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Plasmids of the marine *Roseobacter* clade carry important genetic traits, like genes for the aerobic anoxygenic photosynthesis or the catabolism of phenylacetate [3, 4]. Our completely sequenced model organism *Phaeobacter gallaeciensis* DSM 17395 harbours three plasmids with sizes of 262 kb, 78 kb and 65 kb. The smallest plasmid includes a conspicuous wealth of genes for polysaccharide metabolisms, e.g. for mannose and rhamnose synthesis. The same polysaccharides are involved in symbiotic adhesion of *Rhizobia* [1] and may also be responsible for biofilm formation of *P. gallaeciensis* and symbiotic interactions with algae.
To investigate the function of the 65 kb plasmid, we generated the respective knock-out mutant based on plasmid incompatibility. Extrachromosomal elements harbour specific modules for autonomous replication and partitioning systems, but similar systems are incompatible and these plasmids cannot coexist within the same cell. The 65 kb plasmid contains a RepA-replication system and we cloned the homologous module from

Dinoroseobacter shibae for our knock-out strategy [2]. The construct including a gentamycine resistance cassette was transformed into strain DSM 17395 and thus induced the loss of the indigenous plasmid.
The plasmid knock-out mutant and the wild type strain were compared using the Phenotype MicroArray™ technology (PM; Biolog, USA). With this system nearly 2000 cellular phenotypes can simultaneously be tested in a microtiter plate format. To establish PM data for *P. gallaeciensis* and other members of the *Roseobacter* clade, we optimized the assay conditions regarding salt concentration, vitamins and micronutrients. We focused our initial experiments on plates PM 1 and 2, in which 190 different carbon sources are tested, and analyzed the respective kinetics.
Comparing the metabolic activity of the mutant and the wild type strain in PM 1 and 2, we observed kinetic differences in the conversion of many substrates and it was revealed that the mutant was unable to metabolize four carbon sources. Surprisingly, rhamnose was not converted at all. The mannose metabolism showed a reduced metabolic response in case of the mutant. The functional role of the 65 kb plasmid in *P. gallaeciensis* will be investigated by comparing mutant and wild type strain via biofilm assays.

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MDP020
The influence of plant surface characteristics on naturally colonizing bacterial communities of *Arabidopsis thaliana* leaves

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The phyllosphere of plants represents a suitable habitat for microorganisms. In our study we aim to characterize the role of specific plant surface characteristics of *A. thaliana* and its natural bacterial inhabitants. Several studies have indicated that plant trichomes may affect microbial colonization of the phyllosphere. In a first project, the *A. thaliana* ecotype Col-0 and its *gl1* mutant, devoid of trichomes on the leaf surface, were compared with regard to bacterial community diversity on their leaf surfaces. Comparative analyses were performed using cultivation independent denaturing gradient gel electrophoresis (DGGE). DGGE banding patterns and sequencing of representative DGGE-bands showed only minor differences between the two plant lines suggesting that the presence of trichomes *per se* does not affect bacterial diversity. In a second project, we seek to investigate whether the very-long-chain alkanes known to be present on the *A. thaliana* cuticle serve as a substrate for bacterial growth. Bacterial communities derived from *A. thaliana* ecotype Ler leaf surfaces were grown in enrichment cultures with mineral salts medium overlaid with C_{16} alkane or with addition of C_{22} alkane as a sole carbon source. 16S rRNA gene sequencing and phylogenetic analyses of bacterial isolates from the enrichment cultures classified most of the isolates as *Rhodococcus* species, which are well known alkane degraders occuring in mineral oil contaminated environments. Our future efforts are directed towards analyzing the capability of isolated phyllosphere bacteria to metabolize and to degrade other cuticular wax constituents.

MDP021
Analyzing the biofilm-forming microbiota in a biogas processing plant by 454-Pyrosequencing

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The resident microbiota was analyzed by 454-Pyrosequencing in a mesophilic, continuously operating biogas digester supplied with food-leftovers, stale bread and liquid pig manure. Substrates like food-leftovers and stale bread possess low structure but provide a high energy potential in terms of carbohydrates, proteins as well as fat. Fast fermentation on the other hand poses a high risk to overload the whole process. In fermentation experiments at laboratory scale (4 parallels, 10 L digester) 5 g/l of straw of 2 cm length was supplied as additional surfaces for biofilm formation. These amendments stabilized the biogas processes especially at increasing organic loading rates. With that purpose an adequate amount of straw as a biofilm carrier was added to a fermenter (300 m^3) of a full-scale biogas plant. The 454-Pyrosequencing study was used to compare the microbial composition attached to the straw to them with the liquid fermenter content.
Therefore, the sample A was taken from the fermenter before the straw was added to the process. Simultaneously, a labscale fermentation was started and operated continuously. After 21 days of fermentation the sample B was taken from labscale biogas experiment and sample C was taken from the full-scale biogas plant. The fluid fermenter content form samples A, B and C was sieved and the microbial biomass attached to particles (A) and the straw (B and C) was removed with a sterile swap. The six samples obtained were used for genomic DNA preparation and amplification of bacterial and archaeal 16S rDNA.
The 454-Pyrosequencing of pooled 16S rDNA products resulted in a total of 101269 16S rDNA sequences. Allover 44 % belong to bacteria and 56 % to the archaea. About 52 % of the sequences belong to the samples of the fluid fermenter content and 48 % to the samples of biofilm-forming microbiota attached to straw or particles.

MDP022
The effect of forest management intensity on the diversity of wood-decaying fungi and deadwood decomposition

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Dead wood, also referred to as coarse woody debris (CWD), is a key habitat element in all forest ecosystems and is decayed by various types of organisms such as insects, bacteria and fungi. Among the latter, chiefly Basidiomycota (white- and brown-rot fungi) and a few Ascomycota (soft-rot fungi) are the main wood decomposers. To accomplish lignocelluloses degradation, they actively secrete different sets of oxidative and hydrolytic biocatalysts. Here, we report on the change in dead wood fungal diversity along a forest management intensity gradient and its influence on wood decay and ecosystem processes such as lignin degradation and secretion of oxidative enzyme activities.
We selected around 200 CWD locks of different decay stages and diameter in the Biodiversity Exploratories in Germany (Schorfheide-Chorin, Hainich-Dün and Schwäbische Alb). In each Exploratory are nine plots with different management intensities (unmanaged, age-class forest and selection forest). We took from all locks up to five samples. These samples were analysed for Klason Lignin and water-soluble fragments with aromatic properties (UV absorption band at 280 nm). Also the enzyme activities of laccases and peroxidases (manganes in- and dependent) were measured.
In this poster, we focus on the following questions: How do the content of lignin and water-soluble aromatic fragments of CWD as well as enzyme activities of laccases and peroxidises in CWD vary in dependency on colonization patterns of wood-decaying fungi and on the different decay stages?

MDP023
Molecular biodiversity of *Mycobacterium tuberculosis* isolates from patients with pulmonary tuberculosis in Bulgaria

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Introduction: Tuberculosis remains an important public health issue for Bulgaria and other Balkan countries located in the world region with contrasting epidemiological situation. The rate of multidrug-resistant strains among newly diagnosed TB patients in Bulgaria was estimated to be 10.7% that is much higher than in the neighbouring countries. The prediction of drug resistance by molecular tools presents a correct and rapid detection of resistant strains for timely anti-TB therapy and constitute one of the priorities of the national TB control program.
Methods: The study set included all available DNA samples isolated in several provinces across Bulgaria were analyzed by various molecular

methods (IS*6110*-RFLP, 24-MIRU-VNTR and spoligotyping). Drug resistance mutations analysis was apply for *rpoB* hot-spot, *katG315*, *inhA* promoter region, and *embB306*.

Results and Discussion: The availability of the international database SITVIT2 permitted us to view our results in the context of the globally and locally circulating *M. tuberculosis* clones. Comparison with SITVIT2 showed that spoligotype ST53 is found in similar and rather high proportion in the neighboring Greece and Turkey and almost equally distributed across different regions of Bulgaria. Contrarily, ST125 is not found elsewhere and is specific for Bulgaria; furthermore it appears to be mainly confined to the southern part of the country. Novel 15/24-loci format of MIRU-VNTR typing was found to be the most discriminatory tool. Three types of the *rpoB* mutations were found in 20 of 27 RIF-resistant isolates; *rpoB* S531L was the most frequent. Eleven (48%) of 23 INH-resistant isolates had *katG* S315T mutation. *inhA* -15C>T mutation was detected in one INH-resistant isolate and three INH-susceptible isolates. A mutation in *embB306* was found in 7 of 11 EMB-resistant isolates. A monoresistance was found in 15 of 37 drug-resistant isolates and may be an additional indication of the somewhat insufficient anti-TB control in Bulgaria.

Acknowledgements: This work was partly supported by European Social Found, Bulgaria, Development of human resources-BG051PO001-3.3.04/32 and National Science Fund, Bulgaria,'Young Researchers'DMU 02/1.

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MDP024
Bacteria and archaea involved in anaerobic digestion of distillers grains with solubles

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Cereal distillers grains with solubles (DDGS), a by-product from bioethanol industry, were tested as a possible substrate for biogas production in mesophilic laboratory scale anaerobic digesters. The effects of various organic loading rates (OLR), iron additives for sulfide precipitation, as well as aerobic substrate pretreatment on microbial community structure and performance were investigated. Five continuously stirred tank reactors were run under constant conditions and monitored for biogas production and composition along with other process parameters such as pH, volatile fatty acids (VFA) and ammonium. The microbial communities in the reactors were investigated for their phylogenetic composition by terminal restriction fragment length polymorphism (T-RFLP) analysis and sequencing of 16S rRNA genes. Iron additives for sulfide precipitation significantly improved the process stability and efficiency, whereas aerobic pretreatment of the grains had no effect. The bacterial subcommunities were highly diverse, and their composition did not show any correlation with reactor performance. The dominant phylotypes were affiliated to the phylum *Bacteroidetes,* among them various members of the *Porphyromonadaceae*. Furthermore, members of the *Actinomycetales* seemed to play a significant role, whereas *Clostridia* were less abundant. The archaeal subcommunities were less diverse and correlated with the reactor performance. The well-performing reactors operated at lower OLR and amended with iron chloride were dominated by aceticlastic methanogens of the genus *Methanosaeta*. The well-performing reactor operated at a high OLR and supplemented with iron hydroxide was dominated by *Methanosarcina* ssp. The reactor without iron additives was characterized by lowest biogas yield, accompanied by VFA accumulation and high hydrogen sulfide content, and was dominated by hydrogenotrophic methanogens of the genus *Methanoculleus*. Our results show that distillers grains are a valuable feedstock for biogas production but the use of iron additives is needed to ensure high biogas yield.

MDP025
Thermophilic microbial community for methane production at high temperatures

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The development of an efficient and sustainable bioethanol production plant based on waste biomass requires the integration of various biological and non-biological processes. After the fermentation of raw wheat straw to ethanol and the distillation process large amounts of lignocellulose and yeast cell material remain untreated (stillage). In this project an attempt was made to identify the microbial community, which is involved in the bioconversion process to methane at elevated temperatures (55°C to 70°C). By several molecular biological and microbiological methods e.g. Denaturing Gradient Gel Electrophoresis (DGGE) and 16S rDNA different species of the genera *Methanobacterium* and *Methanosarcina* were identified and pure cultures were isolated. Optimal growth was obtained at a temperature range between 55°C and 70°C; most isolates were sensitive to chloramphenicol and required hydrogen for growth. The establishment of a robust and defined thermophilic microbial community will contribute to the development of a more efficient biogas production technology. This concept will be developed in collaboration with partners from academia and industry (Biorefinery2021) and is supported by the Graduate School 'C_1-Chemistry for Resource and Energy Management' of the Landesexzellenzinitiative Hamburg (LEXI).

MDP026
Impact of fungal aldehyde dehydrogenase on ectomycorrhizal symbiosis

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Ectomycorrhizal fungi are known to improve plant growth, the supply of nutrients and protect plants from pathogens during symbiosis. We isolated a fungal aldehyde dehydrogenase (ALDH) encoding gene *ald1* from the basidiomycete *Tricholoma vaccinum*. ALDHs catalyze the conversion of aldehyde to carboxylic acid in alcohol metabolism.
*Ald1 s*hows specific expression in ectomycorrhiza during interaction with the compatible host spruce (*Picea abies*). It has a function in the detoxification of alcohols and aldehydes occurring in mycorrhizal biotopes and is involved in phytohormone production. By using competitive and real-time RT-PCR, *ald1* was shown to be induced in response to alcohol- and aldehyde-related stress. Overexpression of *ald1* in *T. vaccinum* resulted in an increase in ethanol stress tolerance of the fungus. Phylogenetic analyzes revealed duplication events within the specific fungal ALDH family, which we verified with *T. vaccinum* ALDH sequences (*ald1*, *ald2* and *ald3*).

MPV001
A metaproteomics approach to study host-pathogen interactions between *Pseudomonas aeruginosa* and *Caenorhabditis elegans*

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Pseudomonas aeruginosa, a Gram-negative opportunistic pathogen, causes life-threatening and chronic infections in immunocompromised patients or people suffering from cystic fibrosis and employs an *N*-acyl homoserine lactone -mediated quorum sensing (QS) system to coordinate e.g. the expression of virulence factors in a cell-density dependent manner. Non-mammalian infection models such as *Caenorhabditis elegans* are well-established tools to obtain first insights into molecular mechanisms underlying bacterial pathogenicity. State-of-the-art gel-free, semi-quantitative proteomics based on unique spectral counting allows investigating the „infectiosome" defined as global changes in protein expression in both the host and the pathogen during the infectious-like process (ILP).

Here, we present a comparative proteome analysis of the highly pathogenic *P. aeruginosa* strain PA14 during colonization of *C. elegans* and growth on NGMII-agar (control), respectively. To this end, the nematodes were homogenized after 24 h of „infection", paramagnetic beads coupled to anti-*Pseudomonas* sp.-antibodies were used to enrich bacterial cells, and proteins were extracted and trypsin-digested. The resulting mixture of bacterial and nematode-derived peptides was analyzed by reverse-phase liquid chromatography coupled to electrospray ionization tandem mass spectrometry (MS). MS data were searched against a database containing protein sequences of *P. aeruginosa* PA14 / PAO1 and *C. elegans*. A total of 3940 *C. elegans* and 1500 *P. aeruginosa* proteins were identified from the „infected" nematode, while 2952 bacterial proteins were found in the control. Numerous QS-regulated proteins like proteins involved in phenazine biosynthesis or iron sequestration were found to be highly expressed during the ILP. Overall these findings strikingly confirm the central role of QS-regulated protein expression for *P. aeruginosa* pathogenicity.
The obtained data are currently validated by testing *P. aeruginosa* mutants defective in selected proteins that were exclusively or highly expressed during the ILP in the *C. elegans* pathogenicity model. In a next step, we will extend our metaproteome analyses to a chronic murine infection model system to identify specific pathogenic traits underlying mammalian *P. aeruginosa* infections.

MPV002
A typical eukaryotic lipid in prokaryotic membranes: Synthesis and necessity of phosphatidylcholine in *Agrobacterium tumefaciens*

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The typical eukaryotic membrane lipid phosphatidylcholine (PC) is also present in rather diverse bacteria. A number of pathogenic and symbiotic PC-containing bacteria like *Bradyrhizobium japonicum*, *Brucella abortus* and *Legionella pneumophila* require PC for an efficient interaction with their respective hosts [1].
A PC-deficient *Agrobacterium tumefaciens* mutant lacking *pmtA* and *pcs* is unable to elicit plant tumors [2]. This virulence defect is based on the impaired expression of genes encoding the type IV secretion machinery. This machinery is responsible for transfer of the oncogenic T-DNA into plant cells. PC-deficient mutants are hyper-sensitive towards the detergent SDS and show a growth defect at elevated temperature. Furthermore, the PC-deficient mutant is reduced in motility and enhanced in biofilm formation [2, 3].
PC in *A. tumefaciens* is synthesised via two pathways: the methylation pathway and the phosphatidylcholine synthase (Pcs) pathway. The methylation pathway involves a three-step methylation of phosphatidylethanolamine catalysed by the phospholipid *N*-methyltransferase PmtA. *S*-adenosylmethionine (SAM) provides the methylgroup and is converted to *S*-adenosylhomocysteine (SAH) during transmethylation [3]. In a second pathway the membrane protein Pcs condenses CDP-diacylglycerol with choline to form PC.
PmtA was recombinantly produced in *E. coli* and purified via affinity chromatography. PmtA is a monomer and methyltransferase activity is inhibited by PC and SAH. Moreover, SAM binding depends on lipid binding and PmtA activity is stimulated by phosphatidylglycerol, one of the main phospholipids in *A. tumefaciens* [4]. By combining *in silico* analysis and point mutagenesis the SAM-binding pocket was identified in the N-terminal part of PmtA.

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[3] Klüsener, S. et al (2009): J Bacteriol 191, 365-374
[4] Aktas, M. and F. Narberhaus, F. (2009): J Bacteriol 191, 2033-2041.

MPV003
Virulence properties of *Legionella pneumophila* GDSL lipolytic enzymes: Proteolytic activation of PlaC acyltransferase activity

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Legionella pneumophila infects both mammalian cells and environmental hosts, such as amoeba, and expresses a multitude of lipolytic enzymes belonging to three different lipase families. One of these families, the GDSL hydrolases, comprises enzymes of prokaryotic and eukaryotic origin with phospholipase, acyltransferase, and hemolytic activities. Enzymatic activity depends on a conserved nucleophilic serine embedded into the GDSL motif as well as on the residues aspartate and histidin together building up the catalytic triad. The *L. pneumophila* genome codes for three GDSL-hydrolase genes: *plaA, plaC* and *plaD*. The three enzymes show lysophospholipase A (LPLA) and phospholipase A (PLA) activity with PlaA being the major secreted LPLA. The sequences of PlaA and PlaC harbour N-terminal signal peptides for Sec and subsequent type II-dependent protein export, whereas the secretion mode of PlaD is still unclear. PLA/ LPLA activity of GDSL-enzymes therefore contributes to the hydrolysis of eukaryotic membrane phospholipids and leads to the release of toxic lysophosphatidylcholine, which can function as signal transducer and stimulator of inflammatory response. We here aimed further characterization of *L. pneumophila* PlaC which in addition to PLA and LPLA activities, transfers free fatty acids from phospholipids to cholesterol and ergosterol. This GCAT activity is post-transcriptionally regulated by ProA, a secreted zinc metalloprotease and we here studied the specific cleavage site of ProA within PlaC. Since cholesterol is an important compound of mammalian cell membranes and egosterol of amoeba membranes, GCAT activity might be a tool for host cell remodelling during *Legionella* infection. As phospholipases are important virulence factors that have been shown to promote bacterial survival, spread and host cell modification or damage, we here also aimed to investigate the contribution of GDSL enzymes to *L. pneumophila* virulence and further addressed the impact of GDSL-enzyme combinations on host infection.

MPV004
A *yjbH*-homologue in *S. aureus:* a new role of a thioredoxin-like protein in ß-lactam resistance

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Bacteria have to adapt to a variety of environmental stresses and thereby trying to keep their cellular reduction/oxidation (redox) status in balance. Using low-molecular-weight thiols, like thioredoxins, help aerobic bacteria to maintain a reducing cytoplasm in which protein cysteines are kept in their thiol (-SH) state. The breakage of unwanted disulphide bonds is often mediated via a conserved active site loop motif (CXXC) within these thioredoxins by interprotein exchange reactions [1]. As *Staphylococcus aureus* lacks alternative thiol redox systems, the thioredoxin system is therefore essential for growth [1].
YjbH as a thioredoxin-like protein has been describe in *Bacillus subtilis* as a novel effector within the disulphide stress regulation. Cells lacking *YjbH* show pleiotropic defects in growth, sporulation and display a reduced sensitivity to the thiol oxidant diamide [2].
Hence, the current research project deals with the characterisation of the *YjbH*-homologue in *Staphylococcus aureus*, its active site motif (CXC) and the role in responding to thiol oxidants. In addition, the defined knock-out mutant (Δ*yjbH*) showed an influence on beta-lactam resistance and peptidoglycan cross-linking. Taken together, these results indicate an additional functionality of the *YjbH* protein in *Staphyloccus aureus.*

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[2] Larsson, J.T.et al (2007): Mol. Micro. 66.

MPV005
Iron-limitation triggers the virulence of *Pseudomonas aeruginosa* in urinary tract infections

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Urinary tract infections are one of the most common bacterial infections. Uncomplicated infections are mainly caused by *Enterobacteriaceae*. However, in case of complicated urinary tract infections *Pseudomonas aeruginosa* was identified as one of the most frequent pathogens. The progressive course of these infections is due to the remarkable ability of P. *aeruginosa* to adapt to hostile environments, its multifactorial virulence and its high intrinsic antibiotic resistance.
An *in vitro* growth system mimicking the conditions in the urinary tract was established to investigate the physiology of *P. aeruginosa* during urinary tract infections. Comprehensive transcriptome, proteome and metabolome analyses showed a general change in metabolic processes indicating that *P. aeruginosa* suffers from nutrient starvation and energy limitation. Moreover, in response to iron-limitation and osmotic stress a fine-tuned regulation controls the expression of several important virulence factors.
In summary, the results indicate that the adaptative response of *P. aeruginosa* to the specific conditions in the urinary tract activates a regulatory network inducing the production of virulence factors.

MPV006
Metabolomic priming by a secreted fungal effector

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A successful colonization of plants by pathogens requires active effector-mediated suppression of defense responses. Here we show that the biotrophic fungus *Ustilago maydis* secretes an enzymatically active chorismate mutase Cmu1. This enzyme is taken up locally by infected plant cells and then spreads to neighboring cells. Nonregulated enzymatic activity of the fungal chorismate mutase and interactions with cytoplasmic plant chorismate mutases are likely to be responsible for a re-channeling of the shikimate pathway. The comparison of the metabolomes of maize plants infected either with cmu1- deletion mutant or its progenitor strain showed significant changes in phenylpropanoid pathway derivates and phytohormone levels. Based on these findings, we propose a model in which the virulence factor Cmu1 actively reduces salicylic acid levels, thereby allowing the suppression of PAMP-triggered defense responses. Through this „metabolic priming", the maize plant is prepared for a successful infection by *Ustilago maydis*. Our study describes a novel strategy for host modulation that might be used by a wide range of biotrophic plant pathogens.

MPV007
SACOL0731, a new regulatory link between central carbon metabolism and virulence determinant production in *Staphylococcus aureus*

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Carbon catabolite repression (CCR) in bacteria is a widespread, global regulatory phenomenon that allows modulation of the expression of genes and operons involved in carbon utilization and metabolization in the presence of preferred carbon source(s). In low-GC gram-positive bacteria, CCR is mediated mainly by the catabolite control protein A (CcpA), a member of the GalR-LacI repressor family. In *Staphylococcus aureus*, CcpA has been shown to modulate the expression of metabolic genes and virulence determinants in response to glucose. A second regulator that links carbon metabolism and virulence factor production in this organism is CodY, a sensor of carbon and nitrogen availability that responds to intracellular concentrations of branched-chain amino acids (BCAA) and GTP.
Here we show that *S. aureus* produces a third regulatory molecule, SACOL0731 (a member of the LysR family of transcriptional regulators with homology to CitR of *B. subtilis*) that links central carbon metabolism with virulence determinant production. By deleting this putative *citR* homolog in *S. aureus*, we could show that the inactivation of this gene resulted in a decreased *citB* (encoding the tricarboxylic acid [TCA] cycle enzyme aconitase) transcription, which was also illustrated by a strongly reduced aconitase activity of the mutant under growth conditions that lack glucose. This regulatory effect was also confirmed by NMR-spectroscopy studies, which revealed an elevated citrate content in SACOL-0731 mutant cells. In line with previous findings showing that inactivation of the TCA cycle influences virulence determinant production of *S. aureus*, we found that the transcription of virulence factors such as *capA* (encoding capsular polysaccharide synthesis enzyme A), *hla* (encoding α-hemolysin), and of *RNAIII*, the effector molecule of the *agr* locus, were significantly affected by the SACOL0731 mutation.

MPV008
Characterization of methionine auxotrophic clinical *Pseudomonas aeruginosa* isolates

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Patients with the genetic disorder cystic fibrosis (CF) suffer from increased mucus production in the upper airways. This mucus is rich in nutrients as e.g. amino acids and is colonized by a heterogeneous microflora, which causes persistent infection. Infections with the opportunistic pathogen *P. aeruginosa* are associated with a poor prognosis due to the failure of antibiotic treatment. *P. aeruginosa* colonizes CF mucus and adapts to the CF lung environment by mutation. Auxotrophic *P. aeruginosa* strains are frequently isolated but their contribution to persistent infection is poorly understood.
Most auxotrophic strains require the amino acid methionine for growth. Interestingly, the methionine metabolism of *P. aeruginosa* is closely connected to the formation of the N-acyl-homoserine lactones (AHLs) the quorum sensing molecules.
Here, we investigated and characterized 28 methionine auxotrophic *P. aeruginosa* isolates to elucidate the underlying adaptation strategies. We identified that methionine auxotrophy was caused by a mutation in the *metF* gene in 12 out of 28 clinical *P. aeruginosa* isolates. To elucidate the phenotype of a *metF* mutant, we constructed and characterized a knockout mutant in *P. aeruginosa* PAO1. Growth experiments in M9 caseinate were performed and oxygen consumption during growth was determined for *P. aeruginosa* PAO1 wild type and the *metF* mutant. While we did not observe any growth differences between both strains, we noticed strongly reduced production of the virulence factors pyocyanin and the siderophore pyoverdin in the *metF* mutant. Since pyocyanin production is dependent on quorum sensing, we checked AHL production in the *metF* mutant strain. Unexpectedly, no difference to the PAO1 wild type strain was observed. This indicates that pyocyanin production is reduced in the *metF* mutant strain by a quorum sensing independent pathway. Microarray and metabolome analysis experiments are currently applied to elucidate the respective phenotype of the *metF* mutation.

MPV009
Connecting cell cycle to pathogenic development-regulatory cascades during pathogenesis of *Ustilago maydis*.

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In the smut fungus *Ustilago maydis*, the regulation of cell cycle and morphogenetic switching during pathogenic and sexual development is orchestrated by the *a* and *b* mating-type loci. Activation of either mating-type locus triggers a G2 cell cycle arrest as a prerequisite for the formation of the infectious dikaryon, which is released only after penetration of the host plant. The bE/bW heterodimeric transcription factor encoded by the *b*-mating type locus coordinates a regulatory network consisting of different transcription factors. The C2H2 zinc finger transcription factor Rbf1, as a master regulator, is required for the expression of most (>90%) b-regulated genes. Rbf1 is independently from bE/bW sufficient to initiate pathogenic development, however, further development requires (1) additional factors as the bE/bW dependently expressed Clp1 protein for cell cycle progression and (2) additional bE/bW regulated genes to establish the biotrophic interface. We show that both bW and Rbf1 interact with Clp1. Clp1 interaction with bW blocks *b*-dependent functions, such as the *b*-dependent G2 cell cycle arrest, dimorphic switching and pathogenic development. The interaction of Clp1 with Rbf1 results in the repression of the *a*-dependent pheromone pathway, conjugation tube formation, and the a-induced G2 cell cycle arrest. Thus, the concerted interaction of Clp1 with Rbf1 and bW coordinates *a*- and *b*-dependent cell cycle control to ensure cell cycle release and progression at the onset of biotrophic development.

MPV010
The molecular basis of symptom formation in *Sporisorium reilianum*

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Sporisorium reilianum and *Ustilago maydis* are closely related biotrophic maize pathogens that cause different symptoms. *S. reilianum* infects young plants, spreads systemically and causes symptoms at the onset of flowering. Symptoms include the formation of spores and leaf-like structures in inflorescences, and an increase in the number of ears formed by the plant. In contrast, *U. maydis* can infect the plant via all aerial organs and rapidly induces the formation of spore-filled tumors near the site of infection. The genomes of *S. reilianum* and *U. maydis* are highly syntenic and most encoded proteins are well conserved. However, a few divergence regions were identified that encode only weakly conserved proteins[1]. The largest divergence region is located on chromosome 19 (cluster 19A) and encodes more than 20 potentially secreted proteins. Deletion of cluster 19A in *S. reilianum* leads to dramatically reduced virulence, a change in the number of ears per plant, and early senescence of inoculated leaves. By subdeletion analysis we were able to show that different proteins of cluster 19A contribute to specific symptoms. Thus, symptom formation is modulated by effectors of the divergence region cluster 19A of *S. reilianum*.

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MPV011
Secreted proteins of the dermatophytic fungus *Arthroderma benhamiae* and their contribution to pathogenicity

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To study the mechanism of keratin degradation and to elucidate host colonisation strategies, the secretome of the dermatophytic fungus *A. benhamiae* was analysed in terms of composition and contribution of particular proteins to pathogenesis. Protein secretion is highly up-regulated during growth on keratin and the secretome primarily consists of proteases repesenting all major functional classes such as fungalysins and subtilisins. By contrast, the hydrofluoric acid - extractable cell wall fraction contained two abundant proteins: the constitutively produced hydrophobin Hyp1 and the keratin-inducible integral cell wall protein Kip1. To characterise these proteins, deletion mutants of the respective genes were generated.
The *kip1* deletion mutant did not show an apparent phenotype. By contrast, strains constitutively expressing Kip1 induced an increased interleukin 8 response in human keratinocytes during infection, indicating a pro-inflammatory effect of this so far uncharacterised cell wall protein. Analysis of the *kip1* promoter region suggests a regulation of the gene by the pH response transcription factor PacC. In *A. benhamiae*, deletion of *pacC* led to a strain severely affected in morphology and retarded in hyphal growth, showing that in this species the transcription factor exhibits global regulatory functions. The mutant was still able to grow on keratin but showed prominent alterations of the secretome, suggesting a multiplicity of secretory proteins being de-regulated in this mutant. The conidial hydrophobin of *A. fumigatus* was described to serve as a protectant against the immune recognition by host cells [1]. Inspired by this finding, we characterised the hydrophobin Hyp1 of *A. benhamiae*. Analysis of the *hyp1* gene deletion mutant indicated that also in *A. benhamiae* hydrophobin serves as a masking protein. Phenotypically *Δhyp1* showed a reduced mycelial hydrophobicity and altered adhesivity of conidia to miscellaneous surfaces. Interestingly, the *Δhyp1* strain was recognized more effectively by keratinocyte and macrophage cell lines and by neutrophil granulocytes (PMN). This data was confirmed by cytokine response measurement and, for the PMN, by neutrophil extracellular trap (NET) - induction.

[1] Aimanianda et al (2009): Nature 460:1117-1121.

MPV012
Generation and functional characterization of truncated *Bartonella henselae* BadA mutants

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The humanpathogenic bacterium *Bartonella henselae* causes cat scratch disease and vasculoproliferative disorders (e.g., bacillary angiomatosis). Expression of *Bartonella* adhesin A (BadA) is crucial for bacterial autoagglutination, adhesion to host cells, binding to extracellular matrix proteins and proangiogenic reprogramming. BadA belongs to the class of the lollipop-like structured trimeric autotransporter adhesins (TAAs) and is modularly constructed consisting out of a head, a long and repetitive neck-stalk module and a membrane anchor. Until now, the exact biological roles of these domains in the infection process remains unknown. Here, we analyzed the functions of certain BadA domains in greater detail. For this purpose, deletion mutants were produced by truncating the repetitive neck-stalk module and deleting different head subdomains of BadA. Like wildtype bacteria, a mutant with a nearly completely truncated stalk exhibited autoagglutination, adhesion to collagens and endothelial cells (ECs) and induced the secretion of proangiogenic cytokines (VEGF). Remarkably, *B. henselae* expressing only parts of the stalk bound fibronectin. Deletions of several head subdomains revealed no specific attribution of domain-function relationships. Our data revealed that the fibronectin binding ability of the BadA is located in the stalk domain. The adhesion to collagen and ECs and the secretion of proangiogenic cytokines is mediated by the neck-stalk module and by the single subdomains of the BadA head. These findings suggest overlapping functions of certain BadA domains in the infection process of the host.

MPV013
Interaction of *Yersinia* spp. with invertebrates

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The capability of yersiniae to interact with and to kill insect larvae and bacterivorous predators such as nematodes has only recently been uncovered. Due to the biphasic pathogenicity of *Y. enterocolitica* and *Y. pseudotuberculosis* towards invertebrates and humans, these species represents the transition from entomopathogenic to humanpathogenic bacteria. This is of particular interest because the associations of microbes with non-vertebrates might be a yet neglected source of human pathogen

transmission. Furthermore, bacteria-invertebrate interactions obviously have contributed to the evolution of microbial strategies to colonize eukaryotic hosts, and to withstand their immune system.
The model organisms *Galleria mellonella* (the greater waxmoth) and *Manduca sexta* (the tobacco hornworm) have been used to decipher genetic determinants that play a role in the interaction of *Y. enterocolitica* with invertebrates. The most prominent factor identified to be essential for insecticidal activity is a toxin complex comprising the components TcaA, TcaB, TcaC and TccC. The latter one is biologically active upon ADP-ribosylating of actin and RhoA. The expression of TcaA is regulated in a temperature-dependent manner and requires a novel autoregulated LysR-like inductor. Using the nematode *Caenorhabditis elegans* for infection with *Y. enterocolitica*, we demonstrated that a successful exploitation of this host requires multiple activities including adhesion, colonization, proliferation, toxin release and subsequent bioconversion. Genome comparison revealed a large set of factors that are assumed to specifically contribute to these steps.

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MPV014
Pseudomonas aeruginosa virulence analyzed in a *Dictyostelium discoideum* model of infection

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Pseudomonas aeruginosa is a major opportunistic human pathogen which produces a large variety of secreted and cell-associated virulence factors. Since *P. aeruginosa* infections are difficult to treat due to the emergence of highly antibiotic resistant strains, alternative drug targets including virulence factors are currently being under investigation. Recently, it has been shown that *P. aeruginosa* uses similar virulence factors when infecting mammalian systems or non-mammalian hosts like the social amoeba *Dictyostelium discoideum*, the nematode *Caenorhabditis elegans* or the fruit fly *Drosophila melanogaster* (1,2). In this study, we used a comprehensive *P. aeruginosa* PA14 transposon mutant library to screen for mutants with reduced virulence towards *D. discoideum*. A total of 198 *P. aeruginosa* PA14 transposon mutants were identified to have decreased virulence in this host model system. In addition to mutants with insertions in the type III secretion system (TTSS), we identified genes involved in type IV pili biosynthesis and function, PQS production, tryptophan synthesis, transport, central and amino acid metabolism and gene regulation including quorum sensing, global regulators and two-component regulatory systems. FACS analyses using a gfp-*exoT* reporter construct revealed reduced TTSS activity in several studied mutants including type IV pili, PQS biosynthesis and *cbrAB*, a two-component regulatory system involved in nitrogen and carbon metabolism. Microarray analyses were performed to gain a deeper insight into the interaction of *P. aeruginosa* with *D. discoideum*.

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MPV015
Will be presented as poster with the ID MPP066!

MPV016
Structure/Function Analysis of the Type 3 Secretion System from *Salmonella typhimurium*

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Many infectious Gram-negative bacteria require a Type Three Secretion System (TTSS) to translocate virulence factors into host cells. The TTSS consist of a membrane protein complex and an extracellular needle both that form a continuous channel. Regulated secretion of virulence factors requires the presence of SipD at the TTSS needle tip in *S. typhimurium*. Recently, binding of SipD with bile salts present in the gut was shown to impede bacterial infection.
We showed recently that the TTSS needle protomer refolds spontaneously to extend the needle from the distal end. We developed a functional mutant of the needle protomer from *Shigella flexneri* and *Salmonella typhimurium* to study its assembly *in vitro*. We show that the protomer partially refolds from **a**-helix into **ß**-strand conformation to form the TTSS needle. We also analyzed three-dimensional structures of individual SipD, bound to the needle subunit PrgI, and of the SipD:PrgI in complex with the bile salt deoxycholate. Structures provide insight to the open state of the tip of the TTSS needle. Five copies each of the needle subunit PrgI and SipD form the TTSS needle tip complex. Assembly of the complex involves major conformational changes in SipD. The TTSS needle tip complex binds deoxycholate with micromolar affinity by a cleft formed at the SipD:PrgI interface as shown by isothermal titration calorimetry and crystal structure analysis. In the structure based three-dimensional model of the TTSS needle tip the bound deoxycholate is facing the host membrane. Therefore, binding of bile salts to the SipD:PrgI interface could/may control the TTSS function. Take together our study reveals the molecular assembly mechanisms and the structure of the TTSS at atomic level.

MPV017
The Zwitterionic Cell Wall Teichoic Acid of Staphylococcus aureus Provokes Skin Abscesses in Mice by a Novel CD4+ T-Cell-Dependent Mechanism

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S. aureus is responsible for serious and life-threatening human infections, such as bacteremia, pneumonia, and endocarditis. However the most prominent S. aureus infections are skin and soft-tissue infections (SSTIs). In contrast to other types of infections, the microbial factors involved in the pathogenesis of skin infections provoked by *S. aureus* and the underlying host response mechanisms have yet to be studied in detail. Therefore, a comprehensive understanding of the molecular events taking place during the course of a staphylococcal skin infection remains largely elusive.
Recently, the dogma of adaptive immune system activation was challenged by studies that demonstrated the ability of certain microbial zwitterionic polysaccharides to be processed and presented via the MHC II pathway much like peptide antigens [1]. Cell wall teichoic acid (WTA) of *S. aureus* is a zwitterionic polymer, and we demonstrate that purified WTA is able to stimulate CD4+ T-cell proliferation in an MHC II-dependent manner [2]. We show in both in vitro and in vivo experiments that the zwitterionic charge of WTA is crucial for this activity. The results of T cell transfer experiments and CD4+ T cell deficient mouse studies clearly demonstrate that T cell activation by WTA in *S. aureus* infected tissue strongly modulates abscess formation. The primary effector cytokine produced by WTA activated T cells is IFN-y which is responsible for promoting the early phases of abscess formation. The later stages of abscess progression and clearance rely on a Th17 type response, indicated by high IL-17 levels in the abscess tissues at late timepoints. Our study is both novel and highly important for understanding the molecular basis of the complex pathology of staphylococcal SSTIs. In addition, it provides unique insight on the role of staphylococcal glycopolymers in bacterial virulence, emphasizing the importance of investigating these surface molecules from a new perspective.

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MPV018
Functional characterisation of hemolysins of *Aspergillus fumigatus*

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The ascomycete *Aspergillus fumigatus* is a saprobic mold commonly found in soil and compost piles. *A. fumigatus* is the most important airborne pathogenic fungus causing severe infections in immunocompromised patients. The development of reliable diagnostic methods and identification of new antifungal drugs is of utmost urgency for improved therapy of infections with *A. fumigatus*. Potential new virulence determinants of *A. fumigatus* are hemolytic acting proteins. In the secretome of *A. fumigatus*, two proteins with predicted hemolytic function were identified, namely Asp-hemolysin (Asp-HS) and HS-like. Previous studies already showed that purified Asp-HS has hemolytic and cytotoxic activity. It is assumed that these proteins lyse erythrocytes to attain essential (micro-) nutrients, e.g., iron.
In our study, we aimed at further characterisation of the hemolysins, with special focus on their role during pathogenesis. For that purpose, single and double deletion mutants of the Asp-HS and HS-like encoding genes were generated. Phenotypic characterisation of the mutant strains revealed no differences with regard to growth, morphology of hyphae and spores, sporulation and germination. Although *A. fumigatus* culture filtrate induced hemolysis of rabbit erythrocytes, the hemolytic activity of the culture supernatant of the hemolysin-mutants was not decreased. Hence, it is questionable whether Asp-HS and HS-like proteins are involved in hemolysis. Furthermore, culture supernatant of the wild type and of the deletion mutants revealed no differences in cytotoxicity towards murine alveolar macrophages. Finally, in a mouse infection model for invasive aspergillosis virulence of neither the ΔHS nor the ΔHS/ΔHS-like double-mutant was attenuated when compared to wild type and reconstituted strains. Taken together, the hemolysins Asp-HS and HS-like are dispensible for hemolytic activity of *A. fumigatus* culture filtrate and play no role in virulence of the fungus.

MPV019
Regulating early infection and *in planta* development of *Ustilago maydis*

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In the corn smut fungus *Ustilago maydis*, sexual development is initiated by fusion of two haploid sporidia, generating a filamentous dikaryon that is capable to infect the plant. To get insight into the processes that precede plant infection, we performed microarray analysis of *U. maydis* cells grown on the plant surface. Two of the genes specifically induced in a pathogenic strain are a C2H2 zinc finger transcription factor and a homeodomain transcription factor named *biz1* and *hdp2*, respectively. Whereas D*hpd2* strains are completely blocked in appressoria formation, D*biz1* cells are severely reduced in their ability to form appressoria and to penetrate the plant. Hdp2 appears to be required for expression of most genes induced on the plant surface, while Biz1 regulates about 30% of all genes induced on the plant surface. For 19 of these genes, Biz1 was found to be both required and sufficient for induction. Systematic deletion analysis of these genes led to the identification of *pst1* and *pst2*, encoding potentially secreted *U. maydis* specific proteins. D*pst1/pst2* cells are still able to penetrate the plant surface, but subsequently fail to invade and colonize the plant, resembling the *biz1* deletion phenotype. In contrast to wild type strains, for both D*biz1* and D*pst1/pst2* strains reactive oxygen species (ROS) can be detected at site of penetration, suggesting a function in suppression of plant defence for Pst1/Pst2. Further microarray analysis revealed that 76 of the *biz1*-dependent genes are induced during various stages of pathogenic development. This data suggest that Biz1 is not only a regulator of genes required for plant penetration, but also for genes with impact on pathogenicity at later stages. Currently we are performing deletion mutants of those genes and analyzing their distinctive role during pathogenic development.

MPV020
Functional analysis of the S-layer protein of *Paenibacillus larvae*

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Honey bees are the most important pollinators in agriculture providing about 90% of the commercial pollination of those crops and fruit which depend on insect pollination [1]. Honey bees are attacked by numerous pathogens and parasites causing considerable economical losses to apiculture and agriculture. The notifiable honey bee epizootic American foulbrood (AFB) is a bacterial disease solely affecting the brood of the European honeybee (*Apis mellifera*). The causative agent of AFB is the Gram-positive bacterium *Paenibacillus larvae* [3], which forms extremely resilient spores serving as the transmission stage of the bacterium. In Europe, outbreaks of American Foulbrood are caused by the two differentially virulent *P. larvae* genotypes ERIC I and ERIC II [2]. We applied comparative proteomics to unravel putative factors which will help to explain the observed virulence differences between the two genotypes. 2D-SDS-PAGE revealed the expression of an S-layer protein in ERIC II but not in ERIC I strains. Sequence analysis confirmed that the S-layer protein gene is non-functional in ERIC I due to an ERIC I-specific frameshift-mutation. Knock-out mutants were constructed to functionally analyze the S-layer protein. The S-layer-ko-mutant 04-309-101Δs differed from the wildtype 04-309wt in colony morphology, adhesion capacity and behaviour in infected larvae.

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MPP001
Recombination-based *in vivo* expression technology (RIVET) for avian pathogenic *Escherichia coli* (APEC): Construction and screening of the APEC RIVET library in chickens

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APEC are group of *E.coli* strains causing systemic disease in poultry known as avian colibacillosis. The disease manifests itself initially with septicaemia then either sudden death or localized multiple organ inflammation. The disease is associated with major economic losses to the poultry industry worldwide. Host and bacterial factors influencing and/or responsible for carriage and systemic translocation of APEC inside the host are poorly understood. Identification of such factors could help in the understanding of its pathogenesis and subsequently development of control strategies.
In this study RIVET strategy (Camilli *et al.,* 1994) was developed and used to isolate host-induced APEC promoters in order to investigate APEC pathogenesis in chicken. Random chromosomal DNA fragments from APEC genome were transcriptionally fused upstream to a promoterless *cre* gene to create APEC RIVET library in a promoter trap plasmid. The reporter strain was constructed by integrating the *loxP* sites (in direct orientation) flanking the neomycin resistance marker (*neo*) for positive selection and streptomycin sensitivity gene (*rpsL*) for negative selection into APEC genome. Fused active promoters cause expression of Cre recombinase which subsequently cause recombination of the two *loxP* sites, deleting the cassette and permanently changing the bacterial phenotype such that could be detected after gene expression had ceased.
The APEC RIVET library was pre-selected on kanamycin and ampicillin antibiotics to eliminate *in vitro* active promoters. The bacteria were then administered in chicken host via intra-tracheal route. After screening, 288

kanamycin sensitive clones were isolated from chicken indicating that the *loxP-rpsL-neo-loxP* (LoxP) cassette could be deleted due to *in vivo* active promoters. PCR analysis and sequencing demonstrated a range of insert sizes (1-2kb) suggesting that the screening is functional and the plasmid could stably be maintained in the bacteria even after infection. Further analysis of the DNA fragments' sequences revealed that 27 different APEC genes were induced inside the chicken during infection. With these results APEC RIVET library could be adapted and the strategy showed to be functional for the screening of host-induced APEC genes/ promoters in chickens.

MPP002
New insights into pyomelanin formation of *Aspergillus fumigatus*

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Aspergillus fumigatus is an opportunistic fungal pathogen that causes severe systemic infections in immunocompromised patients. The biosynthesis of dihydroxynaphthalene melanin is an important virulence determinant. Recently, an alternative type of melanin, pyomelanin, was described in *A. fumigatus*. Pyomelanin is produced by polymerisation of homogentisic acid, the central intermediate during degradation of phenylalanine and tyrosine. Four enzymes that are involved in tyrosine degradation are encoded in a gene cluster in *A. fumigatus*. This cluster also contains a gene of unknown function that we designated *hmgX*. Disruption of *hmgX* prevented pyomelanin formation as well as growth of *A. fumigatus* on tyrosine as the sole carbon or nitrogen source, indicating that HmgX is essential for complete tyrosine decomposition. Ectopic integration of *hmgX* into the genome of the Δ*hmgX* mutant strain restored the wild-type phenotype. Interestingly, a mutant in which no homogentisic acid was formed due to deletion of the gene encoding the dioxygenase HppD showed the same phenotype as the *hmgX* knock-out strain. Analysis of culture supernatants by HPLC confirmed that the *hmgX* mutant still converted tyrosine to *p*-hydroxyphenylpyruvate, whereas the next intermediate homogentisic acid was not formed. These data as well as measurement of enzymatic activities suggest that HmgX is involved in the conversion of *p*-hydroxyphenylpyruvate to homogentisic acid that is catalysed by HppD.

MPP003
Strain-specific differences in pili formation and the interaction of *Corynebacterium diphtheriae* with host cells

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Corynebacterium diphtheriae, the causative agent of diphtheria, is well-investigated in respect to toxin production, while little is known about *C. diphtheriae* factors crucial for colonization of the host. In this study, we investigated strain-specific differences in adhesion, invasion and intracellular survival and analyzed formation of pili in different isolates.
Adhesion of different *C. diphtheriae* strains to epithelial cells and invasion of these cells are not strictly oupled processes. Using ultrastructure analyses by atomic force microscopy, significant differences in macromolecular surface structures were found between the investigated *C. diphtheriae* strains in respect to number and length of pili. Interestingly, adhesion and pili formation are not coupled processes and also no correlation between invasion and pili formation was found. Using RNA hybridization and Western blotting experiments, strainspecific pili expression patterns were observed. None of the studied *C. diphtheriae* strains had a dramatic detrimental effect on host cell viability as indicated by measurements of transepithelial resistance of Detroit 562 cell monolayers and fluorescence microscopy, leading to the assumption that *C. diphtheriae* strains might use epithelial cells as an environmental niche supplying protection against antibodies and macrophages.
The results obtained suggest that it is necessary to investigate various isolates on a molecular levelto understand and to predict the colonization process of different *C. diphtheriae strains*.

MPP004
Streptomyces lividans inhibits the proliferation of *Verticillium dahliae* on seeds and roots of *Arabidopsis thaliana*

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The vascular disease in more than 200 dicotyledonous plants is due to the ascomycete fungus *Verticillium dahliae*. As documented by video-microscopy, the soil bacterium *Streptomyces lividans* strongly reduces the germination of *V. dahliae* conidia, and the subsequent growth of the fungal hyphae. Quantification by the use of DNA-intercalating dyes and Calcofluor-staining revealed that during prolonged co-cultivation bacterial hyphae proliferate to a dense network, provoke a poor development of the fungal vegetative hyphae, and an enormous reduction of fungal conidia and microsclerotia. Additional studies allowed identifying proteins and the corresponding genes of *S. lividans*, which play a role in this interaction. Upon individual application to seeds of the model plant *Arabidopsis thaliana*, either the *Streptomyces* spores, or the fungal conidia germinate at or within the mucilage, including its volcano-shaped structures. The extension of hyphae from each individual strain correlates with the reduction of the pectin-containing mucilage-layer. Proliferating hyphae then spread to roots of the emerging seedlings. Plants, which arise in the presence of *V. dahliae* within agar or soil have damaged root cells, an atrophied stem and root, as well as poorly developed leaves with chlorosis symptoms. In contrast, *S. lividans* hyphae settle in bunches preferentially at the outer layer near tips and alongside roots. Resulting plants have a healthy appearance including an intact root system. *A. thaliana* seeds, which are co-inoculated with *V. dahliae* and *S. lividans,* have preferentially proliferating bacterial hyphae within the mucilage, and at roots of the outgrowing seedlings. As a result, plants have considerably reduced disease-symptoms.

MPP005
Regulation of tyrosine degradation in the human pathogenic fungus *Aspergillus fumigatus*

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The saprotrophic fungus *Aspergillus fumigatus* is the most prevalent airborne fungal pathogen, causing severe disseminating infections with invasive growth in immunocompromised patients. This mould possesses specific physiological and molecular characteristics including the biosynthesis of a distinct type of melanin, i.e. pyomelanin. This dark-brown pigment is formed by oxidative polymerization of homogentisate, an intermediate of the tyrosine degradation pathway. Interestingly, the genes involved in tyrosine catabolism are organized in a cluster in the genome of *A. fumigatus*, suggesting that they are regulated in a common manner. Here, we present data on the functional characterization of *hmgR*, a gene localized within the cluster, that encodes a putative transcription factor with a $Zn(II)_2Cys_6$ DNA binding domain. Fluorescence microscopy showed that an HmgR-eGFP fusion protein concentrates in a region in the nucleus, which is not yet identified in further detail. Analysis of *hmgR* deletion mutants and complemented strains revealed that HmgR is essential for tyrosine induced transcription of the cluster genes. These data strongly suggest the hypothesis that HmgR is a transcriptional activator of the tyrosine degradation cluster. Interestingly, tyrosine degradation and pyomelanin formation seem to be dispensable for pathogenicity of *A. fumigatus* as deletion of *hmgR* did not affect virulence in a mouse infection model.

MPP006
Characterisation of the PomA/B Stator Complex of the Sodium-dependent Flagellum of *Vibrio cholerae*

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V. cholerae is a humanpathogen gram$^-$ bacteria which causes the acute diarrheal disease cholera mainly in third world countries. Its virulence is achieved by different virulence factors and the ability to colonize the intestine of its host. *Vibrio* is motile because of its single sodium ion-

dependent polar flagellum. The torque generating unit in the flagellum consists of the proteins PomA, PomB, MotX and MotY in which the PomA/B complex plays a role in the transport of Na^+. It has been shown that this complex consists of two PomA homodimers and one PomB homodimer [1]. Biochemical studies of the native complex have not yet been reported. In this work I will focus on the characterisation of PomA and PomB of the flagellar stator complex. In a recent publication [2] the expression and purification of PomA with a N-terminal His-tag and PomB with a C-terminal Strep-tag was described. Here, the purification of the PomA/B complex using a new construct with a Strep-tag to the N-terminal end of PomA and a His-tag to the C-terminal end of PomB is reported. In addition, a vector introducing a GFP fusion to the N-terminus of PomB was constructed.
The vector functionally complemented a *pomAB* deletion strain, indicating that PomA-GFP-PomB complex was inserted into the stator complex. Polar localization of the complex was confirmed by fluorescence microscopy. Introducing the D23N mutation in PomB did result in a non-motile phenotype of *V. cholerae*, demonstrating a functional role of D23 for stator function.

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MPP007
Localization and function of ubiquinone-8 in the Na^+-translocating NADH: quinone oxidoreductase (Na^+-NQR) from *Vibrio cholerae*

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The sodium ion-translocating NADH:quinone oxidoreductase (Na^+-NQR) from *Vibrio cholerae* is a respiratory membrane protein complex that couples the oxidation of NADH and the reduction of membrane-bound quinone to the transport of Na^+ across the bacterial membrane [1]. The Na^+-NQR is composed of the six subunits NqrA-F and contains at least five redox active cofactors: FAD and a 2Fe-2S cluster on NqrF which also harbours the binding site for NADH, covalently attached FMN on NqrC, and covalently attached FMN and riboflavin on NqrB.
A specific binding site for quinones was identified on the peripheral NqrA subunit by modification with photoactivatable, biotinylated quinone which was prevented by ubiquinone-8 (Q_8). From a titration which monitored the fluorescence of 1-anilino-naphthalene-8-sulfonate (ANS), a dissociation constant of 76 mM for ubiquinone-1 (Q_1) was determined. This indicated that the affinity of NqrA towards short-chain ubiquinone was high. CD spectroscopy revealed pronounced structural changes of NqrA upon binding of Q_1. In our new model describing electron transfer in the Na^+ -NQR, ubiquinone-8 on subunit NqrA is proposed to act as the ultimate acceptor.

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MPP008
Biosynthesis of the membrane lipid phosphatidylcholine in bacteria interacting with eukaryotes

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Phosphatidylcholine (PC, lecithin) is the most abundant membrane-phospholipid in eukaryotes, whereas many prokaryotes lack PC. Based on *in silico*-studies, about 10 % of all bacteria synthesize PC [3]. Most of the PC-synthesizing bacteria are known to interact with eukaryotic hosts in a commensalic, symbiotic or pathogenic manner. Amongst others, PC and the PC-synthesizing enzymes were identified in the plant-pathogen *Agrobacterium tumefaciens* and the soybean-symbiont *Bradyrhizobium japonicum* [1]. Here, PC plays a crucial role in the bacterium-plant-interaction and is important for the virulence and symbiosis [2, 4]. In bacteria, PC-biosynthesis is carried out via two pathways, the *N*-methylation pathway and the phosphatidylcholine synthase (Pcs) pathway. The stepwise methylation of phosphatidylethanolamine (PE) to PC via the intermediates monomethyl-PE (MMPE) and dimethyl-PE (DMPE) in the *N*-methylation pathway requires phospholipid *N*-methyltransferases. While the *N*-methylation route in *A. tumefaciens* is accomplished by a single PmtA, in *B. japonicum* this pathway involves five phospholipid *N*-methyltransferases with distinct substrate specifities [1].
Our recent data provide evidence for PC biosynthesis in several plant-commensalic and plant-growth promoting bacteria (*Methylobacterium extorquens*, *Pseudomonas fluorescens*) as well as in different plant-pathogenic bacteria (*Xanthomonas campestris*, *Pseudomonas syringae* pv. tomato) that cause diseases of different economically important plants. The investigated Pseudomonads seem to prefer the Pcs pathway. Heterologous expression of the *M. extorquens* and *X. campestris* pv. campestris *pmt*-genes in *Escherichia coli* clearly suggest a bipartite *N*-methylation pathway similar to the one in *B. japonicum*.

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MPP009
Trimeric autotransporter adhesin-dependent adherence of *Bartonella henselae*, *Bartonella quintana* and *Yersinia enterocolitica* to matrix components and endothelial cells under static and dynamic flow conditions

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Trimeric autotransporter adhesins (TAAs) are important virulence factors of Gram-negative bacteria responsible for adherence to extracellular matrix (ECM) and host cells. Here, we analyzed three different TAAs [*Bartonella* adhesin A (BadA) of *Bartonella henselae*, variably expressed outer membrane proteins (Vomps) of *Bartonella quintana*, *Yersinia* adhesin A (YadA) of *Yersinia enterocolitica*] for mediating bacterial adherence to ECM and endothelial cells. Using static (cell culture vials) and dynamic (capillary flow chambers) experimental settings, adherence of wildtype bacteria and the respective TAA-negative strains were compared. Under static conditions, ECM adherence of *B. henselae*, *B. quintana* and *Y. enterocolitica* was strongly dependent on the expression of their particular TAAs. YadA of *Y. enterocolitica* did neither mediate bacterial binding to plasma nor cellular fibronectin both under static and dynamic conditions. TAA-dependent host cell adherence appeared more significant under dynamic conditions although the total number of bound bacteria was diminished compared to static conditions. The herein described results allow to dissect the biological role of particular TAAs in ECM and host cell adherence and to identify differences in bacterial binding under static and dynamic conditions. Dynamic models expand the methodology to perform bacterial adherence experiments under more realistic blood-stream like conditions.

MPP010
In vitro production of neutrophil extracellular traps against *Aspergillus fumigatus*

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The opportunistic pathogenic mold *Aspergillus fumigatus* is an increasing cause of morbidity and mortality in immunocompromised and in part immunocompetent patients. The very small conidia, acting as infectious agent, infiltrate the lungs and get in contact with alveolar macrophages and neutrophil granulocytes, which repesent the first line of defense. Both are phagocytic cells and kill the conidia via phagocytosis. Besides, neutrophils are able to form neutrophil extracellular traps (NETs) against *A. fumigatus*

hyphae as well as conidia. This reactive oxygen intermediates (ROI) dependent mechanism results in sticky filaments consisting of nuclear DNA decorated with histones and fungicidal proteins. Coincubation of *A. fumigatus* with neutrophils revealed that the intensity of NET formation of unstimulated, human neutrophils is strain- and morphotype-dependent. Furthermore the killing of A. *fumigatus* conidia was not influenced by the amount of released extracellular DNA. However hyphae seemed to be damaged by NETs after a longer incubation time of 12h. Our data suggest that NETs prevent further spreading, but apparently do not represent the major factor for killing. We are currently investigating strain- and mutant-dependent NET formation that will be discussed.

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MPP011
Pathogenicity factor of the *Streptomyces* strains causing potato scab disease other than thaxtomine

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A few *Streptomyces* species are pathogenic on some plants such as potato. Main pathogenicity factors among this species on potato are thaxtomine, concanamycin and a compound named as FD-981. Potato scab disease is one of the most important diseases in potato growing area in hamedan province. Potato tubers shown raised, netted, shallow and deep pitted lesion symptoms were collected from many potato fields and the *Streptomyces* strains were isolated. Based on the phenotypic features and induced symptoms the isolated *streptomyces* strains were not uniforms. They induced symptoms on the tested plants including potato, parsnip, horse radish, carrot and other tested plants. Most of the tested strains harbored a linear plasmid exemined by pulsed field gel electrophoresis and they had sequences related to insertion sequences, nec1 and thaxthomin biosynthetic genes. Raised and netted scab disease inducing strains produced thaxtomin determined by thin layer chromatography but not pitted lesion inducing strains. The last strains which did not produced thaxtomin also did not hybridized to thaxtomin biosynthesis gene probes. Deep pitted inducing representatives strains produced disease inducing toxins other than thaxtomin.

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MPP012
Phenotypic properties of clinical relevance of *Stenotrophomonas maltophilia* isolates in relation to their genetic subgroups

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Stenotrophomonas maltophilia is a highly versatile bacterial species, belonging to the γ-β subclass of proteobacteria. It is ubiquitously distributed in the environment, but recently its role as nosocomial pathogen became more evident. In our previous work we analyzed genetic diversity of *S. maltophilia* by rep-PCR fingerprinting and *gyrB* gene sequencing, for a collection of 171 environmental and clinical strains. This revealed 11 genetic subgroups for *S. maltophilia*. A subset of 50 representative isolates for these groups was then used for further investigation of phenotypic properties. With respect to the increasing importance as an opportunistic pathogen, potential virulence traits, as the production of extracellular proteases, haemolysins and siderophores were investigated. Furthermore, factors supporting colonization of a human host were examined by swimming and twitching motility and biofilm assays. Virulence was tested by co-culturing the bacteria with the amoebae *Dictyostelium discoideum* and *Acanthamoeba castellanii* as model organisms. After testing twenty different antibiotics on a small subset of strains, gentamicin, vancomycin, norfloxacin, tetracycline and co-trimoxazole, were chosen to determine MICs for the 50 *S. maltophilia* isolates.

Nearly all investigated isolates produced proteases and haemolysins and all of them produced siderophores. Motility assays revealed differences in swimming and twitching motility. Biofilm formation generally differed, but did not correspond to their genetic subgroups of the isolates. An exception is that all isolates from environmental group E2 showed only slight potential for biofilm formation. Virulence for amoebae was shown for about one third of the tested isolates and was in no relationship to clinical or environmental origin. All isolates were resistant to vancomycin and most to gentamicin. Most of them showed intermediate MICs for norfloxacin and tetracycline, and all isolates were susceptible to co-trimoxazole.
For motility assays, biofilm formation, virulence and antibiotic resistance generally no correlation to the previously defined genetic groups was found. In this context it was expected that housekeeping genes and rep-PCR fingerprints are not suitable markers to determine phenotypic properties of *S. maltophilia*.

MPP013
Human Formyl Peptide Receptor 2 Senses Highly Pathogenic *Staphylococcus aureus*

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Virulence of the emerging Community-Associated Methicillin-Resistant *Staphylococcus aureus* (CA-MRSA) and other highly pathogenic *S. aureus* depends on the recently discovered Phenol-Soluble Modulin (PSM) peptide toxins, which combine the capacities to attract and lyse neutrophils. The molecular basis of PSM-stimulated neutrophil recruitment has remained unknown. We demonstrate that the human FPR2/ALX receptor, whose function has previously remained elusive, senses PSMs at nanomolar concentrations and initiates the exuberant proinflammatory neutrophil responses to CA-MRSA. Specific blocking of this receptor or deletion of PSM genes in CA-MRSA led to severely diminished capacities of neutrophils to detect CA-MRSA. Thus, the innate immune system uses a previously unrecognized mechanism to sense highly virulent bacterial pathogens. This G protein-coupled receptor represents an attractive target for new anti-infective or anti-inflammatory strategies.

MPP014
Effects of increasing concentrations of antibiotics on the resistance situation of selected human pathogenic bacterial genera

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Since the development and use of antibiotics the number of resistant bacteria increases constantly. Multidrug-resistant pathogens become more and more problematic, especially due to nosocomial infections. These bacteria, as well as antibiotics and their metabolites, enter waste water by human excretions. To the present state of the art no or no complete degradation of antibiotics and other drugs takes place in waste water treatment plants. Also the retention of drugs and bacteria, e.g. by adsorption to sludge particles, is insufficient, so in WWTP effluents already antibiotic concentrations in excess of the effective concentration were measured (2, 3, 5). By the use of sewage sludge and manure as fertilizers antibiotics and bacteria also reach soil and surface water (1, 4, 11). Moreover, bacteria have the ability to develop resistances at sub lethal concentrations of antibiotics and to exchange resistance genes with other bacteria via horizontal gene transfer. So the aim of our study was the investigation of the development of resistances in the genera *Acinetobacter sp.*, *Enterococcus sp.*, *Klebsiella sp.* and *Staphylococcus sp.* from sewage sludge towards 4 selected antibiotics

(ciprofloxacin, gentamicin, sulfamethoxazole/trimethoprim, vancomycin) in lab-scale treatment plants with culture-dependent methods. Under laboratory conditions with artificial sewage according to OECD before adding of antibiotics only enterococci established probably due to insufficient growth, leaching from the plants, loss of cultivability of the other genera or protozoan grazing, as described in the literature (6-8, 10). The addition of antibiotics had no influence on the total count of bacteria and only led to a decrease in the number of enterococci by one log-level with subsequent increase in the same order of magnitude. Without adding antibiotics there was also an increase in the number of enterococci by one log-level, probably by proliferation, which was shown in municipal oxidation ponds (9). Against the selected antibiotics, which were added in increasing concentrations until 7.5 mg/L each, the enterococci developed no resistance, what was shown by comparison with isolates from a control system without antibiotics. However, from a concentration of 5 mg/L of each antibiotic a decrease of the nitrification rate was found.

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MPP015
Anthrax Toxin as Trojan Horse: An N-Terminal His-Tag Promotes Binding and Protective Antigen Dependent Uptake of C2I and Edin into Cells

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Binary toxins of the AB_7-type are among the most potent and specialized bacterial protein toxins. We report that the addition of an N-terminal His_6-tag increased the binding of LF and EF to the PA_{63}-channels. Interestingly, a similar effect could not be observed for the binding affinity to the C2II-channel from a His_6-tag either added to C2I nor to LF and EF. His_6-tags attached to unrelated proteins, such as the amino acids 684-1132 of Lambda phage tail protein (gpJ) or the epidermal cell differentiation inhibitor EDIN of *Staphylococcus aureus* promoted their binding to PA_{63}-channels. Here, we made the important observation that His_6-EDIN exhibited voltage-dependent increase of the stability constant for binding by a factor of 25 when PA_{63} and His_6-EDIN were added to the *cis*-side of a membrane and the *trans*-side was at -70 mV, which reflects the *in vivo* situation. Further *in vivo* experiments show that Anthrax- and C2-toxin translocate into human endothelial cells. Addition of an N-terminal His_6-tag to C2I leads to its translocation into HUVECs via PA where it induces its cytotoxic effects. Finally, we demonstrated that EDIN as well as His_6-EDIN enters cells through PA_{63}-pores but not through C2II-pores. Our results revealed that a His_6-tag could induce a PA_{63}-dependent translocation of proteins unrelated to the AB_7-type of toxins, possibly opening a new way to import proteins of medical interest into cells.

MPP016
Cross-Reactivity of Anthrax and C2 Toxin

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Binary toxins are among the most potent bacterial protein toxins performing a cooperative mode of translocation and exhibit fatal enzymatic activities in eukaryotic cells. Anthrax and C2 toxin are the most prominent examples for the AB_7 type of toxins. To investigate the mechanism of translocation of these toxins into target cells and possible cross-reactivity of toxin binding and translocation we performed various *in vitro* and *in vivo* experiments by interchanging the respective A and B components. Although the binding and translocation components Anthrax protective antigen (PA_{63}) and C2II of C2 toxin share a sequence homology of about 35%, the results presented here unravel biochemical and functional differences. *In vitro* black lipid bilayer measurements proofed that Anthrax edema factor (EF) and lethal factor (LF) have higher affinities to bind to channels formed by C2II than C2 toxin's C2I binds to Anthrax protective antigen (PA_{63}). Furthermore, we could demonstrate *in vivo* that PA in high concentration has the ability to transport the enzymatic moiety C2I into target cells, causing actin modification and cell rounding, whereas C2II is not able to efficiently transport Anthrax EF or LF. Our findings support the commonly accepted mode of translocation of AB_7 type of toxins. In addition, we present first-time evidence that a heterogenic combination of enzymatic and translocation components of different AB_7 toxins exhibit toxicity to primary human endothelial cells (HUVECs).

MPP017
Influence of different classes of gyrase inhibitors on the SOS response in *Staphylococcus aureus*

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The increase of multi-resistant bacteria is a major problem in health care systems. Therefore, it is important to understand not only the mode of action of antibiotics but also the mechanisms leading to the development of resistance. Different gyrase inhibitors bind to different moieties of the gyrase which results in arrest of DNA replication. It is well documented that the widely used chinolone ciprofloxacin also induces the bacterial SOS response through activated RecA. RecA dependent cleavage of the LexA repressor results in error prone repair, which favours mutations and therefore resistance development. A different class of gyrase inhibitors, namely the aminocoumarines bind the GyrB subunit of gyrase which leads to competitive inhibition of the ATPase activity of gyrase. We have analysed different gyrase inhibitors and their combination with regard to antimicrobial resistance and SOS response in the human pathogen *Staphylococcus aureus*. Therefore, site-specific mutants (*recA*, *lexA*) were constructed and the influence of different gyrase inhibitors analysed by growth experiments, Northern Blot analysis, promotor assays and real-time RT-PCR. *RecA* but not *lexA* mutants were shown to be more sensitive towards several classes of antibiotics. Ciprofloxacin results in a *recA* dependent derepression of LexA target genes such as the error prone polymerase SACOL1400. In contrast the aminocoumarine novobiocin leads to transcriptional inhibition of *recA* as well as *sarA,* but induction of *gyr* and *fnbA*. These phenomenons are lexA independent and thus not related to the SOS response and probably due to other important regulators. Interestingly, the combination of ciprofloxacin and novobiocin results in abrogation of the ciprofloxacin induced induction of RecA. Thus, combination of gyrase inhibitors may be beneficial to suppress the unfavourable SOS induction accompanied by chinolones. The mechanisms of action for novobiocin needs to be further studied, e.g. by Microarray analysis and similar drug targets could be developed.

MPP018
Fibronectin binding ability of *Staphylococcus lugdunensis* is associated with internalization.

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Question: Bacterial invasion of non-phagocytic host cells is an important pathogenicity factor for escaping the host defense system. Several Staphylococci, such as *Staphylococcus aureus, Staphylococcus saprophyticus* and *Staphylococcus epidermidis,* are internalized in eukaryotic cells and this mechanism is discussed as an important part of the infection process. Fibronectin binding proteins were discussed as an important prerequisite for internalization described for *S. aureus*. For *S. epidermidis* a fibronectin independent mechanism has been described. Two fibrinogen binding proteins were previously described for *S. lugdunensis*. Fibrinogen binding proteins support the adhesion of bacteria to eukaryotic cells, but were not considered as prerequisite for the internalization process for *S. aureus*. To date a fibronectin binding protein for *S. lugdunensis* has not been described. We establish this study to question whether *S.*

lugdunensis is generally internalized into epithelial and endothelial cell line cells (5637 and EA.hy 926) and whether this internalization is also fibronectin dependent.
Methods: We characterized several clinical strains of *S. lugdunensis* and compared the fibrinogen and fibronectin binding ability to the internalization of *S. lugdunensis* by use of a recently described FACS-assay and transmission electron micrography.
Results: We could show for the first time that clinical isolates of *S. lugdunensis* that bound to fibronectin were internalized into human urinary bladder carcinoma cell line 5637 and the endothelial cell line EA.hy 926.
Conclusion: The discovery of the internalization attribute of *S. lugdunensis* and a possible linkage to a fibronectin dependent internalization mechanism is an important step in the understanding of the pathogenicity of this pathogen.

MPP019

Will be presented as oral presentation with the ID MPV020!

MPP020

Functional properties of the putative sodium/proline transporter PutP of *Helicobacter pylori*

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Helicobacter pylori is a Gram-negative, pathogenic, microaerobic bacterium colonizing the gastric epithelium of about 50% of the world population. It is responsible for type B gastritis, peptic ulcers, and for increasing the risk of gastric carcinoma [1]. Successful interaction of the pathogen with its host does not only require specific virulence factors, but depends also on its capability to cope with nutrient supply and stress conditions found in the host. Recent analyses revealed that genes encoding L-proline transport (*putP*) and metabolizing proteins (*putA*) are essential for gastric colonization [2, 3]. This research focuses on the mechanisms underlying the particular physiological significance of L-proline and L-proline-specific systems for *H. pylori* and its interactions with host cells. The gene *HpputP* from strain P12 was cloned and heterologously expressed in *E. coli*. HpPutP was shown to complement an *E. coli putP* mutant, thereby transport was stimulated by external sodium. Kinetic parameters of the sodium/proline symport process were determined and found to be in the same order of magnitude as the EcPutP parameters. HpPutP was purified by affinity chromatography and reconstituted into proteoliposomes. Functional analyses with proteoliposomes demonstrated that the activity of HpPutP depends on an electrochemical sodium gradient. Furthermore, sites known from EcPutP to be of functional significance were investigated in HpPutP. By this means, residues potentially involved in sodium or proline binding and/or translocation were identified in HpPutP. Next, analyses of proline transport in *H. pylori* will be performed.

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MPP021

In vivo SigB-activity does not influence kidney gene expression pattern in a murine *Staphylococcus aureus* infection model

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Staphylococcus aureus, persistent commensal of about 20% of the human population, can be transmitted to the blood after body injury or by medical devices like catheters. An elementary model to mimic blood stream infection is the *intra-venous* infection of laboratory animals.
The influence of staphylococcal *i. v.* infection on murine kidney gene expression was analyzed in an *in vivo* model with BALB/c mice using the wild type strain RN1HG and its isogenic *sigB* mutant.
RT-qPCR of mixed eukaryotic and prokaryotic RNA from infected tissue resulted in the detection of SigB-dependent higher expression levels of *asp23* and *clfA* in the wt strain *S. aureus* RN1HG, whereas, in agreement with *in vitro* data, expression of *hla* and *aur* was higher in kidney tissue from animals infected with the *sigB* mutant strain.
Although the virulence of *sigB* deficient strains is often reported to be similar to that of wt strains, the pathomechanism of different infection settings might vary. Therefore, the rationale of this study was to investigate whether the deletion of *sigB* would lead to a different reaction of the infected host. Gene expression profiling indicated a highly reproducible host kidney response to infection with *S. aureus*. The comparison of infected with non-infected samples at 120 h post infection revealed a strong inflammatory reaction. This included e. g. TLR signaling, complement system, antigen presentation, IFN and IL-6 signaling, but also counter-regulatory IL-10 signaling. However, the results of this study did not provide any hints for differences in the pathomechanism of the *S. aureus* strains RN1HG and Δ*sigB* in the selected model of *i. v.* infection in mice, since the host response did not differ between infections with the two strains analyzed. If really existing, such differences might be transient and only apparent at earlier time points. Effects of SigB might also be compensated for in *in vivo* infection by the interlaced pattern of other regulators. Effects of missing activation and missing repression by SigB in the mutant might neutralize each other.
The study supports the conclusion that SigB might possess only to a lesser extent characteristics attributed to virulence factors and might act *in vivo* more like a virulence modulator and fine-tune bacterial reactions. SigB possibly might be important in special niches during infection.

MPP022

RNase Y in the human pathogen *Staphylococcus aureus*

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Bacteria are able to cope with dramatic environmental changes by rapidly altering gene expression. In this regulation, RNA decay, processing and maturation play an important role. RNA decay is crucial in determining intracellular levels of RNA species. RNA processing takes place within complex operons and permits the tuning of protein ratios of the co-transcribed genes. Each of these processes requires the action of ribonucleases (RNases). The involved RNases were elucidated in *Escherichia coli*. In Gram-positive bacteria, RNA metabolism, so far best studied in *Bacillus subtilis*, seems to be different. Sequence homologues of some of the *E. coli* enzymes that play major roles in mRNA decay, e.g. RNase E, cannot be identified in firmicutes. Recently, a new essential endoribonuclease, RNase Y, was identified in *B. subtilis*. This enzyme plays a key role in mRNA turnover, in the initiation of riboswitch decay and is involved in the processing of the gapA operon. RNase Y is not essential in *S. aureus* and, therefore, we were able to construct and characterise a *rny* deletion mutant. As a model for RNase Y action, we used the processing of the global virulence regulator *sae*PQRS. The major transcript of the *sae* operon is originated from an endonucleolytic cleavage of the primary transcript. The *rny* mutant showed both defects in *sae* cleavage and altered transcription levels of the *sae* target gene. Moreover, other operons were also affected by RNase Y suggesting that this enzyme is of general importance for mRNA processing. Microarray analysis revealed that 269 transcripts were significantly upregulated and 300 were significantly downregulated in the RNase Y mutant compare to the wild type (foldchange >2). RNase Y was previously identified as a gene affecting the virulence of *S. aureus* through a silkworm infection model. Thus, the characterization of RNAse Y mode of action and the identification of its targets is likely to enhance our understanding of the virulence of *S. aureus*.

MPP023

GliT a novel thiol oxidase - implications in self resistance and biosynthesis of gliotoxin

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Aspergillus fumigatus and other pathogenic fungi have developed various chemical strategies to distress, weaken or even kill their plant or animal hosts. In invasive aspergillosis, the leading cause for death in immunocompromised patients, the fungal secondary metabolite gliotoxin plays a critical role for virulence. Gliotoxin is the prototype of a small family of epipolythiodioxopiperazines (ETPs), which features unique transannular di- or polysulfide bridges. Extensive molecular studies have revealed that this rare structural motif is indispensable for bioactivity and is the key to the deleterious effects of gliotoxin.
Here, we describe the function of GliT, an enzyme of the gliotoxin biosynthesis pathway. We could reveal the activity of GliT both *in vivo* by means of feeding experiments and *in vitro* by heterologous overproduction and further biochemical characterisation of GliT. We proved that GliT is essential for biosynthesis of gliotoxin and therefore may play a critical role in virulence of *A. fumigatus*. Furthermore, GliT confers self resistance of *A. fumigatus* against gliotoxin. These investigations led to the discovery of an entirely new mechanism how microorganisms could prevent self poisoning by their own toxins.

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MPP024

Staphylococcal serogroup L phage Φ187 use extraordinary polyglycerolphosphate wall teichoic acid as adsorption receptor

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The gram positive bacterium *Staphylococcus aureus* is a human pathogen causing several diseases including nosocomial infections such as sepsis and endocarditis. One very important factor for colonising, resistance to antimicrobial peptides and antibiotics is a cell wall anchored glycopolymer known as wall teichoic acid (WTA). *S. aureus* strain PS 187 shows an extraordinary kind of WTA. Here the actual WTA polymer consists of polyglycerolphosphate modified by D-alanine (D-ala) and N-acetylgalatosamine (GalNAc). Most of the staphylococcal phages can be classified into the serogroups A, B, D, F, G and L. Within these serogroups only serogroup D and L phages are able to infect *S. aureus* strain PS 187. Here we describe for the first time the construction of a WTA deficient mutant in this strain background via deletion of the *tagO* gene responsible for the initial step of WTA biosynthesis. Drastically reduced phosphate content of cell walls isolated from *S. aureus* PS 187 Δ*tagO* confirms complete loss of WTA. Moreover *S. aureus* PS 187 Δ*tagO* is resistant to serogroup D and serogroup L phages shown in a phage susceptibility assay. To show that phage resistance is due to the loss of WTA phage susceptibility could be restored by complementation using a vector expressing the *tagO* gene strongly suggesting that WTA is the receptor of serogroup L phages. Furthermore the question came up if loss of this special kind of WTA can lead to growth deficits. Growth kinetics show that loss of Polyglycerolphosphate WTA is dispensable for growth *in vitro*.

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MPP025

Shigella flexneri induces HIF-1alpha expression and TNF-alpha release in rat hepatocytes

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Shigella flexneri is an intracellular enteric bacteria that can disrupt gut mucosa reaching blood and liver. Hypoxia inducible factor 1alpha (HIF-1alpha) is a known transcription factor responsible for genic expression related to low oxygen tensions and TNF-alpha is a key factor in the immune response. In this study we investigated the ability of *S. flexneri* to invade cultured rat hepatocytes, and also to induce the HIF-1alpha expression and TNF-alpha release by these cells. METHODS: We cultured rat hepatocytes (Wistar) for 7 days. These cells were exposed to *S. flexneri* infection with multiplicity of infection (MOI) 500:1 ($5x10^8$ bacteria) for 1 hour. The HIF-1alpha expression was detected by immunofluorescence and images were analyzed on confocal microscopy. TNF-alpha released was detected by ELISA. Expression of HIF-1alpha and TNF-alpha in rat hepatocytes were compared with groups submitted to hypoxia at incubator chamber filled with N_2 for 24 hours (final pO_2 of 40mmHg). RESULTS: The intracellular bacteria recovered reached $7x10^4$ after 1 hour of infection. Infected rat hepatocytes expressed HIF-1alpha mainly in the cytoplasm. The supernatant of infected hepatocytes showed higher TNF-alpha levels as compared to non infected cells, reaching 350 pg/ml. In hypoxic groups, HIF-1alpha expression was also observed, and TNF-alpha released decreased to 100 pg/ml. CONCLUSIONS: Our results suggest that *S. flexneri* infection and hypoxia microenvironment were able to induce HIF-1alpha expression in rat hepatocytes cultured. The increase of TNF-alpha release in infected cells was lower in hypoxic condition. This phenomenon may be explained to probable deficient immune response of these cells. Taken together, our results suggest that *S. flexneri* is able to invade rat hepatocytes causing functional alterations in these cells, specially for HIF-1alpha expression and TNF-alpha release (supported by FAPESP).

MPP026

Interactions of oritavancin with Lipid II and interpeptide bridge-containing Lipid II variants

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Oritavancin is a semi-synthetic derivative of the glycopeptide chloroeremomycin with activity against Gram-positive pathogens, including vancomycin-resistant staphylococci and enterococci. In contrast with vancomycin, binding of oritavancin to the cell wall precursor Lipid II appears to involve, in addition to the D-Ala-D-Ala terminus, the interpeptide crossbridges, as seen by nuclear magnetic resonance [1]. We studied the impact of ORI and of its des-*N*-methylleucyl variant (des-ORI), which is unable to bind to the D-Ala-D-Ala terminus, on staphylococcal and enterococcal interpeptide bridge formation and Lipid II transglycosylation/transpeptidation.

[1] Kim et al (2008): J. Mol. Biol.

MPP027

Characterization of the major cell-associated phospholipase A PlaB of *Legionella pneumophila*, the first member of a novel phospholipase family

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The lung pathogen *Legionella pneumophila* expresses a variety of phospholipases potentially involved in disease-promoting processes and development of pneumonia. The recently identified major cell-associated phospholipase A (PLA)/ lysophospholipase A (LPLA) with an additional hemolytic activity, designed PlaB, shares no homology to previously described phospholipases. So far, it was shown that PlaB utilizes a typical triad of Ser-Asp-His for effective hydrolysis of phospholipids located within the N-terminal half of the protein for cleavage of phospholipids, such as phosphatidylglycerol (PG) and -choline (PC) as well as the respective lysophospholipids. We further determined that PC- but not PG-hydrolyzing

PLA activity is directly linked to the hemolytic potential of PlaB. The first characterized member of a new family of lipases also plays an important role as virulence factor in a guinea pig infection model and is mainly expressed and enzymatically active during the exponential growth phase. Until now, the function of the C-terminal half is unknown, but it contributes to lipolytic activity. Interestingly, the analysis of three C-terminally truncated versions of PlaB recombinantly expressed in *E. coli* revealed, that a lack of only 5 amino acids (aa) leads to a 90% decrease of PC-PLA activity. The lack of 10 aa at the C-terminus however results in a decrease of 80% PG- and 100% of PC-PLA activity whereas the removal of 15 aa completely abolishes the enzymatic activity. Furthermore, the purification of sufficient amounts of soluble and active PlaB has been pursued for future determination of the crystal structure, which is helpful for further characterization of PlaB properties, function and verification of recent data.

MPP028
Functional expression of truncated *Bartonella* adhesin A (BadA) in *E. coli*

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The trimeric autotransporter adhesin *Bartonella* adhesin A (BadA) plays a decisive role in infections with *Bartonella henselae*. Expression of BadA is crucial for bacterial autoagglutination, adhesion to host cells, binding to extracellular matrix proteins and the induction of proangiogenic reprogramming via activation of hypoxia inducible factor (HIF)-1. BadA is constructed modularly consisting of a head domain, a long and repetitive neck-stalk module and a membrane anchor domain. To analyze the function of particular BadA domains in detail, the generation of BadA deletion mutants would be highly desirable. However, because of the slow growth of *B. henselae* and limited tools for genetic manipulation, we established a recombinant expression system for BadA mutants in *E. coli* to allow functional analysis of certain BadA domains. Therefore, we used (i) a truncated BadA mutant lacking the neck-stalk module (BadA HN23) and exchanged additionally (ii) the BadA HN23 signal sequence with the *E. coli* OmpA signal sequence, (iii) the BadA HN23 membrane anchor with the *Yersinia* adhesin A (YadA) membrane anchor or (iv) exchanged both of these modules. Using a set of expression vectors, these constructs were cloned in several *E. coli* expression strains to analyze the biological functions of such BadA-hybrid proteins. Expression of BadA HN23 hybrids was detected via immunoblotting and fluorescence microscopy. All BadA HN23 hybrids were expressed on the surface of *E. coli* strains although quantitative differences were observed. No differences in collagen-binding between *E. coli* expressing Bad HN23 hybrids and controls were detectable. However, we have preliminary evidence that *E. coli* expressing BadA hybrid-proteins adheres significantly more to endothelial cells than control strains although the total amount of bound bacteria is less than *B. henselae* wildtype. Further experiments using recombinantly expressed BadA-hybrids should allow to investigate BadA-mediated bacteria-host cell interactions in greater detail.

MPP029
Phenotypic and genotypic characterization of *Pseudomonas aeruginosa* isolates from technical water systems

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Pseudomonas aeruginosa is an opportunistic pathogen that can persist in biofilms of man-made water systems. *P. aeruginosa* cells released from biofilms contaminate the water phase and pose a potential threat to human health.
During a period of seven years (2003 - 2010), 77 *P. aeruginosa* strains were isolated from water and biofilms of drinking water distribution systems (52), public swimming pools (13), and industrial water systems (12). Clonal relationship, colony morphology, pigment production, hemolysis, cell surface hydrophobicity, biofilm formation, resistance against antibiotics and occurrence of virulence genes of these isolates and additional seven *P. aeruginosa* reference strains were analysed in order to reveal possible correlations between water source and genotypic as well as phenotypic characteristics.
Genetic diversity assessed by pulsed field gel electrophoresis of SpeI restricted genomic DNA of the 77 environmental strains was high; they belonged to 42 different clonal variants. 64% of these 77 strains and the seven reference strains showed the typical colony morphology for *P. aeruginosa*, 92.8 % produced at least one of the pigments pyocyanin, pyoverdin, pyorubin and pyomelanin, 98.8 % displayed b-hemolysis and were sensitive to antibiotics of four different classes, 90.5 % had a hydrophilic cell surface and all strains were able to form biofilms on abiotic (polystyrene) surfaces. In all 84 strains, the virulence gene *lasB* was detected as well as either the virulence genes *exoS* (63 %) or *exoU* (38 %); only one strain contained both *exoS* and *exoU*.
For the first time an extensive pool of *P. aeruginosa* isolates from different types of technical water systems were characterized phenotypically and genotypically. No correlation between phenotypic and genotypic traits, and between these characteristics and the origin of the strains were detected. The phenotypic characteristics and the occurrence of virulence genes seemed to be distributed in a relatively homogeneous way among the clonally unrelated strains from diverse man-made water systems.

MPP030
Staphylococcus lugdunensis SLUSH peptides - more than haemolysins

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Coagulase-negative staphylococci (CoNS) are becoming more and more important in nosocomial and community-acquired infections such as bacteremia, nosocomial neonatal sepsis, endocarditis, and meningitis and often these bacteria are resistant to several antimicrobial agents.
Staphylococcus aureus and *Staqphylococcus epidermidis* have been shown to secrete phenol-soluble modulin peptides, which can be sensed by dedicated receptors of the innate immune system and lead to neutrophil responses such as chemotaxis, calium ion flux, and IL-8 release. To further elucidate the ability of neutrophils to sense further CoNS we analyzed the response of human primary neutrophils and monocytic cell lines to culture supernatants from different CoNS species. We found most CoNS species to elicit calcium ion fluxes in leukocytes.
One of these CoNS namely *S. lugdunensis* is outstanding since it behaves more like *S.aureus* than other CoNS regarding its virulence and clinical manifestation in infections. In fact, this pathogen has often been implicated in severe inflammatory infections in recent years. These may proceed aggressively and with severity similar to that of *S.aureus*.
S. lugdunensis secretes three small peptides, SLUSH-A, SLUSH-B, and SLUSH-C, which exhibit synergistic haemolytic activity with *S.aureus*. In order to characterize whether the elevated virulence of *S. lugdunensis* is linked to the presence of these peptides we examinined the response of human neutrophils, monocytes and monocytic cell lines to synthetic SLUSH peptides.
Due to its enhanced virulence *S. lugdunensis* is not a typical CoNS species and deserves more attention regarding its interaction with the adaptive immune system.

MPP031
Role for the cysteine, histidine-dependent amidohydrolase/peptidase (CHAP) domain of the *Staphylococcus aureus* autolysin/adhesin Aaa in adherence to fibrinogen, fibronectin, and human endothelial cells

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Question: Staphylococcus aureus is a frequent cause of serious and life-threatening infections, such as endocarditis, osteomyelitis, pneumoniae, and sepsis. Its adherence to various host structures is considered crucial for the establishment of diseases. Adherence may be mediated by a variety of adhesins, among them the autolysin/adhesins Atl and Aaa. Aaa possesses three N-terminal repeated sequences homologous to a lysine motif (LysM)

that can confer cell wall attachment, and a C-terminally located cysteine, histidine-dependent amidohydrolase/peptidase (CHAP) domain having bacteriolytic activity in many proteins.
Method: To characterize the functional domain structure of Aaa, we contructed Aaa subclones expressing the N-terminal or C-terminal Aaa-domains in Escherichia coli and analyzed the functions of the respective purified proteins in various adherence assays and zymographic analysis.
Results: We found that not only the bacteriolytic activity, but also adherence to fibrinogen and fibronectin is mediated by the CHAP domain, thus demonstrating for the first time an adhesive function for this domain. In contrast, efficient adherence to endothelial cells and vitronectin requires the whole Aaa. Adherence of an S. aureus aaa mutant and the complemented aaa mutant is slightly decreased and increased, respectively, to vitronectin, but not to fibrinogen and fibronectin, which might at least in part result from an increased expression of the autolysin/adhesin Atl. Moreover, an S. aureus atl mutant showing enhanced adherence to extracellular matrix proteins and endothelial cells revealed increased aaa-expression and production of Aaa. Thus, the redundant functions of Aaa and Atl might at least in part be interchangeable and furthermore be regulated by so far unknown mechanisms.
Conclusion: In conclusion, the adhesive properties of Aaa might promote S. aureus colonization of host extracellular matrix and tissue and thus might play an important role in the pathogenesis of serious S. aureus infections with this pathogen.

MPP032
Antibiotic treatment provokes activity of IS*256*r in several *S. aureus* strains

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Mobile elements are wide-spread in nearly all bacterial species. After the first description of insertion sequences forty years ago, more than 500 insertion sequences in 159 bacterial species have been described and characterised. Here we focus on IS*256*, a common element of staphylococci.
IS elements have been shown to create mutations by insertion into and excision from the genome, to confer genome plasticity and to confer resistance against antibiotics by insertion into promoter sequences or open reading frames.
In order to test whether the presence of antibiotics leads to the mobilisation of IS elements in *S. aureus*, a system that measures the transposition frequency of IS*256* was employed. This system comprised an IS*256* element that had been tagged by an erythromycin marker (IS*256*r) and an inactivated IS*256* for control purposes [1].
Treatment with subinhibitory concentrations of clinically relevant antibiotics (linezolid, ciprofloxacin and vancomycin) resulted in increases of transposition frequency of IS*256*r which was highest in the presence of ciprofloxacin in *S. aureus* RN1-HG (restored *rsbU*). In conclusion, there seems to be a correlation between antibiotic stress and mobilisation of IS*256*. Interestingly, we observed that there is a higher transposition rate in SigmaB deficient strains like *S. aureus* 8325.
The mechanism behind the activation of transposition is still poorly understood. In order to elucidate this phenomenon, a putative SigmaB antisense promoter in the IS*256*r element was inactivated by site directed mutagenesis. The resulting clone showed an upregulation of transposition activity. Furthermore, the significance of a second putative antisense promoter is still under investigation.
([1] Valle, J. et al (2007): J. Bacteriol., 2886-2896.)

MPP033
Variation within a field population of *Dickeya chrysanthemi* in permissiveness for broad host-range plasmids

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Horizontal gene transfer through broad host-range plasmids has the potential to provide sufficient genetic flexibility to populations of *Dickeya chrysanthemi* to keep its phytopathogenic lifestyle efficient despite evolving plant defences. However, foreign DNA often is deleterious for the individual cell. We investigated whether plasmid uptake varied among individual strains of a field population to balance the benefit from genetic flexibility and the cost on population-level. The transfer frequency of broad host-range IncP-1 plasmids between an *Escherichia coli* donor and *Dickeya chrysanthemi* strains significantly differed among isolates from a field population. Transfer frequencies for two IncP-1 plasmids, pTH10 and pB10 of the divergent a- and b-subgroups, respectively, correlated well. *D. chrysanthemi* strains, which differed in permissiveness for these plasmids by orders of magnitude, were indistinguishable by other phenotypic traits, genomic fingerprints, or by *hrpN* gene sequences. Such strains were isolated in close vicinity. Spatial aggregation of subpopulations with increased permissiveness for plasmids was not observed, indicating a reasonably fast genetic mechanism of switching in permissiveness. In contrast to IncP-1 plasmids, transfer frequencies for the narrow host-range LowGC-type plasmid pHHV216 were similar among strains suggesting that the mechanism underlying the differential permissiveness did not target foreign DNA in general.

MPP034
Staphylococcal teichoic acidis regulate targeting of the major autolysin Atl.

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Staphylococcal cell separation depends largely on the bifunctional autolysin Atl that is processed to amidase-R(1,2) and R(3)-glucosaminidase. These murein hydrolases are targeted via repeat domains (R) to the septal region of the cell surface, thereby allowing localized peptidoglycan hydrolysis and separation of the dividing cells. We could show that targeting of the amidase repeats is based on an exclusion strategy mediated by wall teichoic acid (WTA). In Staphylococcus aureus wild-type, externally applied repeats (R(1,2)) or endogenously expressed amidase were localized exclusively at the cross-wall region, while in Δ*tag*O mutant that lacks WTA autolysin was evenly distributed on the cell surface, which explains the increased fragility and autolysis susceptibility of the mutant. WTA prevented binding of Atl to the old cell wall but not to the cross-wall region suggesting a lower WTA content. In binding studies with ConcanavalinA-fluorescein (ConA-FITC) conjugate that binds preferentially to teichoic acids, ConA-FITC was bound throughout the cell surface with the exception of the cross wall. ConA binding suggest that either content or polymerization of WTA gradually increases with distance from the cross-wall. By preventing binding of Atl, WTA directs Atl to the cross-wall to perform the last step of cell division, namely separation of the daughter cells.

MPP035
Comparative proteomics within the species *Paenibacillus larvae*, a bacterial honey bee pathogen

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Recently, four different genotypes (ERIC I - ERIC IV) of *Paenibacillus larvae*, the causative agent of American Foulbrood (AFB) of honey bees, have been described [3]. The phenotypical differences between these genotypes included differences in metabolism [4], in colony and spore morphology, and in virulence [2]. To identify factors (genes and proteins) putatively responsible especially for the observed differences in virulence we applied comparative genomics via Suppression Subtractive Hybridization [1], 2009) and comparative proteomics via 2D-SDS-PAGE-analysis [5] followed by mass spectrometric identfication of differentially expressed proteins. We here present our data on the successful development of (i) a protein extraction method for *P. larvae* suitable for subsequent 2D-SDS-PAGE analysis and (ii) reproducible 2D-SDS-PAGE-analyses of these protein preparations. Based on the obtained master protein patterns of the four *P. larvae* -genotypes isolated from liquid bacterial cultures, we identified several differentially expressed proteins presumably linked to the observed phenotypic differences.

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[2] Genersch, E. et al (2005): Strain- and genotype-specific differences in virulence of *Paenibacillus larvae* subsp. *larvae*, the causative agent of American foulbrood disease in honey bees. Appl. Environ. Microbiol. 71, 7551-7555.
[3] Genersch, E. et al (2006): Reclassification of *Paenibacillus larvae* subsp. *pulvifaciens* and *Paenibacillus larvae* subsp. *larvae* as *Paenibacillus larvae* without subspecies differentiation. Int. J. Syst. Evol. Microbiol. 56, 501-511.

[4] Neuendorf, S. et al (2004): Biochemical characterization of different genotypes of *Paenibacillus larvae* subsp. *larvae*, a honey bee bacterial pathogen. Microbiology. 150, 2381-2390.
[5] O'Farrell, P. H.(1975): High resolution two-dimensional electrophoresis of proteins. J. Biol. Chem. 250, 4007-4021.

MPP036
Chitin-binding proteins of *Paenibacillus larvae* and their role in pathogenesis

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American foulbrood (AFB) is considered the most contagious and destructive infectious disease in honeybees, caused by the Gram-positive, spore-forming bacterium *Paenibacillus larvae* [1]. Despite the growing impact of this disease, molecular mechanisms involved in pathogenesis still remain elusive. It has been shown that *P. larvae* spores ingested by young bee larvae proliferate massively in the midgut lumen and that breaching the epithelium is one of the last steps in the disease process [2]. However, to achieve their way through the gut, the bacteria must first penetrate the peritrophic matrix, a chitin-rich protective layer of the larval gut. Therefore, we hypothesized that chitin-binding proteins play a major role in both attachment and local degradation of the peritrophic matrix.
Here, we present our data on two chitin-binding proteins secreted by *P. larvae*, which we identified as enhancin and a chitinase-like protein. Knowing that enhancins target insect intestinal mucin[3] while chitinases disrupt chitin, which both are the two major components of the peritrophic matrix, we were prompted to functionally characterize them in infected larvae. We show an expression profile during *P. larvae* infection focused on the production of chitinase and enhancin. Transcriptomic, proteomic and histological studies are combined, both *in vivo* and *in vitro*, to elucidate the role of these chitin-binding proteins during *P. larvae* infection.

[1] Genersch, E. et al (2006): Reclassification of *Paenibacillus larvae* subsp. *pulvifaciens* and *Paenibacillus larvae* subsp. *larvae* as *Paenibacillus larvae* without subspecies differentiation. Int. J. Syst. Evol. Microbiol. 56, 501-11.
[2] Yue, D. et al (2008): Fluorescence *in situ* hybridization (FISH) analysis of the interactions between honeybee larvae and *Paenibacillus larvae*, the causative agent of American foulbrood of honeybees (*Apis mellifera*). Environ. Microbiol. 10, 1612-20.
[3] Fang, S. et al (2009): *Bacillus thuringiensis* bel protein enhances the toxicity of Cry1Ac protein to *Helicoverpa armigera* larvae by degrading insect intestinal mucin. Appl. Environ. Microbiol. 75, 5237-43.

MPP037
Molecular identification of bamboo-inhabiting and degrading fungi

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Bamboo as a fast growing woody grass is of increasing interest for the sustainable production of a material with many potential applications for buildings and industrial utilization. Bamboo has generally a low natural durability and is easily attacked by fungi during storage, transport, processing and final use. Little is known about the fungi inhabiting and degrading the bamboo. Furthermore, for applications it is important to know which fungi might cause harm to potential products. We therefore isolated many Deuteromycetes, Ascomycetes and Basidiomycetes inhabiting and degrading the bamboo and identified them by molecular methods (rDNA-ITS sequencing). Construction of a database with the obtained ITS sequences from bamboo provides a future tool for a fast identification of the fungi even in early stages of colonization. Such knowledge is needed for a better utilization of bamboo and sustainable protection measures.

MPP038
The tetraspanin FgPls1 is involved in fitness and pathogenicity of *Fusarium graminearum*

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Tetraspanins are a family of small membrane proteins specific to animals and fungi. These proteins with characteristic secondary structures are involved in a broad range of biological processes. They behave as „molecular facilitators" interacting with other membrane proteins such as integrins, adhesion proteins, metalloproteases and proteins with Ig domains in animals. In fungi, three different families of tetraspanins were characterized. Pls1 is present in ascomycota and basidiomycota while Tsp2 is unique to basidiomycota, and Tsp3 is unique to ascomycota. Pls1 null mutants from plant pathogenic fungi such as *Magnaporthe grisea*, *Botrytis cinerea*, and *Colletotrichum lindemuthianum* are non pathogenic on plants being defective in appressorium mediated penetration. In this study, we identified *FgPLS1,* the functional orthologue of *MgPLS1* in the wheat scab fungus *F. graminearum*. Null mutants obtained by targeted gene replacement displayed defects in pathogenicity and additional phenotypes (altered mycelium growth, highly reduced production of macroconidia) not observed in other fungal *PLS1* mutants. These results demonstrate that this gene is important for vegetative growth, sporulation, and pathogenicity in *F. graminearum*. Therefore, although Pls1 tetraspanins control cellular functions involved in infection conserved among fungal plant pathogens, they have been recruited to control cellular functions involved in growth and sporulation specifically in *F. graminearum*.

MPP039
Genome sequencing of a *vanA*-negative, high-level vancomycin resistant *Staphylococcus aureus* mutant reveals multiple genetic polymorphisms

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The glycopeptide antibiotic vancomycin remains the main therapeutic agent for the treatment of serious infections caused by methicillin-resistant *S. aureus* (MRSA) that are refractory to other clinically used antibiotics. However, MRSA strains with reduced susceptibility to vancomycin have emerged during the last decade. In times of increased antibiotic treatment failure, there is an obvious need to understand how bacteria respond to the presence of antibacterial compounds and develop resistance.
Characterization of clinical and laboratory vancomycin-intermediate resistant *S. aureus* strains (VISA) identified multiple, resistance-associated changes most probably due to stepwise mutations [1, 2]. In a previous study, the mutator strain *S. aureus* RN4220Δ*mutS*, a *mutS* gene deletion mutant of the parent strain RN4220, was subjected to a stepwise vancomycin selection procedure [3]. Multiple passaging in the presence of increasing concentrations of vancomycin resulted in the generation of the mutant strain RN4220Δ*mutS*-VC40 that exhibits a significantly higher resistance level to vancomycin compared to the non-resistant parent, indicating full resistance of strain VC40 (MIC: 64 µg/ml versus 2 µg/ml, respectively).
In this study, the full genome sequence of strain *S. aureus* VC40 and its parent strain *S. aureus* RN4220Δ*mutS* was determined which revealed multiple genetic polymorphisms in genes related to cell wall metabolism, transport and gene regulation, including the two-component regulatory systems VraSR and WalKR. Further in-depth analysis of strain VC40 by transcriptomic and proteomic studies as well as mutational analyses will complete the genomic data in order to gain a better understanding of the mechanisms underlying glycopeptide resistance development in *S. aureus*.

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[3] Schaaff et al (2002): AAC 46:3540-3548.

MPP040
Influence of increases soil temperature on community structure of *Fusarium sp.* and the corresponding antagonists

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Based IPPC models, climatic change will result in many regions of central Europe in increased temperatures in soil. However the consequences of warmer soil temperatures on microbial life in soil are poorly understood. Mainly questions addressing the influence on complex interactions pattern between plant - plant pathogens and the corresponding antagonists, which have the potential to act as natural biocontrol agents have not been studies so far.
Therefore we focussed in our study on the consequences of increased soil temperature on phytopathogenic fungi of genus *Fusarium*, which are one of the most important pathogens on cereals worldwide, resulting in reduced crop quality and yield due to the contamination with different mycotoxines. Most of those mycotoxins are highly toxic to mammials and thermally stable. In addition we investigated response pattern of increased soil temperature of selected antagonists, including different *Trichoderma* species. Also questions related to changes in plant fitness and immune response have been addressed. The soil samples were taken from a lysimeter experiment where different soil types were incubated at ambient as well as with increased soil temperature (+3 °C) since 10 years.
In the presentation first data from this study will be presented and discussed.

MPP041
Monitoring of pIP501 based conjugative transfer in Gram positive bacteria

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Conjugative plasmid transfer is one of the most important mechanisms for the spread of antibiotic resistance genes and thereby the emergence of multiple resistant pathogenic bacteria. pIP501 is a 30,599-bp plasmid with the broadest known host range for a conjugative plasmid originating from Gram positive bacteria. The ransfer region is organized in an operon comprising a complete type IV secretion system (T4SS).
For monitoring horizontal gene transfer between bacteria, we constructed two plasmids, designated pVA-GFP (encoding *gfp* gene) and pVA-RFP (encoding *rfp* gene). To measure the transfer rate of the constructed GFP plasmid, mating experiments were performed using *E. faecalis* OG1X (pIP501, pVA-GFP) as donor and *E. faecalis* JH2-2 as recipient. The mobilization rate was $7.1x\ 10^{-4}$ [1]. FACS (fluorescence activated cell sorting) analysis was used for quantification of transfer. 92. 4% of *E. faecalis* OG1X (pVA-GFP) cells showed a green fluorescence and 74.5% of *E. faecalis* OG1X (pVA-RFP) cells showed a red fluorescence. Only 18.5 % of *E. faecalis* cells harbouring both plasmids (pVA-GFP and pVA-RFP) exhibited red and green fluorescence. In constrast to the mobilisable plasmid pVA-GFP, the immobilisable plasmid pVA-RFP containing the *rfp* gene revealed low fluorescence activity upon nisin induction in different Gram-positive bacteria.
Therefore we will construct a new immobilisable plasmid, which contains a constitutive promoter to express the *rfp* gene carrying replicons for both G+ and G- bacteria. For that, we obtained the plasmid pT183-S3 (kindly provided from B. Krismer, University Tübingen [2]), which contains a constitutive promoter for expression of the RFP fluorescence in different Gram positive bacteria.

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MPP042
Adaptation of *Pseudomonas aeruginosa* to changing environments using tRNA-dependent formation of alanyl-phosphatidylglycerol

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When exposed to acidic growth conditions the opportunistic bacterium *Pseudomonas aeruginosa* synthesizes significant amounts of the zwitter ionic 2' alanyl-phosphatidylglycerol (A-PG). Thereby, A-PG contributes up to 6% to the overall lipid content of the bacterium at pH 5.3.
Sequence analysis of *P. aeruginosa* revealed open reading frame (ORF) PA0920 showing 34% sequence identity to a protein from *Staphylococcus aureus* involved in tRNA-dependent formation of lysyl-phosphatidylglycerol. The gene product of ORF PA0920 from *P. aeruginosa* was predicted in bioinformatic approaches to be a transmembrane protein with 13 to 15 helices. The *P. aeruginosa* deletion mutant ΔPA0920 failed to synthesize A-PG. Consequently, the protein encoded by ORF PA0920 was named A-PG synthase. Transcriptional analysis of the corresponding gene in *P. aeruginosa* using a *lacZ* reporter gene fusion under various pH conditions indicated a 4.4-fold acid-activated transcription.
In order to get further insight into the biological role of A-PG synthase catalysis, a phenotype microarray analysis was performed. These experiments revealed that intact A-PG synthase is required to render *P. aeruginosa* resistant to the toxic effect of Cr^{3+} and the cationic antimicrobial peptide protamine sulphate. Furthermore, A-PG-dependent resistance to the β-lactam cefsulodin and the osmolyte sodium lactate were observed.
Heterologous overproduction of A-PG synthase in *Escherichia coli* resulted in the formation of significant amounts of A-PG, otherwise not synthesized by *E. coli.* The protein was identified as an integral component of the inner membrane and partially purified by detergent solubilization. By using an *in vitro* activity assay $tRNA^{Ala}$-dependent catalysis was demonstrated. While aminoacyl-PG synthase from *Enterococcus faecium* showed a relaxed specificity for lysine, arginine and alanine, for the A-PG synthase from *P. aeruginosa* a single, strict specificity for alanine was demonstrated.

MPP043
Immunomodulatory properties of Lipoarabinomannan of non-pathogenic and pathogenic mycobacteria

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Introduction: *Mycobacterium avium* subsp. *paratuberculosis* (MAP), the causative agent of paratuberculosis in ruminants, is suspected to be associated with Crohn`s disease (CD) in humans, but its zoonotic potential remains to be controversially discussed. *Mycobacterium avium* subsp. *avium* (MAA) causes a variety of diseases including avian tuberculosis and disseminated infections in immunocompromised persons. *Mycobacterium phlei* is considered non-pathogenic. Lipoarabinomannan (LAM), an integral part of the mycobacterial cell wall, may represent an important virulence determinant of mycobacteria. Terminal residues of LAM differ between pathogenic and non-pathogenic mycobacteria and seem to influence macrophage responses and thus, the impact of the LAM's on the immune system.
Question: The aim of our study was to compare the effect of LAM from pathogenic and non-pathogenic mycobacteria on cytokine responses of the human monocyte cell line THP-1 under standardized conditions to prove that LAM from diverse mycobacteria influence the immune responses of human macrophages in a different way.
Methods: Gene expression of TNF-α and IL-1β was determined by quantitative real-time PCR. Biologically active proteins were measured by ELISA (IL-1β) and cytotoxicity assay (TNF-α). LAM was isolated after cultivation of mycobacteria using a Triton X-114 phase separation technique and purified by column chromatography and dialysis (Hamasur et al. 1999).
Results: *M. phlei* induced more mRNA of the two proinflammatory cytokines in THP-1 cells than the pathogenic MAA and MAP. The amount of IL-1β and TNF-α proteins produced by macrophages infected with *M. phlei* also was higher than the amounts induced by the pathogenic strains. Induced amounts of mRNA and proteins did not significantly differ between MAA and MAP.

Conclusion: TNF-α is instrumental in inhibiting mycobacterial growth in vitro and IL-1 is involved in immunoregulation and inflammation. Our results therefore support the notion that different LAM structures are implicated in the pathogenesis of mycobacterial infections in humans.

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MPP044
A scavenger receptor on nasal epithelial surfaces-An important player in *Staphylococcus aureus* nasal colonization

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It has been demonstrated that cell wall glycopolymers (CWGs) are an important part of the repertoire of adhesins that Gram-positive bacteria use to adhere to and infect host cells. *Staphylococcus aureus* cell wall teichoic acid (WTA) mediates adhesion to nasal epithelial cells and is a key molecule in a cotton rat model of nasal colonization. However, the appropriate receptor on nasal epithelial cells remains elusive. Novel advances in the field of glycobiology suggest members of the scavenger receptor family as WTA interaction partners on nasal epithelial surfaces. We tested this hypothesis by inhibitors against scavenger receptors in adhesion assays and detected a marked inhibition of *S. aureus* to nasal epithelial cells. Recently, epithelial expression of a receptor belonging to the scavenger receptor family had been described and we demonstrated expression of this receptor in nasal epithelial cells of human origin. Function blocking antibodies to this receptor inhibited *S. aureus* adhesion to human epithelial cells under static and mild sheer stress conditions. We therefore propose that the strong influence of WTA mediated adhesion on nasal colonization is also an effect governed by a scavenger receptor. To further elucidate these in vitro findings in the cotton rat model of nasal colonization we established primary cultures of cotton rat nasal epithelial cells. Thereby, we were able to detect the expression of the mentioned scavenger receptor. Furthermore, we could prove WTA depended specific binding to primary cotton rat epithelial cells by utilizing WTA-labelled latex beads. Planned studies with a function blocking antibody against the mentioned scavenger receptor should reveal its influence on colonization in this in vivo model. Thus, we present here the first nasal epithelial WTA receptor.

MPP045
Response of *Candida albicans* to nitrogen starvation

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Candida albicans is a commensal inhabitant of the intestinal tract of warm-blooded animals, but also able to cause life-threatening oportunistic infections in the debilitated or compromised host. During pathogenesis the fungus disseminates via the bloodstream and infections can manifest within various tissues. Regardless the commensal state, in which *C. albicans* has to cope for nutrients with competing microorganisms, dissemination via the bloodstream with subsequent attack by immune effector cells or growth within tissues, acquisition of nutrients is an essential prerequisite to survive within the host. It has been assumed that phagocytosis by immune effector cells generates a carbon and nitrogen limited environment and *C. albicans* escapes from these cells by switching from the yeast into a hyphal growth form causing mechanical pressure on host cells and eventually leading to the pathogen release. In this respect we monitored the transcriptional response of *C. albicans* when shifted from a nutrient rich to a nitrogen-starved environment. As expected, the translational machinery, mainly resembled by ribosomal proteins was strongly downregulated within the first 30 - 60 min after shifting the cells to starvation. However, within four to six hours a transcriptional steady state for these genes was reached. In contrast, transcription of some genes involved in amino acid metabolism was strongly induced, among them several genes involved in arginine metabolism. This induction was in agreement with the observation of cell clumping and formation of hyphae, which has been shown to be dependent on the induction of genes involved in arginine metabolism. In future experiments we will add different nitrogen sources to nitrogen-starved cells to elucidate specific adaptation mechanisms in response to the respective nitrogen source. These experiments will allow to study nutrient uptake and interruption of this process could cause an attenuation of virulence.

MPP046
Pseudomonas aeruginosa population structure revisited under environmental focus: the impact of water quality and phage pressure

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Pseudomonas aeruginosa attracts research attention as a common opportunistic nosocomial pathogen causing severe health problems in humans. Nevertheless, the primary habitat is the natural environment. Here, we relate the genetic diversity of 391 environmental isolates from Northern Germany rivers to ecological factors such as river system, season of sampling, and different levels of water quality. From representatives of 99 environmental clones, also in comparison to 91 clinical isolates, we determine motility phenotypes, virulence factors, biofilm formation, serotype, and the resistance to seven environmental *P. aeruginosa* phages. The integration of genetic, ecologic, and phenotypic data shows (i) the presence of several extended clonal complexes (ecc) which non-uniformly distribute across different water qualities, and (ii) a disproportionate phage pressure on the ecc which apparently depends on the diverged serotype composition of ecc. For at least one ecc (eccB) we assume the ecophysiological differences on environmental water adaptation and phage resistance to be so distinct to reinforce an environmentally driven cladogenic split from the remainder of *P. aeruginosa*. In sum, we conclude that the majority of the microevolutionary population dynamics of *P. aeruginosa* is shaped by the natural environment and not by the clinical habitat

MPP047
Expression of Plx1, an ADP-ribosylating toxin of *Paenibacillus larvae*

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The Gram-positive bacterium *Paenibacillus larvae* is the causative agent of American Foulbrood, a notifiable epizootic of honey bee larvae. The disease process in individual larvae can be divided into a non-invasive and an invasive phase. The non-invasive phase at the beginning of infection is characterized by massive proliferation of *P. larvae* in the larval midgut lumen. During the invasive phase, *P. larvae* enters the haemocoel by breaching the intestinal epithelium of honey bee larvae accompanied or initiated by rounding-up of epithelial cells [4]. Toxins and proteases are most likely involved in this process. Good toxin candidates are ADP-ribosylating AB-toxins, which are expressed by *P. larvae* (Fünfhaus et al., 2009) and which are known to destroy the cytoskeleton [1] and cell-cell contacts of the host [3]. AB-toxins are comprised of a catalytic A-subunit and a B-subunit which induces translocation and targeting of the host cell. We recently identified Plx1 (*P. larvae* toxin 1) as an ADP-ribosylating AB-toxin specific for *P. larvae* genotype ERIC I [2]. For functional characterization, Plx1 was cloned in an appropriate expression vector, harbouring promotor-, RBS- and start codon- sequences as well as an N-terminal His-tag. The recombinant plasmid was *in vitro*-translated using an *E. coli*-based *in vitro* expression system. SDS-gel-analyses revealed a successful translation of the N-terminal His-tagged protein. Purification of Plx1 was performed by affinity-binding of the His-tag to magnetic nickel particles. After binding, washing and elution of the tagged protein with imidazol it could be visualized on SDS-gels with a mass of about 111 kDa. Purified recombinant Plx1 is now available for functional assays including *in vitro* cell culture- as well as larval-assays to verify toxin function and to identify the cellular target molecule.

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MPP048

Staphylococcus saprophyticus is able to adapt to utilization of D-serine as the carbon and energy source

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S. saprophyticus is the only species of the staphylococci that is typically uropathogenic. Several virulence factors have already been identified, but it has been suggested that also certain metabolic activities may contribute to virulence. The genome of *S. saprophyticus* is the only one of all sequenced staphylococci which possesses a D-serine deaminase, an enzyme which converts D-serine to pyruvate and ammonia. Interestingly, this enzyme is also present in other uropathogenic bacteria like *E. coli* (UPEC). The amino acid D-serine is present in relatively high concentrations in human urine and is toxic or bacteriostatic to several non-uropathogenic bacteria. Therefore the uncommon ability to degrade D-serine may play an important role for the virulence of uropathogens. In addition the presence of D-serine may be used as a cue by uropathogens for the presence in the urinary tract. To analyze the metabolism and to understand the significance of D-serine catabolism of *S. saprophyticus* for virulence, we developed a chemically defined medium. By systematically adding and removing components from this medium, we could show that *S. saprophyticus* is able to use D-serine as the sole carbon and energy source. Remarkably, the lag time is much longer when D-serine is used compared to that when glucose is used as energy source. When *S. saprophyticus* is once adapted to D-serine, it grows immediately without an extended lag phase when it is inoculated into fresh media with D-serine as sole carbon source. Moreover, when *S. saprophyticus* is adapted to D-serine, it grows slower with glucose. These results show that *S. saprophyticus* is able to change its metabolism in the presence of D-serine and to adapt to this nutrient. That leads us to the hypothesis that a similar adaptation could happen within the urinary tract. To get more insights into the kind of adaptation, we conducted 2D-gelelectrophoresis and analyzed the protein patterns of *S. saprophyticus* adapted to glucose and adapted to D-serine.

MPP049

Zwitterionic cell wall polymers with immune modulatory function - important players in CA-MRSA pathogenicity?

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Staphylococcus aureus can cause a large variety of infections but skin and soft-tissue infections are the most common type. Recently, community-acquired methicillin-resistant (CA-MRSA) isolates which often carry the gene for the Panton-Valentine leukocidin (PVL) emerged as the major cause of severe skin and soft-tissue infections (SSTIs) caused by *S. aureus* in the USA. The pathogenic potential of CA-MRSA strains seems to depend on an array of different virulence factors, however the relative activity of these factors is still unclear. We recently demonstrated that the cell wall polymer WTA (cell wall teichoic acid) of *S. aureus* is a major modulator of abscess formation. The immune modulatory activity of WTA depends on its zwitterionic nature and the ability to activate CD4 T cells after presentation on MHC II molecules in antigen presenting cells. In turn the zwitterionic WTA activated T cells influence abscess formation by regulating the local cytokine milieu. Interestingly, we find that highly pathogenic CA-MRSA strains exhibit an elevated WTA amount in their cell walls. Purified protein-free cell wall fractions from CA-MRSA induce T cell proliferation and cytokine production more efficiently than cell wall from non CA-MRSA. In addition we could demonstrate that cell wall fractions of CA-MRSA strains are more active in skin abscess formation. Thus, upregulation of WTA biosynthesis in CA-MRSA contributes to pathogenic potential of CA-MRSA. The major focus of this project is to understand how zwitterionic WTA of CA-MRSA shapes the pathogenic potential and what molecular events are involved on the host side.

MPP050

Why does *Staphylococcus aureus* decorate its wall teichoic acid with N-acetylglucosamine?

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Wall teichoic acid (WTA) glycopolymers are major constituents of cell envelopes in *Staphylococcus aureus* and related Gram-positive bacteria with important roles in cell wall maintenance, susceptibility to antimicrobial molecules, biofilm formation, and host interaction. Most *S. aureus* strains express poly-ribitolphosphate (Rbo-P) WTA substituted with d-alanine and *N*-acetylglucosamine (GlcNAc). WTA sugar modifications are highly variable and have been implicated in bacterial phage susceptibility and immunogenicity but the pathway and enzymes of staphylococcal WTA glycosylation have remained unknown.
Revisiting the structure of *S. aureus* RN4220 WTA by NMR analysis revealed the presence of canonical Rbo-P WTA bearing only a-linked GlcNAc substituents. A RN4220 transposon mutant resistant to WTA-dependent phages was identified and shown to produce altered WTA, which exhibited faster electrophoretic migration and lacked completely the WTA a-GlcNAc residues. Disruption of a gene of previously unknown function, renamed *tarM,* was responsible for this phenotype. Recombinant TarM was capable of glycosylating WTA *in vitro* in a UDP-GlcNAc dependent manner thereby confirming its WTA GlcNAc transferase activity. Deletion of the last seven amino acids from the C-terminus abolished the activity of TarM. *tarM*-related genes were found in the genomes of several WTA-producing bacteria suggesting that TarM-mediated WTA glycosylation is a general pathway in Gram-positive bacteria.
Our study represents a basis for dissecting the biosynthesis and function of glycosylated WTA in *S. aureus* and other bacteria.

MPP051

Construction and characterization of three *fbl* knockout mutants of *Staphylococcus lugdunensis*

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Staphylococcus lugdunensis is a commensal and integral part of the normal skin flora but also a important pathogen that causes several serious infections similar to those caused by *Staphylococcus aureus*, like endocarditis, sepsis, skin and soft tissue infections. In contrast to *S. aureus*, data on pathogenicity factors of *S. lugdunensis* is scarce due to fact that an isogenic genetic manipulation of *S. lugdunensis* has not yet been described. We present the first transformation and directed isogenic genetic manipulation of *S. lugdunensis* described so far. Knockout mutants of the *fbl* gene were constructed from three different strains of *S. lugdunensis* to show that at least in these strains fibrinogen binding is exclusively mediated by Fbl. Only 29 out of 104 (27.9 %) clinical isolates of *S. lugdunensis* bound to fibrinogen although the prevalence of the *fbl* gene was very high (100 %). Strains that showed binding to immobilized fibrinogen also induce a clumping in the short coagulase test. In contrast to their wildtypes isogenic *S. lugdunensis* mutants lacking the *fbl* gene neither bind to fibrinogen nor clump in the short coagulase test.

MPP052

Mutants of *Xanthomonas campestris* pv. *vesicatoria* devoid of aconitase B exhibit reduced pathogenicity on pepper leaves

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Bacterial class A and B aconitases (Acn) are iron-sulfur (FeS) proteins that differ in the organisation of their respective domain structures [1]. AcnA and AcnB each can have two different functions depending on the cellular iron level. If iron is plentiful Acn possesses a labile [4Fe-4S] cluster and is functional in the TCA cycle. If, however, iron is limiting then the enzyme loses its [4Fe-4S] cluster and adopts a post-transcriptional regulatory function as an iron regulatory protein (IRP). In many bacteria the apo-form

of aconitases regulates a number of cellular processes, especially in response to iron limitation and oxidative stress. The plant pathogen *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*) is an obligately aerobic γ- proteobacterium that causes bacterial spot disease on pepper and tomato plants. The genome of *Xcv* encodes three aconitases (2 AcnA and 1 AcnB enzyme). The *acnB* gene and one of the *acnA* genes are divergently organized in the genome of *Xcv*. While this *acnA* gene is monocistronic, the *acnB* gene forms an operon with two small genes *xcv1925* (*orfX*) and *xcv1926* (*orfY*). This operon organistaion of *orfX-orfY-acnB* is highly conserved in the related genera of plant pathogenic bacteria *Xanthomonas* and *Xylella*. The *orfX* gene product has similarity to AbrB-type transition-state regulators while OrfY shares amino acid similarity with virulence-associated proteins (Vap) found in a number of pathogenic bacteria [2, 3]. Although the function of neither protein in *Xcv* is currently known, both are predicted to bind nucleic acids. Mutants lacking *acnB* or *orfX-orfY-acnB* both show normal growth when cultured under laboratory conditions but exhibit reduced growth in planta. They also show a reduced disease progression on susceptible pepper plants and a decreased hypersensitive reaction (HR) on resistant plants. Plate sensitivity assays showed that the mutants exhibit reduced growth in the presence of the superoxide generator menadione, suggesting that AcnB might have a role in sensing superoxide stress, which might possibly be a plant response to pathogen infection. An *orfX-orfY* mutant, in contrast, was unaffected for growth on menadione but synthesized higher levels of AcnB than the wildtype, perhaps suggesting a regulatory role for the OrfX and/or OrfY proteins in controlling the levels of AcnB.

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MPP053

In vitro tests of the essential YycFG (WalRK/VicRK) two-component regulatory system of *Staphylococcus aureus*

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Especially Gram-positive bacteria are characterized by a massive cell wall, which is consisting of multiple layers of peptidoglycan. Since the structural integrity of the cell wall directly affects the viability of the bacterium itself, biosynthesis of peptidoglycan is beyond doubt one of the tightest controlled mechanisms in bacterial growth. This is one of the reasons, why cell wall targeting antibiotics have such an importance in clinical treatment of bacterial infections. While our knowledge of cell wall composition, biosynthesis and mode of action of targeting antibiotics is comprehensive, much less is known in detail about how bacteria control the tricky interplay between degradation and new synthesis of peptidoglycan to coordinate cell division and cell growth.
In recent years it has been shown for *S. aureus* that the essential YycFG (WalRK/VicRK) two-component regulatory system (TCS) plays a major role in the homeostasis of cell wall by controlling the expression of peptidoglycan degrading enzymes (autolysins) [1]. Inside the group of Gram-positive bacteria with low G+C-content the orthologues of YycFG (WalRK/VicRK) are almost ubiquitously distributed [1, 2]. Despite this fact, the specific signal(s) by which this TCS is activated is/are still unknown and *in vitr*o tests of the YycG (WalK/VicK) Kinase have been limited to the use of truncated proteins, which comprised only the cytoplasmic regions of the protein.
For this reason, we established an experimental approach that utilizes the purified full-length kinase in *in vitro* phosphorylation assays. This system has been used to determine the phosphorylation properties of the wild type YycG (WalK/VicK) kinase and two mutated versions of this protein, harboring an amino acid exchange in the cytoplasmic PAS and HATPase_c domains, respectively.

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MPP054

Colonisation of the host plant root by *Magnaporthe oryzae*

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The well known leaf pathogen *Magnaporthe oryzae* has also been shown to be able to invade the plant via the roots. However the fungus undergoes a different hyphal differentiation in this interaction then it does in the leaf infection (Dufresne et.al, 2001, Sesma & Osbourn, 2004). This hyphal differentiation is a crucial step for all root colonising fungi and consequentially it was shown that some of these components are shared with other fungi (Heupel et al., 2010). In our work we are interested in the characterisation of underlying differences in hyphal morphogenetic development which are leading to root colonisation. For this we did a screening for root infection defective insertional mutants and, in a second approach, microarray analysis, comparing root infection with leaf infection. In this transcriptomic approach we found several genes that are apparently specific for the colonisation of plant roots. Many of them are secreted proteins and so are good candidates to be fungal effector proteins. For the most interesting ones further analysis, like creation of knock out strains and *in planta* characterisation are on the way.

MPP055

Phosphorylation activities of the VraSR two-component system of *Staphylococcus aureus* - employing the full-length VraS histidine kinase -

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S. aureus is a major cause of nosocomial infections and clinical therapy becomes more and more difficult because of the increase in antibiotic resistance displayed by this pathogen. Two-component regulatory systems (TCSs), consisting of a sensor histidine kinase (HK) and a corresponding response regulator protein (RR), play an important role in bacterial growth, enabling them to respond and to adapt to environmental stress conditions.
In *S. aureus* the VraSR (**v**ancomycin-**r**esistance **a**ssociated **s**ensor/**r**egulator) TCS is involved in resistance to vancomycin and β-lactam antibiotics [1, 2, 3]. In this context, it has been shown that VraSR is involved in a mechanism, which senses inhibition of bacterial cell wall (peptidoglycan) biosynthesis [4].
Signal transduction via phosphoryl group transfer follows a general scheme in TC systems. Upon activation by a specific signal the HK (VraS) undergoes autophosphorylation at a conserved His residue and in a second step the phosphoric group is transferred to a conserved Asp residue of the RR (VraR), thereby changing its activity in controlling DNA transcription.
Since VraS is a membrane protein, that possesses two TM domains at the N-terminus, purification of the entire protein is difficult. For that reason, previous experiments [4] have been carried out with truncated versions of VraS. Here, we want to present the recombinant expression of the full-length VraS kinase and VraR response regulator protein of *S. aureus* NCTC8325 and their activity tests in *in vitro* phosphorylation assays. For this purpose a detergent-micelle system was used, which has been previously established for initial studies of the phosphorylation behavior of the essential YycFG (WalRK/VicRK) TCS of *S. aureus*.

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MPP056

Staphylococcus saprophyticus: localisation of fibronectin binding in the A domain of SdrI

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Staphylococcus saprophyticus is a gram-positive and coagulase-negative pathogenic staphylococcus causing urinary tract infections in young women. It is hydrophobic, able to bind fibronectin, laminin and collagen and

hemagglutinates sheep erythrocytes. Some of its surface proteins have been characterised in the early past. The serine-aspartate repeat protein I (SdrI), contains the longest SD repeat region described so far (854 aa) and a LPXTG-motif for cell wall anchoring. It is a member of the MSCRAMM protein family and shows a typical ABB domain structure. Within the A domain a specific amino acid sequence (TYTFTNYVD) is found. This motif or it variants is also found in many other fibronectin or collagen binding proteins from staphylococci.
Previous experiments have shown that the purified A domain is able to bind to fibronectin coated on microtiter plates. To localise the area of binding the A domain was divided into three parts: N1, N2 and N3. N2, N3 and N2+3 were cloned into the pQE30Xa vector for overexpression and purification using a n-terminal His-Tag. The parts of interest were N2 and N3 because in N2 the TYTFTNYVD is located. Bindingassays were carried out with N2, N3, N2+3 and the whole A domain as a positive control were done using a coated microtiter plate. The bound protein was detected by an ELISA. As expected the results showed a high binding activity for N2, a slightly reduced binding for N2+3 compared to N2 and althougth N3 alone showed no binding at all. N2 was further subjected to x-ray structure analysis but data analysis is still ongoing. We expect to gain new insights into the A domain conformation of SdrI and its implications for the uro-adherence of *Staphylococcus saprophyticus*.

MPP057
Investigations into the metabolism of *Legionella pneumophila*

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Legionella pneumophila is an aquatic bacterium that replicates within protozoa (amoeba), but can also infect human alveolar macrophages causing Legionnaires' disease, an atypical pneumonia. Many virulence factors of *L. pneumophila* have been reported, but less is known about nutrition of the bacteria, especially inside host cells. When nutrients become limiting, a regulatory casdade triggers the differentiation from the replicative form, with high metabolic activity, to the transmissive and virulent form. *L. pneumophila* uses amino acids as primary energy and carbon sources; glucose although assimilated, is thought not to be important for bacterial growth. We used ^{13}C-isotopologue profiling in combination with nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) we demonstrated the use of glucose for de novo-biosynthesis of several amino acids. We found high ^{13}C-incorporation rates for the amino acids alanine, aspartate, glutamate, glycine, and proline as well as for 3-hydroxybutyrate in *L. pneumophila*. The tricarboxylic acid cycle is complete and active. Furthermore, experiments with [1,2-$^{13}C_2$]glucose state the importance of the pentose phosphate pathway for glucose catabolism. In addition, we identified an active glucoamylase, which is secreted by *L. pneumophila* and degrades starch and glycogen. Moreover, we present an experimental background for in vivo-studies of the bacterial metabolism inside *Acanthamoeba castellanii* host cells.

MPP058
Antibiotic translocation through bacterial porins - insights from electrophysiology

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The outer membrane of Gram-negative bacteria contains a number of channel-forming hydrophilic proteins called porins. Such channels allow diffusion of low-molecular weight solutes across the outer membrane. Our method of choice to study the molecular aspects of antibiotic translocation across such membrane channels is electrophysiology using planar lipid bilayers. In this technique, purified porins are individually inserted into the bilayer. Permeation of molecules inside the channel causes fluctuations in the ion current, reflecting the molecular interactions with the channel wall.
We have been able to characterize facilitated translocation of beta-lactams and fluoroquinolones through various outer membrane channels including *Bps*Omp38 of *Burkholderia pseudomallei*, OmpPst1 and OmpPst2 of *Providencia stuartii*, and OmpF and OmpC of *Escherichia coli*. Titration with effective antibiotics revealed concentration-dependent blockages of the ion flow suggesting interaction with the channel. Noise analysis of ion currents through porin in the presence of antibiotics enabled us to determine binding kinetics and transport parameters at single-molecule level. We also characterized the impact of temperature on the antibiotic passage through porins reconstituted into bilayer. *In vitro* activity of antibiotics was determined by MIC assays which correlated with the results obtained from bilayer measurements. Experimental results were compared with molecular dynamics simulations which provide the energy barriers along the diffusion pathway and an atomic description of the antibiotic translocation through porins. Our study of antibiotic translocation at single-molecule level gives new insights to design novel drugs with optimal penetration into bacterial cells.

MPP059
Adhesion of *Ustilago maydis* filaments at the onset of pathogenic development.

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In pathogenic fungi, adhesion to surfaces is considered an important event in disease establishment. Adhesion of the phytopathogenic basidiomycete Ustilago maydis is tightly linked to the formation of filaments on the plant surface that follows the establishment of the infectious dikaryon. Filamentation of the fungus can also be induced solely by contact with a given hydrophobic surface like Polytetrafluoroethylene. The factors propagating hyphal adhesion in U. maydis are still largely unknown, and identification of distinct factors by comparative sequence analyses is hindered by the heterogeneity of involved gene products and their functional redundancies. In an attempt to circumvent these difficulties we adapted a method developed for identification of Candida albicans adhesion-promoting genes. We use a parallel plate shear flow assay to screen a U. maydis cDNA library expressed in adhesion-deficient Saccharomyces cerevisiae cells to identify genes that enhance adhesive capabilities of yeast cells to hydrophobic surfaces. The use of a parallel plate flow chamber provides quantitative reproducible measurements of cell detachment from these surfaces by applying a known shear stress under conditions of laminar flow. Identified genes and respective gene products will subsequently be analyzed and characterized in U. maydis. We expect that this approach will lead to the identification of novel adhesins and regulatory elements controlling surface adhesion.

MPP060
Metabolic flux analysis of enteropathogenic *Yersinia pseudotuberculosis*

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The pathogenic bacterium *Yersinia pseudotuberculosis* is the causative agent of self-limiting enteritis, diarrhoea, mesenteric lymphadenitis and autoimmune disorders [1]. It is able to infect animals and humans and is closely related to *Yersinia pestis*. Moreover *Yersinia pseudotuberculosis* is known to have a complex regulatory network and it is therefore a predestinated model organism for pathogens.
The invasion of mammalian cells is controlled by a cascade composed of regulatory RNAs and proteins some of which are checked by nutritional and environmental conditions [2]. Due to this link between virulence and metabolism a coincident change in central carbon fluxes with formation of infection relevant factors is assumed.
Metabolic flux analysis has proven as major technology in industrial biotechnology to perform system-wide pathway analysis and subsequent design-based strain optimization [3]. Despite its beneficial application in this field, ^{13}C metabolic flux studies are rarely found in the medical field. This methodology is, however, promising to gain a quantitative insight into the in vivo activity of enzymes and pathways for analysing the mentioned link between pathogenicity and metabolism.
The quantitative analysis of metabolic fluxes was based on a comprehensive approach combining an experimental and computational part. This involved the development of a reproducible cultivation strategy for *Yersinia*

pseudotuberculosis and the elucidation of its cellular composition to account for anabolic precursor withdrawal.
For flux calculation from ^{13}C labelling data as well as growth and production kinetics, metabolic reaction model was constructed on basis of the KEGG (Kyoto Encyclopedia of Genes and Genomes) database and implemented into the flux modelling platform OpenFlux [4].
As the result of this work, metabolic fluxes through all major pathways of central carbon metabolism in *Yersinia pseudotuberculosis* wild type could be quantified. In future studies, this technology will be applied to investigate mutants with a lack of specific virulence factors towards better understanding of the in vivo link between the pathogenicity and metabolism.

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MPP061
Adaptation of A*cinetobacter* to saline and dry environments

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Acinetobacter is known for its metabolic versatility that includes the ability to use different carbon and energy sources for growth. Little is known about the adaptation of Acinetobacter to dry environments like the human skin which is of particular importance for the pathogen A. baumannii. To test whether or not Acinetobacter is able to adapt to dry environments, growth experiments were performed using the non-pathogen Acinetobacter baylyi ADP1, which exhibits a growth phase dependent natural transformation phenotype, as a model. When grown in complex media, A. baylyi was able to adapt to increasing salinities (NaCl) up to an upper limit of 900 mM NaCl. The increase in salinity lead to an increase of the lag phase and a decrease in growth rate as well as cell yield. Interestingly, KCl was tolerated much better, indicating that cellular Na^+ homeostasis is involved in adaptation to high NaCl concentrations. To analyze the adaptation to low water activities, growth in the presence of sugars was monitored. Again, cells were able to adapt to 900 mM sucrose indicating the general capability of ADP1 to cope with low water activities. Analyses of the natural transformation phenotype during growth in the presence of high salt concentrations revealed 100fold increased natural transformation frequencies throughout the prolonged lag phase in the presence of 900 mM NaCl.
The molecular basis of adaptation to low water activities was first evaluated by genome analyses. ADP1 has the ability to take up glycine betaine from the medium and has a pathway to take up choline from the medium and oxidize it to glycine betaine. In the absence of exogenous glycine betaine or choline, ADP1 is also able to adapt to high salinity, but the maximal concentrations tolerated are much less. Growth is stimulated by the addition of glycine-betaine indicating that it is used as compatilbe solute in A. baylyi. Mutant studies identified possible transporters which are involved in the adaptation process

MPP062
Establishment of genomic approaches to unravel meningococcal serum resistance factors

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Objectives: *Neisseria meningitidis* is an invasive human pathogen. We seeked for bacterial factors involved in resistance to serum complement other than capsule, LPS sialylation and factor H binding protein.
Methods: Three genomic approaches were followed: (1) comparison of serum resistance of genetically closely related strains, (2) impact of global regulators of gene expression on serum resistance, (3) medium throughput screening of a *mutS* mutant for serum resistant variants.
Results: Closely related strains of the clonal lineage ST41/44 from invasive disease and carriage that differed in complement factor C3b and membrane attack complex deposition were analysed for gene content differences. The genomic oligonucleotide microarray hybridisation revealed genetic differences in the Islands of horizontally transferred DNA (IHT) B and C of the two carrier isolates compared to the invasive isolate. The impact of IHT-B and -C on serum resistance is under current investigation. In the second approach we investigated overexpression of *fnr, hfq* and *rpoH* and knock-out of the transcriptional regulators *asnC*, *nrrF*, and *zur* with regard to complement interaction. Only the zinc uptake repressor (*zur*) knock-out mutant showed an decrease in binding of complement components C5b-9 and C3d. Currently, the Zur regulon is under investigation to identify factors responsible for the altered phenotype. Finally, a medium throughput screen of a hypermutating *mutS* strain was established that allows the identification of mutants with enhanced serum resistance. 850 clones have been screened so far. While re-screening is ongoing, the identification and preliminary characterization of serum resistant variants already confirmed the feasibility of the test design.
Conclusion: Genomic approaches have been established to screen for serum resistance factors of *Neisseria meningitidis* which might be of interest as vaccine targets.

MPP063
Hxt1, a Monosaccharide Transporter and Sensor Required for Virulence of the Maize Pathogen *Ustilago maydis*

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The smut *Ustilago maydis*, a ubiquitous pest of corn, is highly adapted to its host to parasitize on its organic carbon sources. We have systematically deleted all hexose transporter genes in *U. maydis*, and have identified the *hxt1* gene as important for fungal virulence. *hxt1* (hexose transporter 1) encodes a high affinity transporter for glucose, fructose and mannose, and, with lower affinity, for galactose and xylose. Deletion of *hxt1* in *U. maydis* leads to decreased symptom development after plant infection. In axenic culture, Δhxt1 strains show reduced growth on glucose, fructose and mannose. On xylose or galactose containing media, however, deletion of *hxt1* results in an increased growth. Expression analysis revealed that in Δ*hxt1* strains monosaccharide-dependent gene regulation is affected, leading to the expression of genes involved in the metabolism of the low affinity substrates of Hxt1. In the *S. cerevisiae* hexose sensor proteins Snf3 and Rgt2, the mutation of a conserved arginine residue results in a constitutively active signaling pathway. Interestingly, over-expression of a Hxt1-derivative carrying an analogous mutation decreased the virulence of Δhxt1 strains even more.
We propose that Hxt1 has a dual function as monosaccharide-transporter and -sensor. While the transport function may likely to be required to feed the fungus *in planta*, the sensor function of Hxt1 may be most important to sense galactose and xylose levels within the plant that may be indicative for the physiological status of the host cells.

MPP064
Proteomic characterization of *Staphylococcus aureus* surviving in human host cells for extended time periods - achievements and challenges

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S. aureus is worldwide known as a commensal and particularly as a pathogen that causes severe infections. About 20 % of the world's population carries *staphylococci* permanently without showing any symptoms. On the other hand, these bacteria are recognized as the most important pathogens of nosocomial diseases and cause skin infections and life threatening illnesses like endocarditis, pneumonia, and sepsis [1,2].
Although S9 human lung epithelial cells are non-professional phagocytic cells, they are able to take up bacteria and are therefore used as a model cell line to investigate host-pathogen interactions. However, the study of the proteomic adaptation of *S. aureus* upon internalization is complicated by the very low number of available bacteria inside the host cells. In a recent study we were able to monitor the proteome changes of internalized *S. aureus* RN1HG in S9 human lung epithelial cells with our newly developed internalization workflow that combines a pulse-chase SILAC approach [4], GFP supported enrichment of bacterial proteins by FACS-sorting, and gel-free mass spectrometry analysis [3]. Using this workflow we identified

about 600 bacterial proteins from only 10^6 cells in a time range from 1.5 to 6.5 hours post-internalization.
With this study we now wanted to extend this time window and monitor the long-term adaptation of *S. aureus* RN1HG during survival within S9 human lung epithelial cells over several days. We optimized our digestion protocol, because bacterial counts consistently decreased after a short term growth phase (up to 6 hours) finally reaching around 500 cfu per ml 6 days post internalization. In order to quantify the changes of the protein composition of internalized *S. aureus*, we added fully SILAC-labeled *S. aureus* control cells as external standard to each time point after FACS-sorting, which allowed the identification and quantitation of about 300 *S. aureus* proteins post-internalization. In addition, small colony variants that appeared at late time points after internalization were investigated.

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MPP065
Will not be presented!

MPP066
TAL effectors from *Xanthomonas* : a novel DNA-binding domain with programmable specificity
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Pathogenicity of most plant pathogenic *Xanthomonas* spp. bacteria depends on the injection of effector proteins via a type III secretion system into plant cells. The translocated effectors manipulate cellular processes to the benefit of the pathogen. TAL (transcription activator-like) effectors from *Xanthomonas* spp. are important virulence factors and function as transcriptional activators in the plant cell nucleus. They directly bind to target promoters via a novel DNA-binding domain and induce expression of target host genes. This domain is composed of tandem repeats of typically 34-amino acids. Each repeat binds to a specific DNA base pair and repeat specificities are determined by a simple two amino acid-code (termed RVD, repeat-variable diresidue). The array of repeats thus corresponds to a consecutive target DNA sequence. The modular TAL repeat architecture enabled the construction of artificial TALs (ARTs) with novel repeat combinations and target specificities. Recognition sequences of ARTs were predicted and experimentally confirmed in a transient reporter system using *Agrobacterium*-mediated expression *in planta*. The ARTs exhibited predicted specificities, indicating that DNA-targeting domains with novel preferences can be generated. TAL repeats with different RVDs exist in nature, but the DNA-specificity of only a few of them is known, so far. We will present novel repeat specificities that allow conclusions about the DNA-binding mechanism of TAL repeats. The use of TALs as programmable gene switches will be shown. The programmable DNA-binding domain demonstrates that TALs are versatile virulence factors for the pathogen and exceptional tools for biotechnology.

NTV001
Protein mobility in bacterial cytoplasm
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Developments in fluorescence microscopy led to tremendous advances in both bacterial and eukaryotic cell biology in the last decades, but the quantitative potential of fluorescent microscopy still remains largely underappreciated. However, systematic quantitative approaches are absolutely required to understand the complexity of biological systems beyond cartoon-type diagrams. The combination of quantitative fluorescence imaging with other quantitative techniques and with computational modelling is thus going to be the next major frontier at the interface of biology and physics. This talk will focus on the application of quantitative FRAP and time-lapse imaging to systematically study mobility of proteins and protein complexes in the cytoplasm of *Escherichia coli*. The role of protein mobility in the controlled self-assembly and partitioning of protein complexes will be discussed.

NTV002
Hologram stacking with PICOLAY: How to get confocal microscopy for free
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A major issue of light microscopy is the low depth of focus, particularly at high magnification. If images are taken as focus series (so-called z-stacks), one can use image processing software to extract sharp zones and combine these to a single image with increased depth of focus. A depth map indicating the z-positions of the sharp patches allows reconstructing the object in its correct spatial dimensions. Normally, only the sharpest pixels in the stack are selected while others are filtered out from the resulting image.
Here I demonstrate the so-called hologram stacking with the freeware program PICOLAY (www.picolay.de, [1]). This can be used to display not only the sharpest, but all pixels with a pre-defined minimum contrast or colour. The program requires a single z-stack, only, and generates stereoscopic 3D images for different observation methods (red-cyan anaglyphs, observation with crossed or parallel eyes, rocking images). It is also possible to freely rotate the objects and visualise structures that remain hidden during the normal stacking routine. The hologram-stacking technique is especially useful for multi-layered transparent objects such as biofilms or diatoms, radiolaria etc., and can be used with various light-microscopic techniques, magnifications and illuminations (bright field, differential interference contrast, phase contrast, reflected-light or epifluorescence microscopy). Thus, one gets confocal microscopy for free, without being restricted to laser illumination and fluorescence images.
Free download: www.picolay.de

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NTV003
Studying fungal development: Utilization of laser capture microdissection and next-generation sequencing techniques
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Fungi are able to produce a number of different cell types and multicellular structures during their life cycle. One prominent example is the formation of fruiting bodies to propagate sexually. Our studies focused on the filamentous fungus *Sordaria macrospora* which produces fruiting bodies within seven days under laboratory conditions. To identify regulators of sexual development, we have generated and characterized several sterile mutants by standard molecular genetic approaches. Recently, next-generation (NGS) techniques have become available and have revolutionized the field of genomics / functional genomics. We employ NGS in different ways to identify developmental genes in *S. macrospora*: First, we use NGS to sequence the genomes of yet uncharacterized sterile mutants that were generated by conventional mutagenesis. Mapping of sequence reads to the recently sequenced genome of the *S. macrospora* wild type and bioinformatics analysis is used to identify the respective mutation causing the developmental defect. This strategy has already led to the identification of a spore color and a developmental gene. Second, we apply laser capture microdissection (LCM) to separate vegetative and sexual structures. Subsequent RNA isolation from these structures followed by RNA amplification and RNA-Seq should enable us to identify genes specifically transcribed in sexual structures. By this approach, we will generate gene expression profiles that are much more accurate than those generated by conventional techniques that use a mixture of vegetative and sexual cells harvested at different time points.

NTV004
A simple method to prepare microorganisms for AFM analysis

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Nowadays AFM becomes a more and more attractive method for microbiologists to investigate Microorganisms. The technique allows imaging over a broad magnitude scale and is not confined by the diffraction limit. Sometimes it is interesting to measure the dimensions of an organism. The other time the question is about surface properties of a cell. The scanning principle makes the AFM technique comparatively slow and the specimen has to be fixed on a flat surface during the scans. It is quite simple to dry the samples on a surface. Drying leads to a good immobilization but also to drying artifacts like denaturation of Proteins and shrinkage of the whole cell due to the loss of water. Therefore it is advantageous for most biological questions to do the imaging in liquids. Immobilization is not trivial as result of the heterogeneous surface properties of different micro organisms. Existing preparation methods are mostly utilizing coated surfaces or lithographicaly prepared surfaces. While lithography is not an option for everyone there is a variety of coatings available for instance poly-L-lysine or gelatine which work quite good with some microorganisms. A new method based on polyelectrolyte coated surfaces combined with centrifugal sedimentation shows promising results regarding the efficiency of immobilization. A variety of micro organisms were tested with the new method showing universality for many organisms. The samples were prepared with and without fixation. Of course fixation simplifies the imaging by enhancing the stability of the samples. But even unfixed Microorganisms can be imaged which opens the field for investigations in respect to cell division or other dynamic processes of living cells.

NTV005
Development of a novel biosensor for the intracellular detection of L-methionine and branched-chain amino acids

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Metabolite detection and quantification in single bacterial cells is one of the great challenges of current research in the field of White Biotechnology. Here, we report the development of a biosensor which enables the intracellular detection of L-methionine or branched-chain amino acids and transforms this information into an optical readout, in this case the production of a fluorescent protein. The described biosensor will be applied to support efforts in strain development for the production of methionine or branched-chain amino acids and is furthermore of great value for the analysis of production strains on a single-cell level. The sensor is based on the Lrp-BrnFE module of *Corynebacterium glutamicum*, consisting of the Lrp-type transcriptional regulator Lrp and its target genes *brnFE* encoding an export system for methionine and branched-chain amino acids. At elevated intracellular methionine concentrations Lrp is in an active state and induces transcription of the divergently transcribed genes *brnFE*. For the development of the biosensor we constructed a sensor cassette including *lrp,* the intergenic region of *lrp* and *brnF*, and the promoter of *brnF* fused to *yfp*, which encodes a yellow-green variant of GFP. Due to the specificity characteristics of Lrp, this sensor is suitable for the detection of methionine and the branched-chain amino acids L-leucine, L-valine and L-isoleucine. By *in vivo* measurements the relative affinity and specificity of the sensor towards its effectors was determined. Fluorescence spectroscopy and fluorescence-activated cell sorting (FACS) confirmed the general suitability of the system to monitor the intracellular production of methionine and branched-chain amino acids. Thus, this sensor represents a valuable tool for efficient strain development in White biotechnology and can furthermore be applied for the study of the population structure of industrial production strains.

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NTP001
Site specific mutagenesis of lysozyme immobilized on magnetic beads as a target for specific interaction and subsequent separation of bacteria for enrichment or isolation from complex matrices by magnetic forces

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The VBNC state of bacteria and low bacterial densities are big challenges for cultivation based pathogen recovery in e.g. drinking water and food industry. Therefore, a new molecular biologic separation method for bacteria using point mutated chicken c-type lysozymes immobilized on magnetic beads for bacteria separation is described. The immobilized mutated lysozymes on magnetic beads serve as baits for the specific capture of bacteria from complex matrices or water and can be separated by using magnetic racks. To avoid the bacterial cell lysis by lysozyme the protein was mutated at amino acid position 35 leading to the exchange of the catalytic glutamate with alanin (LysE35A) and with glutamine (LysE35Q). As proved by turbidity assay with reference bacteria these changes caused the elimination of the muramidase activity from mutated lysozymes but are known to retain their affinity for bacterial cell wall components. The mutated constructs were expressed by the yeast *Pichia pastoris* and secreted into expression medium. Protein enrichment and purification was carried out by SO_3 functionalized nano-scale cationic exchanger particles. For a rapid proof of principle the proteins got biotinylated and immobilized on streptavidin functionalized, fluorescence dye labelled magnetic beads. These constructs were used for successful capture of Syto9 marked *M. luteus* cells from cell suspension as visualised by fluorescence microscopy which provided a first hint for the success of the strategy.

NTP002
Characterization of microbial ecological systems: an industrial application

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Common strategies for the phylogenetic characterisation of microbial ecosystems are based on the „passive" DNA genome (genetic potential). GATC has developed an integrated solution to analyse such ecosystems using the „active" RNA. Experimental outlines will be shown to analyze a complex industrial microbial ecosystem using a combinatory approach of different libraries and sequencing technologies. RNA Step 1: This approach differs from others in using the transcribed RNA („active" genome) as starting material. Reverse transcription to cDNA is followed by a normalisation step. The normalised cDNA samples are sequenced on the Roche GS FLX. Subsequent proprietary bioinformatic analysis allows in silico separation of rRNA and mRNA. rRNA data, is used for phylogenetic analysis. The remaining reads are assembled (de novo) and build the transcript reference for quantification. Step 2: Total RNA starting material is depleted of rRNA. The cDNA, derived from the remaining mRNA is sequenced on the Illumina HiSeq 2000. The resulting large amount of sequence data can be mapped to the transcripts (step 1) and quantified. This combinatorial approach determines microbial diversity and abundance as well as gene content and relative levels of gene expression. DNA For the verification of the approach, the classical standard 16S rRNA analysis using primers derived from conserved 16S rRNA regions is performed. After sequencing on the Roche GS FLX, the data are compared to the results from the RNA experiment. Pacific Biosciences RS With the new Pacific Biosciences PacBio RS real time single molecule sequencer, reads longer than 1,000 bp can be obtained. These read lengths enable the design of different primer sets to achieve longer and more specific 16S rRNA fragments that can be sequenced in one read. Additionally, only one read is needed to sequence long transcripts. This will increase the accuracy of the phylogenetic studies as well as the cDNA analysis. The PacBio RS will be developed for direct RNA sequencing which will lead to more precise analysis of ribonucleic acids. Conclusion The new approach for a simultaneous phylogenetic, qualitative, and quantitative analysis allows for a precise look into the diversity and change in metabolic pathways of microbial ecosystems.

NTP003
Resolution of natural microbial community dynamics by community fingerprinting, flow cytometry and trend interpretation analysis

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Natural microbial communities have generally an unknown structure and composition due to their still not yet cultivable members. Therefore, understanding the relationships between the bacterial members, prediction of their behaviour and controlling their functions is a difficult and often only partly successful endeavour to date. This study aims to test a new idea which allows following community dynamics on the basis of a simple concept. Terminal restriction fragment length polymorphisms (T-RFLP) analysis of bacterial 16S ribosomal RNA genes was used to describe a community profile which we define as composition of a community. Flow cytometry and analysis of DNA contents and forward scatter characteristics of the single cells were used to describe a community profile which we define as structure of a community. Both approaches were brought together by a non-metric multidimensional scaling for trend interpretation of changes in the complex community data sets. This was done on the basis of a graphical evaluation of the cytometric data, leading to the newly developed Dalmatian-plot tool, which gave an unexpected insight into the dynamics of the unknown bacterial members of the investigated natural microbial community. The approach presented here was compared with other techniques described in the literature.
The microbial community investigated in this study was obtained from a BTEX contaminated anoxic aquifer. The indigenous bacteria were allowed to colonize in *in situ* microcosms consisting of activated carbon. These microcosms were amended with benzene and either of the electron acceptors nitrate, sulfate or ferric iron to stimulate microbial growth. The data obtained in this study indicated that the composition (via T-RFLP) and structure (via flow cytometry) of the natural bacterial community were influenced by the hydro-geochemical conditions in the test site but also by the supplied electron acceptors which led to distinct shifts in relative abundances of specific community members.
It was concluded that engineered environments can be successfully monitored by single cell analytics in combination with established molecular tools and sophisticated statistical analyses, a mélange, which holds great promise for studying and monitoring natural microbial community behaviour.

NTP004
Phosphorylation mechanisms of bacterial organisms show the importance of energy thresholds in living RNA systems.

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The mechanisms of phosphorylation is of vital importance for the living organism, whether at bacterial level or at eukaroytic level. The process occurs in all RNA processes and the actual atomic level process of phosphorylation and the energy requirements for this process provide a large insight into how the living system uses natural energy in cell processes.The detailed energy levels can be calculated,and as will be shown in the presentation,can then be manipulated for comparisons with other intracellular processes and even to extracellular processes.This means that there is a standard to which future phosphorylation mechanisms in RNA can be measured. The detailed method of this will be shown and photographic representations of RNA processes related to phosphorylation will be shown in the presentation.Photographic representations are useful in visualising how the phosphorylation process is progressing in different parts of the RNA activities.

NTP005
Measuring unbiased metatranscriptomics in pelagic aerobic ammonium oxidation zones of the central Baltic Sea

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Microorganisms mediate all geochemical cycles relevant to sustaining life on Earth. An analysis of their metabolism is therefore fundamental to understanding globally important element transformations. However, most microbes are recalcitrant to cultivation, such that culture-independent methods must be used to deduce their metabolic functions. One approach that has already shown great promise in this regard is to analyze the pool of transcripts contained in natural microbial assemblages (metatranscriptomes). Unfortunately, since mRNA is extremely labile and can degrade in less than 30 sec, it is unclear whether the abundance patterns detected in nature are vulnerable to considerable modification *in situ* simply due to sampling procedures. Exemplified on comparisons of metatranscriptomes retrieved from pelagic aerobic ammonium oxidation zones the central Baltic Sea (70-120 m depth) and quantification of the specific transcripts in them, we show that different sampling techniques significantly influence the relative abundance of transcripts presumably diagnostic of the habitat. *In situ* fixation using our newly developed automatic flow injection sampler resulted in an abundance of crenarchaeal ammonia monooxygenase transcripts that was up to 30-fold higher than that detected in samples obtained using standard oceanographic systems. By contrast, the abundance of transcripts indicative of cellular stress was significantly greater in non-fixed samples. Thus, the importance of *in situ* fixation in the reliable evaluation of distinct microbial activities in the ecosystem based on metatranscriptomics is obvious. Taken these results, this could also be the case in attempts aimed at an unbiased analysis of areas below the epipelagic zone, which cover 90% of the world's oceans.

NTP006

Will be presented as oral presentation with the ID NTV005!

NTP007
Live / dead discrimination of biofilm bacteria from a drinking water pilot distribution system

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Formation of biofilms in drinking water distribution networks, including pipelines of households and food industries, are of great concern. Biofilms are potential habitats for all kinds of bacteria, including pathogens, and may be responsible for contaminations of bulk water systems.
Nowadays, DNA-based methods are used for the detection and characterization of bacteria. One of the major disadvantages of these techniques is that they can not distinguish between DNA from live and dead cells. A battery of methods to face this problematic is presented in this work. Conditioned surface water disinfected with ozone/ClO_2 flowed through a pilot scale built up with different pipe materials for biofilm formation. Bacterial population analysis was done by PCR-DGGE, comparing direct samples (total DNA) and samples pre-treated with Propidium monoazide or DNase I (DNA from live cells). Shifts in the DNA patterns observed after DGGE analysis, demonstrated: (i) the applicability of PMA and DNase I treatment in natural biofilm investigation; (ii) detection of DNA from dead bacteria and eDNA was blocked by pretreatment with PMA or DNase I; and (iii) DNase I treatment demonstrated a clearer effect on live/dead differentiation. Traditional cultivation methods and qPCR completed the biofilm analysis.
The results of the bacterial population analysis and the results of the quantification methods that provide an overview of the different physiological states of bacteria: live cells, total amount of cells, and cultivable cells, are presented here.

NTP008

3D chemical and elemental imaging of the purple sulfur bacterium *Allochromatium vinosum* by STXM spectro-tomography

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The scanning transmission X-ray microscope (STXM) at the Canadian Light Source (covering 130 - 2500 eV) images the structure and quantitative distributions (maps) of chemical components for a wide range of samples at high spatial resolution (~30 nm). Recently, STXM spectro-tomography was developed to enable morphological visualization and quantitative chemical mapping in 3D. In this proof-of-principle experiment, spatial distributions of calcite, protein, and polysaccharide in the sulfur-oxidizing bacterium *Allochromatium vinosum* (cultivated in Pfennigs medium) were determined by STXM spectro-tomography at the C 1s and Ca 2p edges. The 3D chemical mapping shows that the sulfur globules are located inside the bacteria with a strong spatial correlation with calcite and polysaccharide, suggesting an influence of the organic components onto the formation of the sulfur and calcite deposits (resulting form the medium). In future, this new and innovative technique will allow more detailed insight into the cellular structure and will enhance our knowledge on sulfur globule formation and sulfur utilization by *A. vinosum.*

NTP009

Global transcription changes upon nutrient limitation in *Synechococcus* sp. strain PCC 7002

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Global transcription analysis in *Synechococcus* sp. PCC 7002 was performed by high throughput cDNA sequencing using the SOLiD™-3 sequencing platform. Transcripts were detected for nearly all of the 3,241 annotated ORFs of the model cyanobacterium *Synechococcus* sp. PCC 7002, with a dynamic range spanning more than five orders of magnitude. RNA was isolated from cells grown under limitation for five major nutrients: CO_2, nitrogen source, sulfate, phosphate and iron. As a basis for comparison, RNA was isolated and sequenced from cells grown under optimal („standard") conditions. A comparison of the relative transcript abundances of the nutrient-limited samples with those for standard conditions revealed that there were generally lower mRNA levels for genes involved in the major metabolic functions, especially protein biosynthesis, photosystems, phycobiliproteins, ATP synthesis and CO_2 fixation. Nutrient limitation further resulted in an increase in transcripts for the *nblA* gene, encoding the phycobilisome degradation protein NblA, which was most prominent under nitrogen limitation. Limiting the supply of a specific nutrient generally resulted in increased mRNA levels for genes encoding the corresponding uptake mechanisms, *i. e.*, transporters for nitrate, ammonia, phosphate, sulfate and iron. CO_2 limitation resulted in increased transcript levels for RuBisCO and carboxysomal proteins, *sbtA*, coding for a bicarbonate transporter, and the genes coding for the so-called inducible CO_2 uptake mechanism, which are related to the Type-1 NADH dehydrogenase complex. Transcriptional profiling further suggested that there might be additional changes in the NADH dehydrogenase complex subunit composition as a result of acclimation to nutrient limitation.

NTP010

A novel genetically encoded FRET biosensor for quantitative detection of oxygen in living cells

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Fluorescent reporter proteins (FPs) like the green fluorescent protein (GFP) from the jellyfish *Aequorea victoria* enable the non-invasive quantitative real-time analysis of complex cellular processes *in vivo*. However, a major drawback of GFP and its variants is their strict limitation to aerobic biological systems. This is primarily due to the fact that the autocatalytic synthesis of the fluorophore depends on molecular oxygen. Therefore, we recently developed a class of fluorescent proteins which can be used under aerobic as well as anaerobic conditions [(1,2,3)]. These FPs carry flavin mononucleotide (FMN) as fluorophore and are thus termed FMN-binding fluorescent proteins (FbFPs). Beside protein labeling, genetically encoded FPs can also be used as molecular biosensors allowing the online *in vivo* measurement of essential parameters or metabolites. For that purpose, two different FPs with overlapping emission/excitation spectra are generally fused together via a sensory linker peptide. Thus, the presence of a certain metabolite can be detected by a biosensor due to Förster Resonance Energy Transfer (FRET) which only occurs after its binding to the sensor domain.
Here, we present a novel FP-based biosensor that allows the detection of molecular oxygen for the first time. The biosensor consist of an oxygen-insensitive FbFP domain and an O_2-sensitive YFP domain. *In vitro* and *in vivo* characterization of the biosensor revealed that FRET from FbFP to YFP only occurs in the presence but not in the absence of oxygen. Therefore, the ratio of the fluorescence emission at 495nm (FbFP) relative to the fluorescence emission at 527nm (YFP) provides quantitative data of the intracellular oxygen levels during microbial growth.

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NTP011

To hear microbes settling down - online detection of microbial biofilm formation by means of acoustic Lamb waves

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Biofilms are a common mode of microbial life in natural as well as industrial and hospital environments. In the case of the latter, early detection of biofilm formation is pivotal in preventing men and machines from life-threatening and costly negative effects. Among the wealth of methods used to monitor biofilm formation, biosensors appear as attractive tools due to the speed of the detection process, suggesting a true online monitoring [1-2]. However, biosensor-based biofilm monitoring still suffers from several drawbacks. For instance, in the case of acoustics, traditional ultrasonic sensors fail in detecting biofilm formation due to the small differences in the acoustic impedance of the biofilm in comparison to water.
Here we present a new macroscopic acoustic approach, aiming at the detection of deposits on the bottom of liquid-filled tubes and containers by means of Lamb waves, i.e. elastic waves propagating in thin solid media such as plates or tubes [3]. Preliminary experiments with gelatine layers as a biofilm substitute proved the feasibility of this approach: Interdigital transducers attached to the outer wall of the liquid-filled container were used to produce and receive the acoustic signals. Signal transmission times and signal amplitudes of short Lamb wave pulses changed significantly with the thickness of the gelatine layer on a test surface and allowed for a reliable detection of layers thinner than 10 µm. Subsequently, a measurement cell equipped with such transducers was passed through with a culture medium inoculated with an overnight culture of biofilm-forming *Stenotrophomonas maltophilia* cells. After 16 hours of percolation at 30°C, cell densities had increased to 10^8 cells / ml. Signal transmission times and amplitudes between the interdigital transducers had changed notably in comparison to

an un-inoculated reference cell, probably due to formation of a biofilm on the bottom of the measurement cell. Biofilm quantification 48 hours after the onset of the experiment corroborated a strong biofilm formation in the measurement cell (10^8 cells / cm^2).
Following the encouraging data presented here, future work will include technical refinement of the sensor prototype and more experimental data acquisition to improve the correlation between biofilm formation and changes in the acoustic signals.

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NTP012
MALDI-TOF Mass Spectrometry as a Diagnostic Tool for Identification of Important Veterinary *Streptococcus Species*

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Streptococcus species and subspecies are known to be associated with infectious diseases of cattle, pigs, sheep, birds, horses, dogs, fish and aquatic mammals. The identification of *Streptococcus species* traditionally relies on the determination of biochemical properties, haemolytic reaction on blood agar and on serological grouping by use of Lancefield antisera. Several *Streptococcus species* are biochemically and serologically almost indistinguishable. During the last few years the use of matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) technique as a diagnostic tool for the identification of bacterial pathogens became more frequent. This technique allows the identification of microorganisms *as a result of* protein fingerprint analysis. In this study we used the MALDI Biotyper system (Bruker Daltonik) for identification and differentiation of 44 different *Streptococcus* field isolates and 17 *Streptococcus* reference strains. Field isolates were previously identified with biochemical and haemolytical tests and on serological properties. The bacteria used in this study included *S. agalactiae*, *S. canis*, *S. dysgalactiae*, *S. uberis*, *S. parauberis*, *S. equi* subsp. *equi*; S. *equi* subsp. *zooepidepidemicus*. A total of 56 (91.8%) isolates were identified to species level and five (9.2%) isolates from species *S. iniae* were not reliably identified. This was due to a lack of *S. iniae* in the database at this time. This species will be added to the database for further improvement of the system. Differentiation between *S. equi* subsp. *equi* and S. *equi* subsp. *zooepidepidemicus* is possible by MALDI-TOF MS, however, more strains have to be analysed for a validation of this finding. MALDI-TOF technique is a promising tool for identification of *Streptococcus species* and might help to clarify the streptococcal infections in different animals.

NTP013
A classification method for *Enterococcus faecalis* after stress using maldi-tof mass spectrometry and subsequent multivariate data analysis

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As demonstrated before, MALDI-ToF/MS in combination with multivariate data analysis represent a powerful tool for mass spectrometric pattern recognition of biological samples. We use this technique to classify the viable but not culturable (VBNC) stage as a survival state of bacteria caused by starvation and cold as well as their reactivation for cultivability in comparison to cells during exponential growth phase.
In this study *Enterococcus faecalis* was selected as model organism. The generated „molecular fingerprint" spectra were subjected to multivariate data analysis without targeting single bacterial molecules or molecule structures and were compared to the corresponding growth curve afterwards. *E. faecalis* was kept in dormancy state for 42 days and reactivated by incubation in BHI media at 37 °C at 150 rpm. After 3 h and 6 h, respectively, an aliquot of bacterial suspension was analysed by MALDI/Tof-MS. As a control, bacteria in the exponential growth phase were analysed. For an effective data analysis a multivariate approach using hierarchical cluster analysis and principle component analysis was applied in order to classify each state in comparison to each other. The mass spectrometric results were compared with those obtained by CFU (colony forming units) and Live/Dead staining.

NTP014
Time resolved protein-based stable isotope probing (Protein-SIP) analysis allows quantification of induced proteins in substrate shift experiments

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The detection of induced proteins after introduction of specific substrates in culture is of high interest for a comparative description of microorganisms growing under different conditions. In the past, protein expression changes were detected by the use of ^{35}S-methionine incorporation and the subsequent detection of labeled proteins by autoradiography. Later ^{35}S amino acid labeling was used in 2-DE studies which allowed a direct comparison of the protein pattern and the protein spot intensities. Other ways to detect quantitative changes in the proteome employ labeling with isotopically labeled amino acids (SILAC) [1].
In this study protein-based stable isotope probing (Protein-SIP) [2] is used for a fast and reliable detection of differentially expressed proteins in a substrate shift experiment. Stable isotope probing (SIP) is an established method in microbial ecology to identify metabolic key players in microbial communities using substrates labeled with stable isotopes, e.g. ^{13}C [3-5]. Besides the applicability for ecological studies Protein-SIP is now used to study protein expression of single cultures. Therefore, *Pseudomonas putida* ML2 cells pre-cultured on ^{12}C-acetate and ^{12}C-benzene, respectively, were incubated with ^{13}C-benzene as a stable-isotope labeled substrate.
Protein samples from early to stationary growth phase were separated by one-dimensional gel electrophoresis (1-DE), subsequently tryptically digested and analyzed by UPLC Orbitrap MS/MS measurements. Identified peptides from proteins involved in aerobic benzene degradation as well as from house-keeping proteins were chosen to calculate the labeling ratio (proportion of labeled protein on total protein) at different times. A comparison of parameters from a nonlinear regression analysis of the calculated data enabled a clear differentiation between induced (proteins from lower degradation pathway, e.g. catechol 1,2-dioxygenase) and constitutively (proteins from upper degradation pathway, e.g. benzene 1,2-dioxygenase) expressed proteins.
Thus, Protein-SIP has proven to be a valuable tool for quantitative analysis of induced proteins in substrate shift experiments.

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NTP015
Rapid identification of cyclic depsipeptides from micro-organisms by means of mass spectrometric techniques

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Rapid screening of secondary metabolites from micro-organisms can be carried out successfully by means of a combination of separation and

structure elucidation techniques such as the direct couplings of HPLC with PDA-MS and -MS/MS or by means of MALDI-TOF-MS and -TOF-TOF-MS, which enable high throughput screening for metabolite libraries. These tasks in combination with biological tests are an essential part of the entire biocombinatorial process for rapid screening of modified natural products.
Our results of the investigation of thousands of extracts from fungi and bacteria strains by using a combination of *on-line* and *off-line* chromatographic and spectroscopic methods showed that more than 30% of the identified metabolites are novel compounds.
This talk presents as examples only the rapid identification of novel biologic active cyclic depsideptides from *Fusarium* and *Xylariaceae* strains as well as from *Pseudomonas* bacteria associated with pathogenic *Phytophthora* species by means of MALDI-TOF-TOF, LC-ESI-Q-TOF-MS and -MS/MS as well as -H/D-Exchange-MS, -MS/MS and -Pseudo-MS^3.

NTP016
Studies on *Chalara fraxinea* infection process of ash plants - Direct and rapid detection of the pathogen *Chalara fraxinea* in plant tissue by means of mass spectrometric techniques

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Since several years, *Chalara fraxinea* has caused a large-scale decline of ash (*Fraxinus excelsior)* in Europe. The disease affects trees of various ages in natural as well as in artificial environments. Infected trees die rapidly independent of their age-class, irrespective of site conditions and regeneration methods. Therefore, EPPO had assigned this new invasive pathogen into its Alert List of potential threats.
Our tests (with different modes of artificial inoculation on 2-year-old ash seedlings) revealed that inoculation with conidia elicits the colonizing of the phloem of the whole shoot by the pathogen within a few weeks resulting in wilting of leaves, drying of buds of canopy, and culminating in death of plant. The course of disease was much stronger in the sample inoculated after flushing, whereas inoculation before flushing resulted in a slow disease outbreak with weak symptoms.
Investigation of cell extracts of *C. fraxinea* grown on MEA, CMA, and PDA by means of LC-MS or MALDI-MS showed a range of unknown secondary metabolites, especially the series of $[M+Na]^+$ at *m/z* 1133.7, 1175.7, and 1217.7 Da. The same metabolites were detected in the tissues of dead plants in nature or after artificial inoculation with the pathogen too, partly in high concentration. A transport of secondary metabolites of *C. fraxinea* or of the pathogen itself from the inoculation spot to plant roots was observed. The highest concentration was found near stem basis, root collar, and primary root whereas these metabolites were not found in segments above inoculation spot. No metabolites of *C. fraxinea* were detected in those plants which did not sicken after treatment with conidia of *C. fraxinea*, e.g. because inoculation occurred before flushing.
For the first time, the pathogen *C. fraxinea* is directly and rapidly detected *in-vitro* in tissues of diseased ash by means of MS techniques. Thus, these MS based *high-throughput-screening* methods can very effectively complete or replace the time consuming and expensive microbiological isolation procedures for detection of the pathogen *C. fraxinea* and can be used to rapidly test ash genotypes for resistance / susceptibility to *C. fraxinea* infection, respectively. They could also be verified in natural regeneration of an ash stand infected by the pathogen in the natural environment.

NTP017
Identification of Microorganisms of Veterinary Origin by MALDI-TOF Mass Spectrometry

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Identification of microorganisms of veterinary origin is usually done by using traditional culture depending and biochemical methods or by semi automated methods. These methods are time consuming, need a lot of consumables and laboratory staff has to be highly qualified. Faster, more reliable, automated and cost effective methods for identification and differentiation of microorganisms of veterinary origins will become more important. In this study we evaluated the MALDI Biotyper system (Bruker Daltonik) for identification and differentiation of 197 different isolates obtained from veterinary routine diagnostic (n = 125) and reference strains (n = 72). 121 field isolates were previously identified with morphological and biochemical tests, *e.g.* API test system. The bacteria used in this study included isolates of genera *Actinobaculum, Actinobacillus, Avibacterium, Bordetella, Brachyspira, Campylobacter, Clostridium, Corynebacterium, Cronobacter, Enterobacter, Enterococcus, Erysipelothrix, Gallibacterium, Histophilus, Janthinobacterium, Kluyvera, Mannheimia, Micrococcus, Moraxella, Nocardia, Ornithobacterium, Pantoea, Pasteurella, Proteus, Salmonella, Staphylococcus, Streptococcus* and *Yersinia*. A total of 169 (85.8 %) isolates were identified to species level, 14 (7.1 %) to genus level and 14 (7.1 %) isolates were not reliably identified. For further improvement of the system bacterial strains from the genera *Avibacterium*, *Brachyspira*, *Riemerella*, *Staphylococcus*, *Streptococcus*, and *Taylorella* will be added to the database. The present results show that MALDI-TOF MS is a fast and reliable automated method for identification of most species of veterinary origin.

NTP018
Modification of Extremozymes by Non-canonical Amino Acids

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Synthetic Biology offers a broad spectrum of techniques for *de novo* design or re-design of enzymes. In this context, genetic code engineering allows the residue-specific replacement of a particular canonical amino acid(s) at all positions in the protein sequence, with non-canonical one(s) without need for DNA mutagenesis [1]. Non-canonical (mainly synthetic) amino acids exhibit distinct features from canonical ones and usually endow substituted (i.e. congeneric) proteins with novel structural and functional features [2]. This concept was applied on the well characterized enzymes from extremophilic microorganisms such as lipase, amylase and cellulase. They are interesting candidates for these modifications, since they show catalytic activity at extremes of pH and temperature and tolerate high solvent concentrations. The lipase from the extreme thermophile *Thermoanaerobacter thermohydrosulfuricus* has been modified and the generated lipase congeners showed enhanced activation and significant shifts of optimal temperature and pH [4].
In this study, we introduce two synthetic enzymes from thermophilic microorganisms by expanding their amino acid repertoire. An α-amylase from the archaeon *Pyrococcus woesei* [5] and a cellulase from an anaerobic thermophile have been cloned and heterologously expressed in strains of *Escherichia coli*. Translation was reprogrammed by introducing different non-canonical amino acid analogs of methionine, proline, phenylalanine and tryptophan to the cultures. A complete substitution of methionine residues by L-norleucin, proline residues by (4-fluoro)proline and phenylalanine residues by D,L-(4-fluorophenyl)alanine was observed. The specific reactions of the obtained enzyme congeners were compared with the wild-type proteins and their substrate spectra. The contribution of synthetic modifications to enzyme activity, stability and efficiency will be presented and discussed.

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NTP019
Identification and metabolic activity of single cells simultaneously measured with NanoSIMS

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HISH-SIMS (Halogen In Situ Hybridization) allows quantitative measurements of microbes without cultivation and pre-selection [1].
Similar to the well known FISH (Fluorescent In Situ Hybridization) method the microbes of interest are selected with a gene-tag. Instead of a fluorescent dye a halogen-marker (e.g. fluorine) is used. In an isotopic enrichment experiment the nanoSIMS is then recording simultaneously element images of F and other isotopes of interest (12C, 13C, 14N, 15N ...) thus allowing identifying the microbes and measuring their metabolic activity with high precision and a lateral resolution down to 50nm.
This contribution shows application examples of the Exchanges of nitrogen and carbon in a dual-species microbial consortium, Ecophysiology of anaerobic phototrophic bacteria and others.
Secondary Ion Mass Spectrometry (SIMS) technique provides direct *in situ* measurement of elemental and isotopic composition in selected µm-size areas of the sample. Similar to a scanning electron microscope (SEM), a primary beam of reactive ions is rastered on the surface of the sample. The material sputtered by the primary beam is collected and mass filtered by a magnetic sector mass analyzer. Up to seven mass selected images of different elements or isotopes can be simultaneously recorded, originating from the exact same sputtered volume, ensuring reliable isotopic ratio and perfect image registration [2].

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OTV001
The C-terminal domain DUF1521 of the *Bradyrhizobium japonicum* protein and its functional stability

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Bradyrhizobium japonicum is known as soybean symbiont. The effector protein NopE1 is secreted via its type III-secretion system and exhibits autocleavage activity in the presence of calcium. NopE1 consists of 484 amino acids and contains two domains of unknown function (DUF1521), each comprising about 170 amino acids [1]. To characterise the minimal protein domain with autocleavage activity, deletion derivatives were created. This revealed that the minimal functional domain covers the 170 amino acids of the DUF1521. To test if this domain can be used as a self-cleaving linker, it was fused with a Strep tag at its C-terminal end and GST at the N-terminal end. The recombinant protein still exhibited self-cleavage after addition of calcium. Then, cleavage was analysed under different conditions. Cleavage took place at room temperature, on ice and partially at 60 ºC. After incubation for 20 min at 75 ºC, followed by incubation at room temperature, the protein still showed partial cleavage. Cleavage was also observed at a pH range from 4.5 to 9. These properties suggest, that NopE1 is useful for the development of a self-cleaving linker for biotechnology purposes. Further tests with alternative fusion partners are ongoing.

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OTV002
Culture Collections' Provision of Continuity for Academic and Industrial Research - Meeting the Emerging Challenges

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Microorganisms provide essential raw material for biotechnology - but to date less than 0.1% of the estimated number of species are described and available from culture collections to be harnessed by man. Microbial culture collections have to reconcile the interests of providers of living biological material (scientists, institutions, countries of origin) and the various kinds of recipients/users of cultures of microorganisms from academia and industry. Providing access to high-quality material, related data and scientific services while, at the same time, observing donor countries' rights (CBD-ABS), intellectual property rights, and biosafety/biosecurity aspects, poses demanding challenges. A similarly challenging task is to keep abreast of developments in taxonomy and systematics, as well as new methods for the authentication and identification, cultivation and maintenance of cultures. Recognising that these challenges are best met by collaborative work, collections organised themselves, e.g., in the European Culture Collection Organisation (ECCO). Before this background of understanding, a number of successful scientific-technical projects emerged (e.g. MINE and CABRI: agreed procedures on quality issues related to biological material and data; EBRCN: information documents to help implementing regulations; MTA: harmonising modes of supply of cultures; EMbaRC: training and research in microbial collection matters). In the Global BRC Network initiative, the partners will - in an era of globalisation - work toward common policies and principles with a view to existing and emerging legal frameworks and in-house procedures when handling living biological material. The recently launched ESFRI initiative MIRRI (Microbial Resources Research Infrastructure) will complement this global effort on the European level. Both latter initiatives take up common interests between culture collections and researchers to bring issues forward for discussion and initiate better interaction of the culture collections' and the scientific and biotechnology communities.

OTV003
Authenticity of Microbiological Material - The Impact in the Research Environment.

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The dictionary definition of science indicates that it relates to knowledge. In biology that knowledge is directly linked to testable explanations and predictions about the organisms we study. While much emphasis is now being put on adequate record keeping and archiving of data (obtained during the study of biological entities) in order to combat elements such as forgery or falsification of results, little attention is actually given to the fate of the objects that we actually study. Both the literature and databases are full of errors based on the incorrect/mistaken identity of the biological material under study. While in most cases the erroneous data has not been collected with malicious intent, the consequences are significant because they are not highlighted by scandals and they are often only identified by experts, with the broad masses often blissfully unaware of the problem. The consequences may well be incorrect interpretation of results that make reference to the incorrect data or significant financial effort being put into research either to verify or disprove the published data. Strangely little attention is given to very basic, simple aspects in research with microorganisms relating to the longterm storage and routine checking of the identity of strains used in research projects. In instances where data is published or deposited in databases the fate of the strains under study beyond the duration of the project or the career of the scientist involved is rarely considered by those who are providing funding. The problem is accentuated by the deceasing numbers of experts to cover the myriad of aspects of a hugely diverse range of organisms coupled with an exponentially growing dataset.

OTV004
Capacity Building, Transnational Access and Encouragement for the Deposit of Microbiological Material - the EU Project EMbaRC

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European Consortium of Microbial Resources Centers (EMbaRC) is an EU project funded under the Seventh Framework Programme Research Infrastructures (INFRA-2008-1.1.2.9: Biological Resources Centers (BRCs)) for microorganisms. It aims to improve, coordinate and validate microbial resource Center (MRC) delivery to European and International researchers from both public and private sectors. The EMbaRC project is a mixture of networking, access, training and research. To ensure

harmonisation of the quality of MRCs, EMbaRC plans to implement the current OECD best practice guidelines and emerging national standards for Biological Resource Centers (BRCs) at the international level. Outreach and training activities will enable not only the EMbaRC consortium but all European collections to operate according to the standards required to deliver products and services of comparable and consistent quality thus meeting customer expectations both present and future. The EMbaRC project takes European collection networking to new heights of coordination and efficiency providing new services and better access for users. The opportunity will be taken to work more closely with the user community. A one-stop access to the collections of EMbaRC and the wider European BRC community via a searchable web portal will be provided, building on the outcomes of the previous EU projects, CABRI and EBRCN, whilst adopting appropriate new IT technologies. Access and high-quality support and training to research teams are offered from the consortium partners via calls for access, enabling trainees to work in the partner facilities accessing staff, resources and technologies. The research part of the EMbaRC project will deliver new methods for strain and DNA preservation, novel techniques for identifying species and high throughput screening for enzymes of industrial interest. The networking elements will give better access to authentic microorganisms and validated associated data and provide a set of business models to increase self-sustainability of BRCs. This project creates the European node of the OECD envisaged Global Biological Resource Center Network (GBRCN) and brings together 10 European microbial resource Centers in 7 countries. Amongst its objectives is to ensure access to biological materials that underpin and validate published information either in journals or electronically, including sequence databases. It will work with the user communities, journal editors and research funders to put in place a strategy for the preservation of biological materials and associated information for the confirmation of results and for further study.

OTV005
The ABS protocol

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On the 30th October 2010, after 10 years of negotiation, the Nagoya Protocol on Access and Benefit Sharing was adopted by the Conference of the Parties (COP) of the Convention on Biological Diversity (CBD). The objective of this Protocol is to implement the principles imbedded in articles 15 and 8(j) of the CBD.
The primary goal of article 15 of the CBD is to facilitate access to biological resources because it is a prerequisite for their sustainable exploitation in knowledge-based bio-economy [1]. The other provisions of article 15 stipulate in what context and how facilitated access should be achieved. Article 8(j) focuses on the involvement of local communities, their role, contributions, and retributions in the CBD. These provisions must be translated in appropriate legal, administrative, and technical measures to secure access to raw biological material.
While the Protocol was eagerly awaited to fill a legal loophole, the final text was characterized by many as a masterpiece in creative ambiguity[2]. Instead of resolving outstanding issues by drafting balanced compromise provisions the contentious references were either deleted from the text or replaced by short and general provisions allowing flexible but also diverging interpretation, possibly too imprecise for univocal implementation.
To formulate more practical terms the COP has established an Open-ended Ad Hoc Intergovernmental Committee for the Protocol to undertake the necessary preparations to operationalize the Protocol. Depending on how these issues are addressed, the Protocol could become a powerful tool for a more balanced implementation of the CBD's three objectives[3].
Since the mission of culture collections is to provide facilitated access to fit-for-use (technically and legally) characterised microbiological resources, the World Federation for Culture Collections (WFCC) is concerned about the impact of the Protocol on research and therefore look for and work to contribute to its balanced implementation. The World Federation for Culture Collections pleads for a simple, cost effective and efficient multi-purpose system that integrates collecting, tracking, managing, and exploiting biological material as well as related information.

[1] Knowledge-Based Bio-Economy can be concisely defined as transforming life sciences knowledge into new, sustainable, eco-efficient and competitive products. New Perspectives on the Knowledge-Based Bio-Economy, Conference Report, European Commission, Brussels 2005. See also http://ec.europa.eu/research/biosociety/kbbe/basics_en.htm
[2] Earth Negotiations Bulletin Vol. 9 No. 544 Page 27 Monday, 1 November 2010
[3] CBD Article 1: *...the conservation of biological diversity, the sustainable use of its components and the fair and equitable sharing of the benefits arising out of the utilization of genetic resources, ...*

OTV006
Tools to implement the Nagoya Protocol on ABS in microbiology

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On 30th October 2010, after 10 years of negotiation, the eagerly awaited Nagoya Protocol on Access and Benefit Sharing was adopted by the Conference of the Parties (COP) of the Convention on Biological Diversity (CBD).
Legal and administrative measures taken to implement the Nagoya Protocol in activities of upstream and downstream research will impact on the transaction costs and therefore must be carefully explored and tested to avoid adverse effects.
Since the mission of culture collections is to provide facilitated access to fit-for-use (technically and legally) characterised microbiological resources, it is not surprising that several initiatives to translate proactively the rules into practices were taken in the culture collections community. The outcomes of these various initiatives represent a set of coherent and complementary tools to implement the ABS concept. Although developed before the Nagoya Protocol, they are still valid and relevant solutions for microbiologists. These solutions will be improved now that the Nagoya Protocol proposes a more precise legal framework to abide by. Main contributions of the culture collections to the practical implementation of the ABS concept are:
1. The Code of Conduct MOSAICC [1] (Micro-organisms Sustainable use and Access regulation International Code of Conduct).
2. The Material Transfer Agreement (MTA) [2].
3. The innovative concept of bundle of rights [3].
4. The combination of the World Data Center for Micro-organisms (WDCM) [4] database system with electronic markers called Globally Unique Identifiers (GUIDs).
5. The Straininfo.net [5] portal designed as an information broker.
6. The design of microbial commons [6] for the exchange of (micro) biological material which would provide basic common use principles for access to both material and information.
The World Federation for Culture Collections works towards the development of a balanced system incorporating these developments. Combining adapted legal concepts developed within existing legal framework, and IT tools imbedded in bioinformatics contribute to build safe, ethical and socio-economically balanced ABS processes at global level, via, among others, the Global Biological Resource Centers Network [7].

[1] See http://bccm.belspo.be/projects/mosaicc/index.php
[2] See recommendations about MTA in MOSAICC at http://bccm.belspo.be/projects/mosaicc/d/code2009.pdf and the core MTA model issued by the ECCO at http://www.eccosite.org/
[3] Dedeurwaerdere T.. Understanding ownership in the knowledge economy: the concept of the bundle of rights. BCCM News Edition 18 - Autumn 2005. See http://bccm.belspo.be/newsletter/18-05/bccm03.htm
[4] See http://www.wdcm.org and http://bccm.belspo.be/projects/mosaics/reports/files/ics_report.pdf
[5] See http://www.straininfo.net/
[6] See http://www.thecommonsjournal.org/index.php/ijc/article/view/215/144
[7] Visit http://www.gbrcn.org/

OTV007
Lipids – The fourth cornerstone in biological chemistry

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As further progress is being made in other areas of the biological sciences it is slowly becoming recognised that there are gaps in our knowledge. One of those gaps Centers around a diverse class of compounds that are collectively known as lipids. These compounds are largely hydrophobic or amphiphilic molecules and are best known for their role as structural components in cell membranes. In prokaryotes there is well-documented evidence, extending back half a century that the range of lipids found in prokaryotes is extremely diverse and it is impossible to state that any one lipid is universally distributed. In addition to that structural diversity it is also becoming clear that lipids are not just structural components but may serve other functions, such as in cell communication or sensory systems. The purpose of this overview is to provide a brief introduction to this topic and to set the scene for the remaining talks in this symposium.

OTV008

Structural analysis of the polar lipids of *Sphingobacterium spiritivorum* and *Pedobacter heparinus*.

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Examination of the polar lipids of *Sphingobacterium spiritivorum* and *Pedobacter heparinus* showed that they had features typical of the aerobic branch of the phylum Bacteroidetes, namely a single diglyceride based phospholipid and numerous non-digylceride based lipids. Mass spectrometric analysis of the isolated polar lipids of these two strains indicated that the majority of the lipids were derived from amino acids rather than glycerol, to which fatty acids were linked, either by an amide linkage or by direct condensation between the fatty acid and the amino acid. Data will be presented outlining the structures of the polar lipids in these two organisms.

OTV009

Short cationic antimicrobial peptides versus multidrug resistant bacteria

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Despite decades of intensive research, antimicrobial peptides (AMPs) have not yet revealed all their secrets; in fact, increasingly they are appearing to be more complex than previously imagined. In recent years, it has become clear that they are not only able to kill Gram-positive and Gram-negative bacteria, fungi, parasites and enveloped viruses, but can also alter immune response in mammals. It has been shown that short cationic AMPs can kill a broad range of multidrug resistant bacteria, indicating a different mode of action as the „classical antibiotics". This feature makes them an ideal candidate for novel antimicrobial drugs that can be used to treat infections with multidrug resistant bacteria.
Little is known about the sequence requirements of short cationic AMPs, especially for short peptides with a length between 9-13 amino acids. With help of our novel technique using an artificially created luminescence producing Gram negative bacterium and peptide synthesis on cellulose (SPOT technology), we investigated the sequence requirements of such peptides. Several thousands of peptides were tested for their ability to kill *Pseudomonas aeruginosa*. Complete substitution analyses of different indolicidin variants as well as a semi-random peptide library with about 3000 members were studied. The complete substitution analysis gave us information about the importance of each single position whereas the peptide library gave us broader information concerning which composition of amino acids resulted in an active antimicrobial peptide. The data is being analyzed using a different quantitative structure-activity relationship approach (QSAR) to A) increase the percentage of active peptides in a library (100000 peptides were screened *in silico*) with very complex descriptors and B) understand the rules by using simple descriptors that discriminate between active versus inactive. For the first time, we now understand the sequence requirements for short antimicrobial peptides.
One critical parameter for the success of such peptides as drugs is the stability in blood serum. Here we report an easy strategy to improve the half life time dramatically. In addition, we also added valuable information for a better understanding of the mode of action. The results of these measurements and analyses will be discussed in detail.

OTV010

Recombinant hydrophobin coated surfaces and their influence on microbial biofilm formation

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Biofilms represent a very successful symbiotic life form of microorganisms. They play an ambivalent role in industrial systems and can not be avoided on a great variety of surfaces. However, the characteristics of a material and its corresponding surface properties affect the biocompatibility and consequently bacterial adhesion and biofilm growth. In this approach recombinant fusion hydrophobins were used for surface modification. Hydrophobins are non-toxic and non-immunogenic fungal proteins which self-assemble on different surfaces into extremely stable monolayers in an amphiphilic manner. Recombinant hydrophobins provide the opportunity to use these surface-active proteins for large-scale surface modification of industrial and medical relevant materials.
Thus, protocols for surface coating with recombinant fusion hydrophobins were developed. Quartz crystal microbalance measurements were used to analyze the adsorption behaviour of the fusion hydrophobins. The hydrophobin coatings were characterized with water contact angle measurements, immunofluorescence microscopy and atomic force microscopy in terms of hydrophobicity and homogeneity. The self-assembly process of the recombinant fusion hydrophobins depended on the incubation temperature and the incubation time. Fusion hydrophobins are as well suited as natural hydrophobins for surface modification.
To test the possible application of hydrophobins for antifouling coatings, the growth behaviour of various microorganisms was studied on hydrophobin modified versus unmodified glass surfaces. Single bacterial strains as well as natural bacterial communities were used to analyse biofilm formation. Apart from conventional plating experiments, fluorescence microscopy and molecular-biological methods such as denaturing gradient gel electrophoresis were applied to determine differences in the biofilm growth. The results demonstrated that the change of surface hydrophobicity and the fusion hydrophobins itself did not affect the biofilm formation.
Due to their self-assembly properties, fusion hydrophobins can be used for effective large-scale surface coating in monolayer manner. To stimulate the effect on biofilm formation the hydrophobins can subsequently be functionalized with already bioactive molecules like antimicrobial peptides to influence the bacterial adhesion

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OTV011

Investigating membrane proteins *in situ* by cryo-electron tomography

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Cryo-electron tomography (CET) of pleomorphic microbiological objects provides unprecedented insight into the structural organization of native cells and complex macromolecular assemblies [1] and opens the way to identify and locate protein complexes and interacting macromolecules in their natural environment [2]. However, a number of technical restrictions limit the usable resolution to about 4 nm and impede the investigation of medium sized macaromolecules in the cellular context and of membrane proteins in particular.
We already improved the tomographical reconstruction of membranes, demonstrating the bilayer structure of mycobacterial outer membranes in intact cells [3]. Here, we present a strategy for investigating single membrane proteins that are embedded in lipid bilayers. Our approach includes improvements of the acquisition of tomographic data, the reliable determination and correction of the contrast transfer function of tilt projections, the classification, alignment and the averaging of subtomograms containing single membrane complexes. We used the mycobacterial outer membrane protein MspA (molecular mass 160 kDa) as a test molecule, reconstituted it in lipid vesicles, and reconstructed these by CET. The 3D model was considerably improved, revealed the lipid bilayer as expected, and allowed us to interprete structural details on a level of better than 1.5 nm.
The benefit of our approach is that it can be applied to single complexes that are embedded in lipid vesicles as well as to (thinned) vitrified cells without the necessity to artificially crystallize proteins two-dimensionally within membranes or to investigate the molecules in solubilized form.

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OTP001

Plasmid Profile and characteristics of Extended-spectrum Beta-lactamases Enzymes in *Pseudomonas aeruginosa* isolated from Intensive Care Units of Tabriz by PCR

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Introduction: Antimicrobial resistance in hospital pathogens is an important concern. Pseudomonas aeruginosa is one of the most common agents in nosocomial infection. Infections caused by bacteria producing extended spectrum beta lactamases enzyme (ESBLs) can enhance it, so they could be plasmid mediated resistant against beta- lactams. This study was conducted to assay ESBL-producing strains of Pseudomonas aeruginosa.
Methods: Samples from tracheal aspirate, urine, blood, bronchial aspirate, sputum, CSF, wound discharge, bone marrow and peritoneal fluid of the patients of 5 hospitals in Tabriz were taken. All of isolates were identified using conventional bacteriologic methods. They were tested for susceptibility and screening of ESBL-producing by Disk diffusion method and E-test, respectively. Plasmid DNA extraction was done by Kado and Liu technique. The presences of bla $_{CTX-M1}$, bla $_{CTX-M2}$ were studied by PCR.
Results: 240 ICU patients, infected by Gram-negative bacilli were studied. Pseudomonas aeruginosa was the second agents in nosocomial infection, 64(26.6%). The susceptibility test showed 22%, 98%, 70% 100% and 100% resistance against Amikacin, Cephoxitin, ceftriaxone, Tetracycline and cefuroxime. The Double Disk Test showed 3.2%, 0% and 3.3% resistance against Ceftriaxone, Cefotaxime, and Ceftazidime. The combined Test showed 36.5% positive result against Cefotaxime / Clavulanic acid and 32.5% against Ceftazidime / Clavulanic acid. By E-test 83.6 % of strains were ESBL-producing. 64.5 % of isolates harbored a single plasmid of 63kb. All of strains lacked either CTX-M-1 or CTX-M-2 gene to confirm the rule for *bla* $_{CTX-M}$.
Conclusion: Pesudomonas aeruginsa was one of the most prevalent bacteria. Highest and lowest rate of resistance was showed against Cefuroxime, Tetracycline and Amikacin. Our results showed that DDT test was not as sensitive as CT and MIC methods and no statistical significant difference was found between results of CT and MIC. Confirming no rules for suspicious genes by PCR, 78% of strains were founded as ESBL producer. Since the genes encoding theses enzymes are mainly located on plasmids, so transmission of the plasmids could disseminate the resistance in future, unless the consumption of cephalosporins are restricted and antibiotics such as imipenem substituded for the third generation cephalosporins, because these antibiotics, especially ceftazidim and ceftriaxone are strong inducers of ESBLs.

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OTP002

Diversity of Epiphytic and Endophytic Microorganisms in some dominant weeds

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A through study was conducted in May 2010 to assess the diversity in epi- and endophytic microorganisms from the local weeds. In the search of diversity and relationship of epi and edophytic microorganism, 46 fungal strains and 19 bacterial strains were isolated from the surface and the inner tissue of four dominant agricultural weeds. A combination of cultural methods i.e. Leaf wash and from homogenized leaf mixture solution respectively were used for the isolations from healthy leaves of four weeds *viz. Chenopodium album, Euphorbia helioscopia, Parthenium hysterophorus, Convolvulus arvensis*. Current study indicated that complex interactions existed between the host and their epi and endophytic microflora. Each weed has specific bacterial community with the reference of epi and endo phyllospere. The number and species of bacterial strains varied not only with their host weed plants but also in epi and endo phyllospere. Sørensen's *QS* of all tested weeds for the endophytic and epiphytic bacterial assemblages was 0.00 that indicated no species overlap/ similarity between the communities. Five fungal genera were common as epi and endophytes in all weeds samples: *Aspergillus* (56% of all isolates), *Drechslera* (10%), *Alternaria* (10%) *Penicillium* (6%) and *Cladosporium* (4%). Frequency of all five common genera differed significantly among weeds. It was also noted that entophytic fungal communities were not noticeably less speciose than epiphyte communities. Sørensen's *QS* of *Euphobia sp.* (0.23), *Chenopodia sp.* (0.37) and *Convolvulus sp.* (0.46) for the endophytic and epiphytic fungal assemblages was intermediate in the range (0.12-0.79) of previous studies. Although in case of *P. hystophorus*, the value for Sørensen's *QS* was 0.00 means no species similarity. The other identified genera were rare, such as *Absidia*, *Cuvularia*, *Phoma* and *Trichoderma.*

OTP003

Structure and molecular dynamics studies of the human anti-bacterial Dermcidin-channel (DCD)

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Human DCD is produced in sweat glands and secreted to the surface of the skin in order to protect humans from bacteria such as *Staphylococcus aureus* and others. The small 48 residues long peptide has been synthesized and crystallized in the presence of Zinc. The 3D architecture of the channel in solution is formed by six elongated, *a*-helical peptides with a strong charge distribution inside the channel and small hydrophobic residues pointing outwards (A,V,L). The internal symmetry is C3 due to the formation of the hexamer from a trimer of anti-parallel monomers. Six Zn-ions are bound at the ends inside the channel, three at each end, stabilizing two peptides in their anti-parallel arrangement. The length of the channel is 8 nm and slightly extends the diameter of a standard membrane. In molecular dynamics simulations, we observed that the channel adopts a tilted orientation in membranes to minimize the hydrophobic mismatch. Using our newly developed computational electrophysiology scheme, a conductance in the range of ~10 pS is predicted together with cation selectivity. Also, we observed unique ion entry and transfer mechanisms.

OTP004

The case of botulinum toxin in milk - experimental data

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Botulinum neurotoxin (BoNT) is the most toxic substance known to man and the causative agent of botulism. Due to its high toxicity and the availability of the producing organism *Clostridium botulinum*, BoNT is regarded as a potential biological warfare agent. Because of the mild pasteurization process, as well as rapid product distribution and consumption, the milk supply chain has long been considered a potential target of a bioterrorist attack. Since no empirical data on the inactivation of BoNT in milk during pasteurization, to our knowledge, are available at the present time, we investigated the activity of BoNT/A and BoNT/B as well as their respective complexes during a laboratory-scale pasteurization process. When we monitored milk alkaline phosphatase activity, which is an industry accepted parameter of successfully completed pasteurization, our method proved comparable to the industrial process. After heating raw milk spiked with set amounts of BoNT/A, BoNT/B or their respective complexes, the structural integrity of the toxin was determined by ELISA and its functional activity by mouse bioassay. We demonstrated that standard pasteurization at 72°C for 15 seconds inactivates at least 99.99% of BoNT/A and BoNT/B,

and at least 99.5% of their respective complexes. Our results suggest that if BoNT or their complexes were deliberately released into the milk supply chain, standard pasteurization conditions would reduce their activity much more dramatically than originally anticipated, and thus lower the threat level of the widely discussed BoNT in milk scenario.

OTP005
Synthesis and characterization of the bacteriocin produced by the *Enteroccocus* VL47 strain in the presence of prebiotics

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The probiotic cultures of lactic bacteria synthesizing bacteriocins are used in the food industry to obtain yoghurt and other dairy products. These peptides have a special practical importance due to the thermostability, which is demonstrated even for small concentrations, in case of peptides isolated by precipitation. This is one of the most important properties of the probiotic strains of lactic bacteria synthesizing bacteriocins. In order to obtain probiotic products, in addition to the capacity of synthesizing bacteriocins, the strains must adhere and colonize the intestinal tract.
The aim of the study was to determine the effect of the prebiotics on the synthesis of a bacteriocin, as well as to provide its partial biochemical characterization. The *Enteroccocus faecium* VL47 strain producing bacteriocins was used. *Bacillus cereus* CMGB 215, *Listeria innocua* CMGB 218 and *Escherichia coli* CBAB2 were used as sensitive strains. The synthesis of the bacteriocin was tested by using the MRS medium, even if the carbon source was replaced by other carbohydrates. In order to determine the prebiotics effect on the synthesis of the bacteriocin, MRS was supplemented by 1% prebiotic. The partial biochemical characterization of the bacteriocin was realized by determining the thermostability (at 60, 80, 100 and 121^{0}C, for 15 minutes), pH (2, 5, 7, 9, 11), enzymes (proteolytic and nonproteolytic) and organic solvents with a concentration of 10%. The partial purification of the bacteriocin was made by adding ammonium sulphate.
Due to the resistance to pH and T, the bacteriocin can be used to obtain products acting on the biological control of the human gut flora. The studies indicated that the used prebiotic influenced directly the inhibiting capacity. It was proved that lactulose with a concentration of 1% determined the maximum inhibiting capacity. The bacteriocin was still active in the presence of nonproteolytic enzymes.

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OTP006
Will not be presented!

OTP007
Virus elimination in the wastewater treatment plant of Herrenhausen in Hannover

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The total virus load of wastewater can be reduced during the wastewater treatment process in a municipal plant. In untreated wastewater an average of 1-80.000 infectious particles per litre is detectable [1]. The current research project is focussed on enhancing the efficiency of the virus elimination in activated sludge systems by adjusting the operating conditions (e.g.: sludge age, sludge loading, pH, temperature etc.) within the limits of maintaining the treatment performance. We were looking for an indicator organism which behaves like pathogenic viruses in wastewater, but is safe to work with in laboratories. Therefore we decided to use bacteriophages *φX174* and *MS2*, infecting *Escherichia coli* instead of human cells. In contrast to human pathogenic viruses bacteriophages can multiply in wastewater treatment plants (WWTPs), so it is necessary to determine their potential of growth to find out if they are suitable indicator organisms for our study.
For this reason, we examined the virus concentration in the effluent of the primary treatment step, in the activated sludge and the effluent of the clarifier. Measuring a concentration of $3{,}98*10^3$ PFU ml^{-1} in the primary treatment step and approximately $3{,}88*10^1$ PFU ml^{-1} in the effluent of the clarifier we observed a virus reduction of about two log levels within the plant. The concentrations in the effluent of the primary treatment were equal to those in the activated sludge. During rainfall events the PFU increased by one log level at all sampling points. The comparison of the host (*E. coli*) CFU with the CFU of all cultivable bacteria in the activated sludge indicated, that a multiplication of the phages should be possible, because of the sufficient *E.coli*-concentrations of ca. $1{,}04*10^4$ CFU ml^{-1}. Additionally the sensitivity of isolated *E. coli* strains from the activated sludge against *φX174* and *MS2* was determined. We observed that ca. 84% of the tested isolates were sensitive against at least one of the bacteriophages. Still both of these issues had no detectable effect on the overall concentration in the activated sludge system. Consequently this indicates that the chosen bacteriophages are suitable indicator organisms to represent the growth characteristics of pathogenic viruses in wastewater.

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OTP008
Recombinant *Clostridium acetobutylicum* expressing *Clostridium perfringens* enterotoxin (CPE) for treatment of pancreatic cancer

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The prognosis for patients diagnosed with pancreatic cancer is very poor. The five-year survival rate is less than 5 % and therefore for most patients only a palliative treatment is possible. Genetically modified clostridia offer a convincing potential for anti-tumor treatment. Clostridial spores only germinate in hypoxic regions of solid tumors. The selective tumor colonisation enables a specific delivery of reactive agents directly to their targets. *C. perfringens* enterotoxin CPE interacts with claudin-4 receptors, which are up to 1000fold overexpressed in pancreatic carcinoma cell lines. The binding of CPE to these receptors results in the formation of pores that finally cause cell death. An engineered *C. acetobutylicum* strain was able to produce and secrete the toxin into the surrounding medium. However, the level of production proved to be too low for therapy in an *in vivo* mouse model. Thus, an advanced CPE expression system for *C. acetobutylicum* is required. First, we tested a number of different clostridial signal peptides, but in *E. coli* the produced fusion proteins led to the death of the host. Therefore, the Tet-system was chosen as a potential expression system which is strictly regulated, so that in *E. coli* no fusion protein will be produced.

OTP009
Distribution and respiratory activity of bacteria in capillary fringes

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The capillary fringe (CF) is a commonly oligotrophic soil ecosystem, which originates from the capillary water suction above the groundwater level. For soil bacteria it offers a broad range of growth conditions, which differ in parameters like water saturation or oxygen concentration. In this study, experiments in thin glass chambers (Hele-Shaw cells, 20 x 20 x 0.2 cm), filled with silica sand or glass beads were conducted. The CF was generated by hanging the Hele-Shaw cell into a tray with bacterial suspension, so that the bacteria were transported into the CF via capillary forces. Three types of bacteria were used: *Pseudomonas putida* (motile), *Corynebacterium glutamicum* (non-motile), *Escherichia coli* (motile, gfp labeled for better visualization).
The tests revealed that the saturated/unsaturated interface region at 60 - 85 % water saturation offers best growth conditions for all bacteria types. Immobile bacteria and bacteria with a hydrophobic surface were not able to reach the upper end of the CF [1]. Cells were mostly suspended in the aqueous phase and only in the interface region attachment of cells to the

mineral surfaces as a biofilm (around 30 %) could be observed after 6 days. The cell density per CF volume significantly correlated with esterase activity (Pearson r = 0.77, measured with Fluorescein diacetate) or the fluorescence intensity (Pearson r = 0.91, measured with GFP-labeled *E. coli*). The respiratory activity of *P. putida* mainly depended on water and oxygen availability. At high water saturation, no oxygen was available for respiration and at low water saturation, below 7.5 % at the very top of the CF, not enough water seemed to be bioavailable. Furthermore the respiratory activity of cells grown on porous sand grains was always higher than of cells grown on smooth glass bead surfaces [2]. At sufficient nutrient supply the interface region in a CF acts as a barrier for oxygen diffusion towards the saturated zone: More oxygen is consumed by bacterial respiration and its diffusion into the water phase is limited.
Our results can help to improve models for biodegradation of organic pollutants or for vertical gas transport across the CF, which presumably is influenced by the activity of aerobic bacteria.

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OTP010

Will not be presented!

OTP011

Recombinant S-layer production induces disordered cell division in *E. coli* filaments

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The rod-shaped bacterium *Escherichia coli* is one of the best studied microorganism with a size of 1.1-1.5 µm x 2.0-6.0 µm. We used *E. coli* BL21 (DE3), one of the most widely used host in genetic engineering, for heterologous expression of surface layer (S-layer) proteins to enable fast and efficient protein production.
S-layer are proteins which cover the outermost of many prokaryotes and are probably the basic and oldest forms of bacterial envelope. These proteins are mostly composed of protein and glycoprotein monomers and have the ability to self-assemble into two-dimensional arrays on interfaces. Several characteristics like their work as molecular sieve, as virulence factor or the protection of the cell from toxic heavy metal ions make S-layer proteins interesting for their usage as ultrafiltration membranes, drug microcontainers, filter materials or patterning structures in nanotechnology.
Surprisingly, the heterologous expression of S-layer proteins of the uranium mining waste pile isolate *Lysinibacillus sphaericus* JG-A12 induced drastic morphological changes of *E. coli* BL21 (DE3) single cells to filaments and single cell enclosing tubes of >100 µm in length. The assumed secretion of tube-stabilizing S-layer proteins was investigated with SDS-PAGE and ß-galactosidase assay. These analyses result in a high S-layer appearance without significant ß-galactosidase activity in the supernatant and the periplasm. The origin and composition of filaments and tubes were analysed by membrane stain studies. We identified that filaments in the exponential growth phase form a continuous intracellular space without partitioning. To investigate the mechanism of filament and tube formation we analyzed GFP/S-layer expressing *E. coli* with DAPI-stain. The staining showed >50 µm long DNA-fibres that cross the filaments and „DNA-free" areas, the latter exhibiting strong GFP-expression. Our results point to a disordered cell division in *E. coli* filaments which is effected by recombinant S-layer expression.
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OTP012

Insights into the active site of the nitrogenase MoFe protein

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Biological nitrogen fixation is an essential process that transforms atmospheric dinitrogen (N_2) into a bioavailable form, ammonium (NH_4^+). This process is catalyzed by the enzyme system nitrogenase, a complex of two metalloproteins that forms under turn-over conditions. The two components of the complex are the Fe- and MoFe-proteins. The MoFe-protein from *Azotobacter vinelandii* is a 230 kDa $\alpha_2\beta_2$-heterotetramer that contains two types of metal centers, the P-cluster [8Fe:7S] and the FeMo-cofactor [7Fe:Mo:9S:X:homocitrate] per αβ-heterodimer [1)]. The FeMo-cofactor marks the active site of the enzyme and is the most complex metal center known in nature so far. Due to its complexity, the reaction mechanism is not known in detail [2)]. High resolution X-ray data of the MoFe-protein revealed the presence of a ligand (X = C, N or O) in the center of the FeMo-cofactor [3)] which is masked by the unique metal environment in X-ray structures solved at lower (> 1.55 Å) resolutions, but which is of vital importance for understanding the mechanism of catalysis. Due to the limited feasibility of X-ray diffraction to discriminate between light atoms, a combined approach between Electron-paramagnetic-resonance (EPR) and high-resolution X-ray crystallography is explored. The crystallographic refinement at < 1.1 Å as well as $^{12}C/^{13}C$-electron nuclear resonance spectroscopy provide new insights into the nature of the cofactor and the character of the central atom.

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OTP013

The special type IV secretion system of *Neisseria gonorrhoeae*: Biochemical characterization of the novel relaxase TraI and the coupling protein TraD

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The human pathogen *Neisseria gonorrhoeae* causes the sexually transmissible disease gonorrhoeae. Approximately 80 % of the clinical isolates of *N. gonorrhoeae* contain a Gonococcal Genetic Island (GGI) which encodes a remarkable type IV secretion system (T4SS) [1, 3]. However the gonococcal T4SS differs from other known T4SS in the way that single stranded chromosomal DNA is secreted into the environment [3]. The secreted DNA can be taken up via natural competence and can be integrated into the chromosome. The high transformation frequency of *Neisseria* leads to a wide spread of genetic information and results in an increase of antibiotic resistance.
T4SSs consists of a membrane spanning complex through which the substrates are secreted. Substrates are targeted to this complex via the coupling protein, a hexameric ATPase. In conjugative T4SSs, the transported DNA is initially cleaved at the *oriT* by the relaxase protein that stays bound to the DNA, and is then transported to the recipient cell.
Remarkably, the neisserial relaxase TraI belongs to a novel family of relaxases. Besides typical relaxase features this family is characterized by special sequence motifs: i) a conserved HD domain, ii) an alternative 3H motif, and iii) a C-terminal DUF1528 domain. These relaxases can be found in Genetic Islands (GIs) as well as in conjugative plasmids and Integrative and Conjugative Elements (ICEs) [2, 4].
To date, the DNA processing mechanism and the targeting mechanism of this large and novel relaxase family has not been characterized. We set up a biochemical approach to characterize the relaxase TraI and the coupling protein TraD to gain insights into the mechanism of the special T4SS of *N. gonorrhoeae*. Both proteins were overexpressed and purified, and here we report a initial biochemical characterization of these proteins.

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OTP014
Interplay between zinc uptake and efflux systems mediates zinc homeostasis in *Cupriavidus metallidurans* CH34

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Cupriavidus metallidurans is adapted to high concentrations of transition metal cations. This bacterium harbors a variety of transition metal efflux systems. Central to metal resistance is the CzcCBA transenvelope protein complex, which probably transports cations from the periplasm directly to the outside. Overexpression of *czcCBA* led to zinc auxotrophy in mutant cells devoid of the zinc importer ZupT. Expression of *zupT* was studied by reporter gene fusions and compared to that of other secondary zinc uptake systems. Production of ZupT and the other systems was regulated by zinc availability. Deletion of *zupT* but not of the other systems led to decreased EDTA (ethylenediaminetetraacetate) resistance and decreased ability to acquire zinc in the presence of EDTA. Thus, prominent function of ZupT is to transport zinc from the periplasm to the cytoplasm under conditions of low zinc availability, such as decreased periplasmic zinc concentrations due to the action of the CzcCBA efflux complex. This provided another piece of evidence in favor of outer membrane efflux (from the periplasm to the outside) as main function of the CzcCBA complex.

OTP015
Assembling Next Generation reads and subsequent genome analysis with BioNumerics

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Next generation sequencing has considerably increased the data throughput. High performance bioinformatics systems are required to process the vast amounts of data generated.
The computationally challenging problem of assembling up to millions of reads is met by the Power Assembler, an assembly pipeline tool in the BioNumerics software, for managing high throughput sequence data. The features and possibilities of this tool will be illustrated using publicly available sequence reads from bacterial genomes.
A power assembly project is essentially a series of actions, which together constitute a project pipeline. Besides a set of predefined actions for frequent manipulations, there is also the possibility to construct user-defined actions. The project results can be overviewed in summarizing reports, and represented as sequence curves displaying e.g. coverage or sequence quality information, summary graphs, or in an assembly view. There is an information flow path from the Power Assembler to the underlying BioNumerics database, allowing further analyses of the resulting contig sequences.
The Chromosome Comparison module allows full genome comparisons and clustering for evolutionary and population genetic studies to be calculated. Both DNA-based chromosome comparisons and CDS-based chromosome comparisons can be performed. Moreover, the annotation of new genomes, mutation analysis and gene selection, and chromosome-wide comparisons can be performed to study the organization and structure of genomes.

OTP016
Fecal indicators in particles of swimming pools

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Particles in public swimming pools can contain pathogenic microorganisms. Therefore, a German standard for bathing water (DIN 19643) sets a minimum circulation volume to be treated by particle removal. Moreover, a minimum chlorine content of 0.3 mg/L in the pool water is fixed to avoid infections by waterborne pathogens. For a revision of the standard DIN 19643, it is planned to reduce the required minimum circulation volume by a factor of 2 in case that ultrafiltration is implemented for particle removal.
To analyze, if particles in swimming pools are really hygienically relevant, the water from swimming pools was investigated in a worst case situation on a hot summer day by filtering volumes of 5L up to 150L for particles > 1 µm. The water itself contained free chlorine in concentrations of more than 0.5 mg/L. The particles were then analyzed for the presence of the indicator bacteria E. coli and coliform bacteria. In 5 of 5 samples coliform bacteria were detected, in 4 of 5 samples also E. coli could be detected. Thus, E. coli could survive in particles despite the presence of 0.5 mg/L free chlorine.
Besides these fecal indicators also pathogenic microorganisms can be present in such particles. Therefore a reduction of the minimum flow through in the circulation has to be seen critically as it increases the turbidity and the concentration of particles in the pool water. As a result, also the concentration of potentially pathogenic microorganisms will considerably increase.

OTP017
Tumor specific promoters of *Salmonella enterica* serovar Typhimurium

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Cancer is the second most frequent cause of death in the industrialized world and still conventional therapies are often limited in effectiveness and exhibit strong side effects. Therefore alternative therapeutic strategies are demanded. The administration of tumor-colonizing bacteria that exert anti-cancer effects is a promising approach that is under increasing investigation since several years. *Salmonella enterica* serovar Typhimurium is one such bacterium and has been used in many animal tumor models as well as in first clinical studies already. It has an inherent tumoricidal effect that should be improvable by using *S*. Typhimurium as a vector to deliver therapeutic agents. In this context, bacterial expression has to be restricted to the tumor to prevent toxic substances to harm healthy tissue. Therefore, an *S*. Typhimurium promoter trap library has been screened in order to define promoters that exclusively drive expression in the tumor tissue. 12 such promoters could be found that show reporter gene expression in tumor but not in spleen and liver. In addition, a sequence motif has been identified that appears to be necessary for the specificity of expression. Now, these tumor specific promoters can be used to express therapeutic proteins in tumor-colonizing *S*. Typhimurium.

OTP018
Structural insights into the activation mechanism of bacterial di-heme cytochrome *c* peroxidases

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Bacterial cytochrome *c* peroxidases (Ccps) are detoxifying enzymes that protect the cells from reactive oxygen species by reducing hydrogen peroxide to water. These proteins are located in the periplasm and show a conserved structure with two domains containing a high potential (HP) electron transfer heme group and one low potential (LP) catalytic heme group. The HP heme group is coordinated by a methionine and a histidine residue whereas the LP heme group shows a *bis*-histidinyl coordination[1]. With the exception of the constitutively active *Nitrosomonas europaea* enzyme a reduction of the HP heme group is required for the activation [2].

This reduction leads to a significant rearrangement of three distinct loops, resulting in an accessible catalytic site [3]. The dissimilatory metal-reducing bacterium *Geobacter sulfurreducens* possesses two genes with sequence homology to bacterial Ccps, whose expression increases dramatically under oxidative stress [4]. The proteins were isolated and crystallized after heterologous expression in *Escherichia coli*. Additional biochemical characterization confirmed peroxidase activity. For a better understanding of the reaction mechanism we created several variants of these two proteins which on one hand mimic critical regions of the *Nitrosomonas europaea* enzyme and one the other hand differ with respect to the ligands of the two heme groups. The crystal structures of these variants provide new insights into the mechanism of bacterial Ccps [5,6].

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OTP019
Characterization and crystallization of YhjA, a predicted cytochrome *c* peroxidase from *Escherichia coli*

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The *yhjA* gene of *Escherichia coli* encodes a putative cytochrome *c* peroxidase (CCP), a protein containing 3 heme groups, with a molecular weight of 53 kDa. The heme groups are covalently attached to the protein chain via two thioether bonds. Cysteine residues occur in the amino acid sequence as a CxxCH heme binding motif. It is known from previous work that the expression of the *yhjA* gene is regulated by the oxygen-sensitive transcription factor FNR and the regulator OxyR [1]. Thus YhjA probably serves to protect the cell against reactive oxygen species (ROS) and acts as a cytochrome *c* peroxidase, by reducing hydrogen peroxide to water [2]. The amino acid sequence shows a high similarity to known diheme CCPs, such as MacA and CcpA from *Geobacter sulfurreducens* [3]. *Aggregatibacter actinomycetemcomitans* contains a homologus triheme cytochrome *c* that catalyzes the peroxidation reaction in the respiratory chain and uses quinol as the physiological electron donor, but this activity could not be detected for YhjA [4].
For the isolation of the gene product it was necessary to express YhjA together with the cytochrome *c* maturation system (ccm) of Escherichia coli, which is encoded by the plasmid pEC86.[5] This system is physiologically active only under anaerobic conditions, but was placed under the control of a constitutive *tet* promoter, allowing for cytochrome *c* expression under aerobic conditions. YhjA shows a low peroxidase activity with $ABTS^{2-}$ [2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)] as an electron donor. The protein could only be crystallized in its reduced state under anaerobic conditions, which points towards conformational changes between the oxidation states, that may be required to activate the enzyme.

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OTP020
Incorporation of the prosthetic heme group into cytoplasmatic and membrane proteins

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Modified tetrapyrroles are complex macrocycles and the most abundant pigments found in nature. They play a central role in electron transfer-dependent energy generating processes such as photosynthesis and respiration. They further function as prosthetic groups for a variety of enzymes, including catalases, peroxidases, cytochromes of the P450 class and in sensor molecules. Heme is a hydrophobic molecule and associates non-specifically with lipids and proteins in aqueous solution where it promotes peroxidations. Due to its hydrophobicity und toxiticity, heme has to be transported to its target proteins by different mechanisms, e.g. transport by transmembrane proteins, heme binding proteins and heme chaperones.
The aim is to identify heme-binding and/or heme-transporting proteins /*in vivo*/ using the */P. aeruginosa/* Bacterial Adenylate Cyclase Two-Hybrid system. The interaction between the probable candidates for heme-binding and/or heme-transport and their target proteins are then further analysed by /in vitro/ translation and further more assays.
A */Lactococcus lactis/ Δ/hemW/* mutant showed accumulation of free heme and failed to respire upon hemin supplementation. Further it was possible to complement a */E. coli/ Δ/yggW/* mutant with */Lactococcus lactis/ hemW/*. To verify that */E. coli/ /yggW/* is a heme-transporting protein, the */E. coli/ Δ/yggW/* mutant was physiologically characterized. The incorporation of a transporter provides the opportunity to monitor the ability of respiration upon addition of heme and its derivatives.

OTP021
Characterization of the electronic properties of the nitrogenase Fe protein [1-3]

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Biological nitrogen fixation is carried out by the enzyme complex nitrogenase. It consists of two metalloproteins, the MoFe protein and the Fe protein. Whereas the MoFe protein is involved in substrate reduction of nitrogen to ammonia, the Fe protein is the physiological electron donor for the MoFe protein. The MoFe protein contains the active site, a molybdenum-iron metal cluster. The Fe protein is a homodimer with a molecular mass of 64 kDa and contains one [4Fe-4S]-cluster. Mechanistic understanding of the reduction of the MoFe protein by the Fe protein depends on the elucidation of the distinct oxidation states of the Fe atoms of the [4Fe-4S]-cluster. Due to the oxygen sensitivity of the protein, an anaerobic purification strategy of the wild-type enzyme from *Azotobacter vinelandii* was established that yields high amounts of protein of high purity. Crystallization of the protein provides the basis for performing X-ray diffraction, single crystal electron paramagnetic resonance (EPR) spectroscopy and a combination of X-ray diffraction and X-ray absorption spectroscopy (XAS) [4] to gain a more detailed insight into the electronic structure of the [4Fe-4S]-cluster. Refining the structure at high resolution in combination with single crystal EPR spectroscopy offers the possibility to correlate the cluster orientation with its g-tensor. XAS offers the opportunity of assigning different oxidation states of the Fe atoms in the [4Fe-4S]-cluster. Combining these techniques may provide new insights into the Fe protein being the unique electron donor for the MoFe protein.

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OTP022

c-type cytochromes from *Geobacter sulfurreducens* as model system for spectroscopic studies

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The genome of the δ-proteobacterium *Geobacter sulfurreducens* includes 111 genes encoding for *c*-type cytochromes that contain between one and 40 heme groups [1]. From these, three small-size cytochromes are chosen as model systems to determine the orientation of heme groups by single-crystal EPR spectroscopy. The selected *c*-type cytochromes from *Geobacter sulfurreducens* are OmcF [2], DHC2 [3] and MacA [4]. A single heme group yields a distinct axial signal in the EPR spectrum. Here we present a new approach for detecting multiple heme group arrangements by monocrystal EPR spectroscopy.
OmcF is an 11 kDa *c*-type cytochrome which contains one single heme group and serves as a reference system. The 11 kDa diheme *c*-type cytochrome DHC2 contains the conserved parallel heme-packing motif. The iron atoms have a short distance of 9.4 Å and thus are expected to be engaged in magnetic coupling. The third model system is the 35 kDa diheme cytochrome MacA containing two domains with one heme group each. The heme iron atoms have a distance of 21 Å and the orientation of the porphyrin plains in MacA is virtually perpendicular.
These model systems can be used as a general basis for further studies on cytochromes containing multiple heme groups in more complex structures.

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OTP023

Inhibition of quorum sensing in *Pseudomonas aeruginosa* and *Serratia marcescens* by a staphylococcal compound

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Quorum sensing is a process of bacterial communication, which is using secreted membrane-diffusible signaling moleculars called autoinducers to regulate intercellular interaction. This process plays critical roles in regulating various physiological activities, including production of antibiotics, secretion of virulence factors, formation of biofilms, swarming motility, bioluminescence, sporulation as well as symbiosis. Similarly, it is found that various bacteria are able to secrete compounds for inhibiting, inactivating or stimulating quorum sensing signals in other bacteria. In our previous study on coinfection of *Staphylococcus* and *Pseudomonas aeruginosa*, we observed that *P. aeruginosa* could repress the growth of pathogenic staphylococcal *species* but not of nonpathogenic staphylococcal *species* by respiratory inhibitors. Meanwhile, to our surprise, two strains of the nonpathogenic staphylococcal *species* exhibit unknown compound X to interrupt the function of quorum sensing-controlled factors in gram-negative bacteria, such as the red prodigiosin pigment in *Serratia marcescens*, the blue-green pyocyanin pigment and biofilm formation in *P. aeruginosa.* Physical analysis using XAD-16 resin demonstrated that the molecular weight of compound X is below 2 kDa. Moreover, compound X resists alkaline and acid pH, high temperature and proteinase K treatment, which might exclude compound X as a peptide. However, the mechanism of compound X expression is still unknown since it is independent of the growth temperature, oxygen, NaCl and glucose concentration in the medium. In further study, not only purification and identification of the compound X using high-performance liquid chromatography (HPLC) and mass spectrometry (MS) are essential. It also needs to indentify the corresponding genes by transposon mutagenesis or cloning random chromosomal DNA of compound producing staphylococcal stain into a nonproducing strain. In the end, investigation of how compound X disrupts the quorum sensing signaling system in gram-negative bacteria would be an important and interesting issue for new generation of antibiotics.

OTP024

Protein-Protein Interactions in PHB-Metabolism of *Ralstonia eutropha* as Revealed by Two-Hybrid Analysis

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Question: *R. eutropha* is the model organism for studying metabolism of poly(3-hydroxyalkanoates) (PHA). Recently, it was found that more polypeptides are present on the surface of PHA-granules in *R. eutropha* and in *Pseudomonas putida* than would be essential for PHA-synthesis [1, 2]. However, little is known whether and how PHA granule associated proteins interact with others proteins. A bacterial two-hybrid system was used to study protein-protein interaction in *R. eutropha*.
Methods: The bacterial adenylate cyclase-based two-hybrid system (BACTH) was used that is based on genetic fusions of two putative interacting proteins with two complementary fragments (T25 and T18) of *Bordetella pertussis* adenylate cyclase. An association of the two-hybrid proteins *in vivo* results in functional complementation between T25 and T18 fragments and leads to cAMP synthesis in an adenylate cyclase deficient reporter strain [3].
To study protein-protein interactions in *R. eutropha* the genes for several proteins involved in PHB-metabolism including PHB-synthase (PhaC1), β-ketothiolase (PhaA), acetoacetyl-CoA-reductase (PhaB), phasins (PhaP1-P4), regulator of phasin expression (PhaR) and PHB-depolymerase (PhaZa1) were cloned into the two-hybrid vectors pUT18C and pKT25 and the corresponding plasmids were co-transformed in the reporter strain *E. coli* BTH101. The efficiencies of interactions between different hybrid proteins were analyzed by colour formation on LB X-Gal and MacConkey maltose/lactose medium and quantified by determination of β-galactosidase activity in liquid cultures.
Results: In this study two-hybrid experiments using nine different proteins of *R. eutropha*, with particular functions in PHB metabolism were performed. Nearly all tested proteins showed a more or less intense ability for homo-oligomerisation. A strong interaction was found between phasin PhaP2 and phasin PhaP4 and other phasins as well as moderate interactions between PHB-depolymerase PhaZa1 and phasins. PHB synthase PhaC1 apparently did not significantly interact with any of the phasins.
Conclusions: Our data indicate that PhaP2 and other phasins play an important role in the spatial organisation of PHB granule associated proteins and that PHB depolymerase PhaZa1 and phasins may influence each other.

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OTP025

Escherichia coli growth and biofilm development are influenced by secreted metabolite products of other *Enterobacteriaceae* species

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In nature, microorganisms are predominantly associated with surfaces and organized in communities known as biofilms. Most of them are complex consortia of different microbial species, where complicated interstrain and interspecies interactions exist. They are often mediated by metabolite products secreted in the environment, including exopolysaccharides (EPS), proteins, etc. Autoinducers (AI), used for cell-to-cell communication, are involved also. All these metabolites are components of biofilm matrix which is a defining feature of microbial biofilms and contributes to their resistance. This study aims at the examination of the effects of sterile late stationary-phase supernatants, containing secretory metabolite products by *Enterobacteriaceae* species (SMP-E), on growth and biofilm development of *E. coli*.
Methods: Eight strains *E. coli* K-12 were tested. Sterile supernatants were isolated from *E. coli* K-12, *E. coli* O157, and *Yersinia enterocolitica*. 96-well microtiter plate assay was applied for estimation of the effect of SMP-E on bacterial growth and biofilm development. The presence of AI-2 in the supernatants was detected using *Vibrio harveyi* bioluminescence assay. The production of the EPS colanic acid (CA) and poly N-acetyl-D-glucosamine (PNAG) was registered by Enzyme-linked lectinosorbent assay. Comparision of the effect of the supernatants and the crude EPS, extracted

from them, was done also. After proteinase K treatment of the supernatants, the influence on their biofilm-modulating capacity was examined.
Results: *E. coli* K-12 strains W1655, 420, and 406 (named as first group strains), produce significantly greater biofilm biomass when grown in M63 medium, supplemented with spent media from the late stationary-phase cultures tested. What is more, strains that do not produce detectable amounts of biofilm in pure M63 medium, like *E. coli* 421 and 446 (named as second group strains), are stimulated for sessile growth by the spent culture media. Only *E. coli* K-12 strains 409, 8341 and W3110, produced no biofilm under the tested experimental scheme. Further, considering the stimulation effect of the supernatants in five of the strains, the separate components of the matrix were examined. The presence of AI-2 in the supernatants suggests cell-to-cell communication between the *Enterobacteriaceae* species. Individual *E. coli* strains showed different response to SMP-E regarding the production of CA and PNAG. The crude EPS, extracted from the supernatants, stimulate biofilm-forming capacity of the first group strains and reduce the influence on the second group strains. In the absence of proteins the stimulating effect is reduced also.
Conclusions: Most of *E. coli* K-12 strains were stimulated by SMP-E. Determination of the components of biofilm matrix and their role in biofilms building will facilitate finding of appropriate inhibitors of bacterial growth in biofilms.

OTP026
Novel application of nitrifying bacterial consortia to ease ammonia toxicity in ornamental fish transport units: Trials with zebrafish

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In ornamental fish trade though it is imperative to supply the customer with high quality live fish, there has not been any concerted effort to improve the transport systems in order to keep the stress factors to a minimum and improve the survival of the fish. During the transportation the accumulation of metabolic wastes from the fish, mainly ammonia - that is responsible for the deterioration of water quality - may cause stress and possibly lead to mortality. Converting this ammonia to less harmful nitrate through the process of nitrification with the help of extraneous nitrifying bacteria may serve as a novel bio-control procedure. Therefore in this study two commercial nitrifying bacterial consortia were applied in the transport systems in order to understand their capacities to reduce the accumulating ammonia. Zebrafish were employed in replicate 72-h experiments, conducted in simulated fish holding units, at densities of 25 fish per liter. The process of nitrification with and without the test consortia was compared based on the water quality parameters and the community structures of ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB).
The nitrifying bacterial consortia tested significantly improved the nitrifying activity that facilitated the removal of the ammonia accumulated in live fish transport systems. The diverse AOB and NOB populations observed could be related to the difference in nitrifying activity. These results imply that the use of nitrifying bacterial consortia during the transportation of ornamental fish could profoundly improve the water quality by containing the ammonia accumulation, thereby possibly reducing stress to fish and improving their survival. Thus exploiting this bioremediation practice for the benefit of ornamental fish could improve the fish welfare.

OTP027
Mode of action of human β-defensin 3 (hBD3)

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Host-Defense-Peptides (HDPs) are important effector molecules of the innate immune system in multicellular organisms. They represent small, cationic and amphipathic peptides that display - in addition to their immunomodulatory functions - direct antimicrobial activity against both Gram-positive and Gram-negative bacteria, fungi and even certain enveloped viruses.
Among these antimicrobial peptides the defensins are an important HDP family characterized by disulfide-stabilized β-sheets as a major structural component (Hancock & Lehrer, 1998; Lehrer & Ganz 2002; Zasloff, 2002).
We have studied the mode of action of human β-defensin 3 (hBD3) against *Staphylococcus aureus* and *Escherichia coli*. It is generally assumed that HDPs act rather unspecifically by permeabilising the cell membrane than via a defined target.
A series of *in vivo* and *in vitro* cell wall biosynthesis assays demonstrated that lipid II is a molecular target for this highly cationic peptide (net charge +11) and that inhibition of cell wall biosynthesis is a major determinant of its mechanism of action against staphylococci.
Further experiments indicate that hBD3 is able to permeabilize both the outer and the inner membrane of *E. coli* and that the susceptibility towards hBD3 depends on the composition of lipopolysaccharides.

OTP028
The effect of CdTe-TGA Quantum dots on bacterial viability

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Quantum dots (QDs) are semiconductor nanocrystals (2-10 nm) with unique and size-tunable optical properties such as narrow and symmetrical emission spectra and high fluorescence quantum yields in the visible and near-infrared spectral region. *In vivo* imaging of cellular processes with QDs like CdTe is well established, primarily for eukaryotic cells. QDs toxicity, as with other nanoparticles, depends on multiple parameters, including size, charge, shape and surface functionalization. However, relatively little is known about the toxicity of QDs, especially for bacteria. The aim of the present study is thus to assess whether QDs are toxic to bacteria and to elucidate the mechanism of QDs toxicity in bacteria.
Toxicity of CdTe-QDs, functionalized at their surface with thioglycolic acid (TGA) molecules, was investigated by growth experiments with *Escherichia coli* DH5α. It was shown that QDs started to interfere with the growth of *E.coli* DH5α in a dose-dependent manner at a concentration of 10 nM. Further analysis demonstrated that Cd^{2+} does not act as the main causative agent of QDs toxicity and indicated that toxicity is not caused by the small size (nanotoxicity) of the particles, but by the release of tellurite formed upon QDs oxidation. We are currently investigating how tellurite is entering the *E. coli* cells.

OTP029
Characterization of the biosynthetic pathway of thienodolin in *Streptomyces albogriseolus* MJ286-76F7

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Thienodolin is a secondary metabolite isolated from a culture broth of *Streptomyces albogriseolus* MJ286-76F7 in 1993 that showed a plant growth-regulating activity and is able to reduce the damage to rice plants caused by some herbicides [1, 2]. Structurally, thienodolin has a thieno-indole skeleton and it is chlorinated in the 6-position of the indole ring. A tryptophan 6-halogenase gene (*thiH* called before *thal*) has been detected, isolated and characterized from the thienodolin producer. *In vitro* assays showed that the tryptophan 6-halogenase encoded by this gene catalysis the regioselective chlorination of tryptophan and this is probably the first step in thienodolin biosynthesis [3].
The tryptophan 6-halogenase gene was used as a starting point for the isolation and sequencing of thienodolin cluster. Several open reading frames probably involved in the biosynthesis of thienodilin were detected. Analysing the proposed functions of the revealed ORFs resulted in two genes which are candidates for catalysing the second step in thienodolin biosynthesis, an amino transferase and an amido transferase. The amino transferase could convert 6-chlorotryptophan into 6-chloro-indole-3-pyruvate which could be the substrate for an enzyme introducing the sulphur atom at the α-carbon followed by a ring closure reaction resulting in the formation of the thiophene ring. In this proposed pathway, the amido transferase, forming the amide group, would be the last enzyme in thienodolin biosynthesis. The amidating activity of amido transferase could be demonstrated using chemically synthesised 6-chlorthieno[2,3-*b*]indole-2-carboxylate as substrate.A putative 2-methylthioadenine synthetase, a cytochrome P-450, an oxigenase and a dehydrogenase were also detected.

To characterize the gene involved in introducing the sulphur atom to the thienodolin structure, a deleted mutant in the 2-methylthioadenine synthetase will be created. Using a PCR mediated system and a conjugative vector the apramycin resistance gene will be inserted directly into this gene and their accumulated products will be isolated and characterized.

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OTP030
The bibartite S unit of an ECF-type cobalt transporter

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High affinity uptake of cobalt ions into prokaryotic cells is mediated in many organisms by members of the energy-coupling factor (ECF)-type of ABC transporters [1, 2, 3]. ECF-type Co^{2+} transporters consist of the bipartite substrate-specific unit (= S unit or core transporter) CbiMN and, like all ECF systems, copies of a universally conserved transmembrane (= T) protein and an ABC ATPase. T and ATPase (= A) units are represented by CbiQ and CbiO, respectively. CbiMN was shown to mediate a basal level of Co^{2+} transport in the absence of CbiQO [2, 4]. Attempts to purify the CbiMNQO holotransporter via an affinity tag on CbiO yielded tripartite CbiMQO complexes but failed to detect CbiN. Likewise, affinity chromatography of CbiMN with a tag on CbiN led to purified CbiN but CbiM was lost. These results indicated that CbiN is only loosely bound to its partners in detergent solution. To overcome the problem of loss of the essential CbiN we constructed a translational *cbi(MN)* fusion. The fusion protein was active and interacted functionally with CbiQO in vivo, but did not copurify with the latter [4]. Thus, we focussed on the bipartite core transporter with fused Cbi(MN) domains. Mature CbiM proteins contain an extremely conserved extracytoplasmic N-terminus containing a His residue at position 2. 16 Cbi(MN) variants with modifications affecting the stretch of nine N-terminal amino acids were constructed and analyzed. Only two of them (representing natural variations) retained activity. The results indicated that the length and sequence of this region are critical for transport activity. Specifically, they pointed to essential roles of His2 and the distance of His2 to the amino group of the peptide chain in metal recognition. Purification of the wild-type and 16 mutant Cbi(MN) variants led to the observation that active proteins appear in two forms after SDS-PAGE while inactive variants give a single band that comigrates with the slower-migrating species. N-terminal peptide sequencing and probing with an antibody directed against the C-terminal tag excluded the possibility that the faster-migrating species of active Cbi(MN) proteins results from proteolysis [4]. We hypothesize that this species represents an unknown modification of Cbi(MN) that correlates with its activity state.

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OTP031
Removal or inactivation - Effect of flocculation parameters on bacteriophages

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Bacteriophages are used as surrogates for pathogenic viruses to determine removal efficiencies of different drinking water treatment steps. Within the frame of the BMBF-project Efficiency of virus elimination by filtration processes in drinking water treatment (02-WT 0945) the f-specific bacteriophage MS2 and the somatic coliphage phiX174 were used for the quantification of the log removal value (LRV=log(c_0/c)) of flocculation processes.
To quantify the actual removal of the bacteriophages it is essential not to inactivate the phages during the process, otherwise the LRV is partly due to inactivation and not to removal. Therefore additionally to the conventional double layer agar method [DIN EN ISO 10705-1/ -2:2001] quantitative PCR (qPCR)-detection for both phages were installed: For the phage MS2 the method according [Dreier, Störmer & Kleesiek 2005] was used, for quantifying the phage phiX174 the method according [Crews, Wittwer & Gale 2008]. Both techniques had to be optimized to use them for the flocculation experiments. The detection limits were $2*10^4$ (MS2) and $1*10^3$ copies/mL (phiX174) respectively.
The phages are dosed into the water and samples are taken before flocculation and after sedimentation/filtration of the metal hydroxide flocs. Moreover, the recovery was determined for the total solution including flocculants and phages directly after dosing.
When determining the LRV of polyaluminum chloride (PACl) and ferric chloride ($FeCl_3$) as flocculants the results for the double layer agar method and the qPCR differed in some cases significantly. The removal efficiency of MS2 as tested by qPCR was lower than the one determined by the double layer agar method, for all water qualities tested (model water and two different natural waters, representative for Germany). Especially for higher PACl concentrations, this phenomenon was more distinct. The recovery analyses also showed that the MS2-phages were already inactivated in the total solution. So far, it is assumed that either the changes in pH in the flocculation process and/or the presence of aluminum ions lead to the inactivation of the phage MS2. In contrast, the phage phiX174 did not show this behaviour, even though undergoing the same procedures. So, phiX174 seems to be the better surrogate to analyze the LRV for flocculation processes.

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OTP032
Molecular biological characterization of the microbial biocenosis in commercial biogas plants during process failure

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Anaerobic co-fermentation of sewage sludge and waste with the objective of producing biogas is of growing interest to generate renewable energy and to reduce greenhouse gas emissions. Up to now, an anaerobic digester is operated as a so called „black box" and process failures such as foam formation, over-acidification or swimming layers occur in various plants. In this context the microbial biocenosis inside the biogas plant plays a decisive role due to its hydrolysing, acidogenic, acetogenic, and methanogenic properties. Changes in the microbial community during over-acidification could already be observed in laboratory scale fermenters as presented in the studies of Marietta Liebrich. However, the alteration in the microbial biocenosis during process failures in large-scale biogas plants is scarcely investigated.
In the studies to be presented, the variances of the microbial community during over-acidification and foam formation in commercial biogas plants were analyzed. To compare the diversification in the microbial community, full genomic deoxyribonucleic acid (DNA) was extracted using a commercial DNA isolation kit. The partial 16S rDNA genes of the two microbial domains Bacteria and Archaea were analyzed by polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) and microorganisms were identified by sequence alignment. Activity measurements and analysis of spatial relationship are planned to be conducted as well using fluorescence *in situ* hybridization (FISH).
First results of the molecular fingerprinting reveal an altered microbial biocenosis during foam formation. Some of the bacteria being present in the digested sludge are absent in the foam. Furthermore, changes in the microbial biocenosis during over-acidification are expected by reason that the optimal living conditions (e.g. pH, volatile fatty acids) highly varied.
Due to the importance of a balanced biocenosis in anaerobic digesters the investigation of alterations in the microbial community during process failure events in biogas plants will help to improve process understanding and to take action in countermeasures against process failures. Presence or absence of certain microorganisms could serve as indicator for the stability of the biogas production process.

OTP033
The acyltransferases KirCI and KirCII involved in Supramolecular Templating of kirromycin biosynthesis

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Streptomyces collinus Tü 365 is the producer of kirromycin, which is a polyketide antibiotic. Kirromycin binds to the elongation factor Tu and inhibits the bacterial protein biosynthesis. The skeleton of this antibiotic is synthesized by a large complex of type I polyketide synthases and non-ribosomal peptide synthetases (PKS I/NRPS complex), encoded by the genes *kirAI-kirAVI* and *kirB*.
KirAI-KirAV, except the NRPS, KirAIII, belong to PKSs type I with „*trans-AT*"-architecture. These megaenzymes possess no acyltransferase domains integrated in the PKS modules. In contrast KirAVI is of the classical „*cis-AT*"-type PKS, of which the ATs are part of the PKS protein. Two separate genes, *kirCI* and *kirCII* with similarity to acyltransferases were identified in the kirromycin gene cluster. To characterize the role of *kirCI* and *kirCII* in kirromycin biosynthesis, mutants in these genes were constructed and analyzed for kirromycin production. The inactivation of *kirCI* (Δ*kirCI*) resulted in a significant reduction of kirromycin production. In Δ*kirCII* kirromycin synthesis was completely abolished. Both mutants were successfully complemented with the wild type genes. The complemented strains produced the antibiotic at levels comparable with the wild type. This data indicate that both genes are involved in kirromycin biosynthesis and the gene *kirCII* is essential for the production of this antibiotic.
For kirromycin assembly, a selective loading of ACPs with the building blocks malonyl-CoA and ethylmalonyl-CoA is required. This function is presumably carried out by KirCI and KirCII, respectively. To confirm this hypothesis and to determine the specificity of KirCI and KirCII an *in vitro* ACP loading assay was developed.
Therefore KirCI, KirCII and two selected ACPs were expressed in *E. coli* and purified. The proteins were used in the *in vitro* assay and the loading of malonyl-CoA, methylmalonyl-CoA and ethylmalonyl-CoA to the ACPs was monitored by autoradiography and HPLC-ESI-MS. The experiments showed that KirCI loads specifically malonyl-CoA onto ACP4 and the second enzyme, KirCII is the first, biochemically characterized „*trans-AT*" with high specificity for ethylmalonyl-CoA and transfers this substrate to ACP5. Thus, the specific recognition mechanism of the ACP of module 4 and 5 by the ATs, KirCI and KirCII, respectively is at least in part determined by the ACP. To our knowledge, such interaction mechanism, where free-standing proteins that provide building blocks, dock site-specific to the „recipient"-protein to achieve structural diversity in polyketides was not characterized until now.

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OTP034
Analysis of the chlamydial amidase AmiA

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For intracellular bacteria there is no need to maintain osmotic stabilization by means of a cell wall and peptidoglycan has not been reliably detected in the obligate intracellular genus Chlamydia so far. Nevertheless, chlamydiae are susceptible to antibiotics that target cell wall biosynthesis, a paradox known as the chlamydial anomaly. A genome-wide search within chlamydiae has identified a nearly complete pathway for peptidoglycan biosynthesis.
Recently, we demonstrated *in vitro* activity of the chlamydial enzymes MraY and MurG that catalize the last two steps of the biosynthesis of the membrane bound cell wall block lipid II. We discussed the hypothesis that maintaining lipid II biosynthesis in cell wall lacking bacteria reflects an essential role of this precursor in procaryotic cell division. [Henrichfreise, Schiefer et al. 2009. Mol Microbiol. 73: 913-23.].
Here, we investigate the fate of lipid II in Chlamydia. To check whether the peptide chain of lipid II is released from its carrier (C55-P) by amidase action, as previously suggested [Ghuysen and Goffin. 1999. Antimicrob. Agents Chemother. 43:2339-2344.], amidase AmiA from *Chlamydia pneumoniae* was overproduced in *Escherichia coli* and purified. Chlamydial AmiA exhibited *in vitro* activity in dye release assays using Remazol Brilliant Blue R-dyed peptidoglycan as substrate.
Our findings strengthen the hypothesis that in chlamydiae lipid II is synthesized and further processed, including recycling of the carrier C55-P. A deeper insight into the peptidoglycan biosynthesis machinery and the chlamydial anomaly on molecular level will provide a basis for the design of novel anti-infective strategies against Chlamydia.

OTP035
Completing the Inventory: Systematic Deletion Analysis of Secondary Zinc Uptake Systems in *Cupriavidus metallidurans* CH34 to Understand Multiple Metal Handling

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Cupriavidus metallidurans is adapted to high concentrations of transition metal cations. The bacterium harbors a variety of metal efflux systems, which are the basis of its metal resistance. It is able to maintain cellular metal homeostasis even at high concentrations of many heavy metals in parallel. *C. metallidurans* contains four CorA paralogs of the metal inorganic transport (MIT) protein family of magnesium transport systems, ZupT of the ZRT/IRT protein family ZIP of zinc/iron transporters and PitA, which imports metal phosphate complexes. Multiple deletion muntants were constructed to characterize the contribution of each system to transition metal import. All of these transporters were regulated by zinc availability. While expression of *zupT* was induced up-regulated under conditions of zinc starvation, that of the other genes was down-regulated at high zinc concentrations. Only $corA_1$ expression was influenced by the magnesium concentration. This identified ZupT as the main zinc uptake system under conditions of low zinc availability, PitA as cation-phosphate uptake system, $CorA_1$ as main secondary magnesium uptake system, $CorA_2$ and $CorA_3$ as back-up systems for metal cation import

OTP036
Structure and function of PilQ, a unique secretin of the DNA transporter from the thermophilic bacterium *Thermus thermophilus* HB27

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Thermus thermophilus HB27 is known for its extremely high competence for natural transformation and its ability to take up DNA from members of the archaea, bacteria and eukarya. A genome-wide genetic screen followed by mutant studies led to the identification of 16 distinct proteins [1]. One of the competence proteins, the secretin-like protein PilQ, was found to be essential for DNA binding and uptake in HB27 [2]. Here we report the isolation, structural and functional analyses of a unique PilQ from *T. thermophilus*. Native PAGE, gel filtration chromatography and electrophoretic mobility shift analyses indicated that PilQ forms a macromolecular homopolymeric complex that binds dsDNA. Electron microscopy showed that the PilQ complex is 15 nm wide and 34 nm long and consists of an extraordinary stable „cone" and „cup" structure and five ring structures with a large central channel. Moreover, the electron microscopic images together with secondary structure analyses combined with structural data of T2SS and T3SS secretins suggest that the individual rings are formed by conserved domains of alternating α-helices and β-sheets. The unprecedented length of the PilQ complex correlated well with the distance between inner and outer membrane of *T. thermophilus*. Indeed, PilQ was found immunologically in both membranes indicating that the PilQ complex spans the entire cell periphery of *T. thermophilus*. This is consistent with the hypothesis that PilQ accommodates a PilA4 comprising pseudopilus mediating DNA transport across OM and periplasmic space in a single step process [3].

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OTP037

Identification of an acidic lipase activity from *Phialemonium curvatum* and comparison with two other fungal lipases in a newly developed two-layer assay

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Lipases catalyse the hydrolysis of long-chain triglycerides at interfaces between oil and water. Additionally they have interesting properties making them useable in different fields of industrial production. Most lipases used in industry are produced by microorganisms. Still the screening and production of lipases with specific properties such as activity at acidic or alkaline pH as well as a wide temperature range is of high interest. Acidic lipases are involved for example in food and flavour industries [1] or as a substitute for gastric lipase in enzyme therapy [2].
A fast and reliable test to analyse the pH range of newly identified or mutated lipases is valuable in lipid research. A two-layer lipase activity assay was established in microtiter plates for rapid activity test as well as on Petri dishes to compare the activity at a specific pH. Two layers were established, bottom was 2 % agar melted in appropriate buffer system, for pH3-5 0.05 M acetic acid-sodium acetate; pH6-7 0.05 M phosphoric acid-sodium phosphate; pH8-9 0.05 M Tris-HCl, top layer contained additionally to the components of the bottom layer 1 % tributyrin as substrate. Two well-characterized lipases were applied to the system, *Rhizomucor miehei* lipase stable between pH7-10 with an optimum at pH8 [3] and *Thermomyces lanuginosus* lipase acting between pH5-9, optimum at pH7 [4], respectively. Cell-free lipase as well as homogenized mycelium of *Phialemonium curvatum*, reported to grow under acidic conditions in minimal medium with plant triglycerides as sole carbon source [5], were used to determine pH dependence. In microtiter plates *R. miehei* lipase, as well as *T. lanuginosus* lipase showed activity between pH4-9. Only lipase of *P. curvatum* showed activity starting at pH3 to pH9. Comparing these results with the same two-layer activity test in Petri dishes determination of the clearance zone diameter led to the exact pH optima. For the lipases of *T. lanuginosus* and *R. miehei* the literature values were confirmed. The newly characterised lipase activity of *P. curvatum* had its optimum at pH7.

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OTP038

Site-Specific Cross-Linking Between the A and T Units of an ECF-Type Biotin Transporter

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Energy-coupling factor (ECF) transporters are a class of micronutrient importers in prokaryotes composed of a substrate-specific transmembrane protein (S unit) and an energy-coupling module. The latter consists of a conserved transmembrane protein (T unit) and pairs of ABC-ATPases (A units) [1, 2]. Although highly diverse on the sequence level, recent elucidation of the 3D structures of the riboflavin-specific S unit RibU [3] and the thiamine-specific equivalent ThiT [D.J. Slotboom, personal communication] uncovered a conserved fold with six transmembrane domains (TMD). Oligomeric-state analyses of BioY, a biotin-specific S unit, in living bacteria suggested that S units are organized as oligomers [4]. Choosing the biotin transporter BioMNY (BioM=A; BioN=T) we have investigated the role of T units. BioN forms stable BioMNY holotransporter complexes, and is contained - in vitro and in vivo - in stable BioMN complexes in the absence of BioY [4]. Two well-conserved three amino-acid motifs with Ala-Arg-Gly as the consensus, found in a cytoplasmic helical loop of T units, are essential for complex stability and intersubunit signaling [5]. In analogy to canonical ABC transporters, we hypothesized that the ARG-containing stretches may function as coupling domains for interaction with the A units. A cysteine-less BioMNY variant was constructed and used to generate sets of double Cys variants with individual Cys residues in the ARS-ARG region of BioN and the Q loop of BioM. Cu-phenanthroline-induced Cys cross-linking in isolated membranes confirmed the predicted BioN-BioM interaction. Specifically, our present data show that (i) both ARG signatures (163-ARS-165; 194-ARG-196) interact with the Q loop(s), (ii) they interact in particular with the N-terminus of the Q loop and (iii) Cys residues adjacent to ARS-ARG do not crosslink with Q loop.

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OTP039

Identification and structure resolution (2.3 Å) of a novel metagenome-derived short chain oxidoreductase (SDR) involved in quorum quenching phenotypes in *P. aeruginosa*

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Here we report on the identification and structural characterization of a novel short chain oxidoreductase from a soil metagenome. The corresponding gene *bpiB09* was identified through a screening for metagenome clones interfering with bacterial quorum sensing. The *bpiB09* gene encoded for a 239 aa protein which was weakly similar (Identity 58 %, blastx E-value 6e-60) to a predicted short-chain dehydrogenase from Acidobacteria. Heterologous expression as a 10x his-fusion protein in *E. coli* resulted in the production of a 30 kDa protein. Additional crystallographic studies established BpiB09 as an NADPH-dependent reductase. Structural and phylogenetic analyses revealed that it belongs to the classical SDR family of proteins. There it falls within the subgroup cP3. Interestingly, expression of *bpiB09* in *P. aeruginosa* PAO1 resulted in significantly reduced pyocyanin production, decreased motility and poor biofilm formation. Furthermore HPLC-MS analyses suggested that autoinducer synthesis of *N*-3-oxo-acyl-L-homoserine lactone was strongly affected in cells expressing the *bpiB09* gene suggesting a possible role of the protein during the early steps of autoinducer biosynthesis.

OTP040

Novel lactonases from *Rhizobium sp.* NGR234

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Quorum sensing (QS) is an important area of application, when it comes to fighting microbial infection. Quenching of QS-signal molecules, such as the autoinducer I- family of N-acyl-L-homoserine lactones, is a useful strategy to inhibit QS-mediated processes e.g. biofilm formation.
Here we report on the analysis of the genes and enzymes involved in autoinducer I hydrolysis in *Rhizobium* sp. NGR234. Using a previously published function-based screening with the biosensor strain *Agrobacterium tumefaciens* NTL4, which carries a *tra*I-*lac*Z gene fusion for the detection of autoinducer I hydrolase genes, we identified a total of five cosmid clones that repeatedly gave positive results in our assay. Two of these loci were located on the megaplasmid pNGR234*b* and three were encoded by the chromosome cNGR234. Subcloning and transposon mutagenesis in combination with blast analyses identified the corresponding ORFs, designated *dlh*R, *qsd*R1, *qsd*R2, *ald*R and *hit*R. Employing recombinant and purified DlhR and QsdR1 protein, we showed that both enzymes inhibited biofilm formation and other QS-dependent processes in *Pseudomonas aeruginosa* PAO1, *Chromobacterium violaceum* ChV26 and *Agrobacterium tumefaciens* NTL4. Using high performance liquid chromatography-mass spectrometry (HPLC-MS) analysis, we demonstrate the cleaving

mechanism, thus these enzymes function as AHL-lactonases. Finally our data suggest that the autoinducer hydrolases are of importance for rhizosphere colonization.

OTP041
The overlapping gene pair *htgA/yaaW* in *Escherichia coli* O157:H7 EDL933

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Overlapping genes are defined as a pair of genes whose coding regions are partially or completely overlapping. Since DNA consists of two complementary strands and triplets code for the amino acids, six open reading frames would be possible in theory. Overlapping genes were first observed in bacteriophages, but in bacteria they are assumed to be rare. A few examples have been reported for *Pseudomonas fluorescens* and *Streptomyces coelicolor*, but most bacterial overlapping genes are not very well characterized [2, 3]. In this work we examine the controversial overlapping gene pair *htgA/yaaW* in *Escherichia coli* O157:H7 EDL933.
htgA has been described as heat inducible in 1993 [1]. Since then, the experimental evidence is scarce and *htgA* is now classified as (obsolete) synonym to *yaaW* in the databases. This is astonishing insofar as *htgA* and *yaaW* are at the same locus, but in opposite orientation. Several records of microarray experiments in the databases suggest differential, strand specific regulation of the *htgA/yaaW*-region.
First, we analyzed the transcription of *htgA* and *yaaW* at six different growth conditions. Next, we cloned the suspected promoter regions of both genes in front of a *gfp* reporter. Third, one of the overlapping open reading frames was destroyed by introducing a mutation causing a stop codon, which is silent on the other frame. Both mutants were tested under several growth conditions for a phenotype. Preliminary results suggest that both, *htgA* and *yaaW,* are weakly transcribed. The latter varies with different growth conditions. The existence of HtgA is still under investigation.

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OTP042
Comparison of two prokaryotic expression systems for the gamma-cyclodextrin glucanotransferase from *Bacillus sp*. G-825-6

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Cyclodextrin glucanotransferases (CGTases) convert starch to a mixture of cyclic and linear oligosaccharides. The cyclic oligosaccharides, known as cyclodextrins have the ability to form inclusion complexes with many organic molecules altering their stability, solubilty, or bioavailabilty. Cyclodextrins are therefore of interest for applications in pharmaceutical, food and cosmetic industries. Gamma-CGTases are microbial glucanotranferases mainly producing gamma-cyclodextrin consisting of 8 glucose units. The gamma-CGTase gene (*cgtS*) of the alkalophilic *Bacillus sp.* G-825-6 was codon-optimized for usage with *E. coli* and *B. subtilis* allowing an expression in both hosts. *E. coli* BL21 (DE3) [pET-20b(+)::cgtS] and *B. subtilis* DB 430 [pHT08::*cgtS*] were used and the efficiency of the systems was compared. The transformation efficiency was analyzed by PCR resulting in 4 positive *E. coli* and 7 positive *B. subtilis* clones. Both vector systems were IPTG-inducible and contained a His-tag. *E. coli* and *B. subtilis* were grown in batch cultures at 37 °C. The induction was performed at an OD_{600} of 1,0 for 14 h at room temperature. Crude protein extract obtained by sonification of the harvested cells was used for determination of starch-hydrolyzing activity by a colorimetric assay. Using *E. coli* as expression system, clones containing starch-hydrolyzing activity could be obtained while no activity was detected with the *B. subtilis* clones. The His-tag fusion protein obtained with the *E. coli* expression system was purified and employed for the synthesis of cyclodextrins. As main end products, beta-cyclodextrin composed of 7 glucose units and gamma-cyclodextrin in a ratio 1:3 were detected by HPLC with pulsed amperometric detection.

OTP043
A Glycogen Synthase Defect Mutant of *Clostridium acetobutylicum* ATCC 824

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The transition phase of the growth of the Gram-positive, spore-forming anaerobe *Clostridium acetobutylicum* is characterized by several morphological changes. At the beginning swollen and cigar shaped cells, clostridial stages, are formed. In the cells a polymeric carbohydrate, so called granulose is accumulated in the form of granules. This macromolecule is defined as an amylopectin-like structure with slightly branched (2 % of 1.6-linkages) glucose molecules. Granulose is expected to be a energy- and carbon storage, putatively serving as a prerequisite for sporulation.
In *C. acetobutylicum,* the synthesis of granulose is believed to be encoded by the gene products of the glg-Operon: GlgC (*cac2237*, Glucose-1-phosphate-adenyltransferase), GlgD (*cac2238*, ADP-Glucose-pyrophosphorylase), GlgA (*cac2239*, Glycogen [Granulose] synthase) and Cac2240 (a protein of unknown function). As only one glycogen synthase is annotated in the genome of *C. acetobutylicum, glgA* was expected to play a crucial role in the biosynthesis of granulose.
Here we report on the construction of a specific *glgA* defect mutant using the ClosTron® technology [1]. Individual mutant strains were selected, which are unable to accumulate granulose. Molecular analysis (Southern Blot and PCR investigations) proved the correct insertion of the „knock out" gene cassette.
Furthermore, results of the phenotypic characterisation are presented. This data include iodine staining of colonies and cells in comparison to the wildtype, growth analysis (optical density, pH, product spectrum) and first comparative sporulation assays.

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OTP044
Changes in abundance and activity of the biocenosis during provoked process failure

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One solution to reduce the effects of climate change due to increasing CO_2 emissions is the anaerobic fermentation of organic material. The knowledge about the interaction of the complex biological processes in biogas plants is limited up to date, e.g. the behaviour of the biocenosis under stress conditions.
Due to the lack of process understanding biogas plants often run below their maximum loading rate. In order to maximise the space-time-yield of a biogas plant changes of the microbial community during shock loading, over-acidification and deacidification were monitored in several lab experiments.
During these experiments the formation of different aggregates was observed, that had an influence on the process stability. The size of aggregates was depended on the amount of additives used to stabilize the process during over-acidification.
The biological samples were examined using different molecular biology methods, to observe changes in the abundance and activity of the microorganisms involved:

- Genetic fingerprinting (*Denaturing gradient gel electrophoresis, DGGE)* for the characterisation of the dominant species of the biocenosis
- qPCR (*quantitative real-time-polymerase chain reaction*) to quantify the metabolic activity of the groups of microorganisms involved
- FISH (*fluorescence in situ hybridization*) to quantify the different groups of microorganisms

OTP045

Penicillin binding protein 2x of *Streptococcus pneumoniae*: A GFP-PBP2x fusion is functional and localizes at the division septum

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Penicillin-binding protein 2x (PBP2x) is one of the six PBPs in *S. pneumoniae* involved in late steps of peptidoglycan biosynthesis. PBP2x catalyse a penicillin-sensitive transpeptidation reaction. Mutations in PBP2x that interfere with beta-lactam binding are crucial for the development of high level penicillin-resistance which involves other PBPs as well. The PBP2x gene is located in a cluster devoted to cell division, and localization of PBP2x at the septum as revealed by immunofluorescence techniques confirmed its role in the division process [1]. However, immunostaining has the disadvantage that cells need to be fixed and have to undergo a damaging cell wall permeabilization treatment. Green fluorescence protein (GFP) fusions can overcome these problems and allow the visualization of fusion proteins in living cells.
To investigate the role of PBP2x during growth and division of *S. pneumoniae* cells, an N-terminal GFP-PBP2x fusion was constructed using plasmid pJWV25 that contains Zn^{2+}-inducible promoter driving *gfp*-fusion gene expression [2]. This plasmid also carries the flanking regions of the nonessential *S. pneumoniae bgaA* gene, facilitating a double cross-over event at this locus. GFP-PBP2x signal was observed at the septum in *S. pneumoniae* cells. Furthermore, the native copy of *pbp2x* gene could be deleted in these cells without affecting cell growth, showing that GFP-PBP2x is functional. This system was applied to study cellular localization of PBP2x protein in strains which contain a reduced amount of PBP2x.

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OTP046

Purification of the MCAP 3-halogenase from pyrrolnitrin biosynthesis in *P. fluorescens* BL915

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Pyrrolnitrin is an antifungal compound [1] first isolated from *Pseudomonas pyrrocinia* [2]. The gene cluster responsible for pyrrolnitrin biosynthesis was identified in *Pseudomonas fluorescens* (BL915) [3, 4] and other pyrrolnitrin producing bacteria. Four conserved enzymes are involved in pyrrolnitrin biosynthesis, named PrnA, PrnB, PrnC, and PrnD, according to their order in catalysis. The tryptophan 7-halogenase PrnA catalyzes the regioselective chlorination of the amino acid tryptophan in 7 position of the indole ring [6]. The second enzyme, PrnB, converts 7-Cl-tryptophan into monodechloroamino-pyrrolnitrin [4]. This intermediate is chlorinated by the third enzyme, PrnC, a second flavin-dependent halogenase. The fourth enzyme, PrnD, oxidizes the amino group to a nitro group, yielding pyrrolnitrin [7].
$FADH_2$-depending halogenases contain two conserved regions - the GxGxxG and the WxWxIP motif, leading to the assumption that the MCAP 3-halogenase PrnC operates by the same mechanism as the well-analyzed tryptophan 7-halogenase PrnA. So far, the MCAP 3-halogenase PrnC could not be purified in active form, precluding further analysis. We now report a novel purification strategy leading to purified and active PrnC. Using the GST-fusion protein strategy it is possible to obtain pure PrnC produced by a recombinant *Escherichia coli* strain. Both, fusion protein and cleaved MCAP 3-halogenase show halogenating activity.

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OTP047

Flavoenzymes of *Escherichia coli* as targets for the riboflavin analog roseoflavin from *Streptomyces davawensis*

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The gram-positive soil bacterium *Streptomyces davawensis* is the only known organism to produce the antibiotic roseoflavin (8-dimethylamino-8-demethyl-D-riboflavin) a riboflavin (vitamin B_2) analog (4). Roseoflavin exhibits antibiotic activity against gram-positive and also gram-negative bacteria if a flavin uptake system is present (2). In the cytoplasm roseoflavin is converted to roseoflavin-5'-monophosphate (RoFMN) and roseoflavin adenine dinucleotide (RoFAD) by the combined activity of flavokinase (EC 2.7.1.26) and FAD synthetase (EC 2.7.7.2) (1). A recombinant *Escherichia coli* strain overproducing the flavin transporter PnuX (from *Corynebacterium glutamacium*) is roseoflavin sensitive. *Bacillus subtilis* naturally contains a flavin transporter and thus is roseoflavin sensitive as well. Both bacteria were cultivated in the presence of riboflavin and sublethal amounts of roseoflavin. The total protein was isolated and analyzed with respect to its flavin content. The total protein obtained from riboflavin grown cells contained FMN and FAD, the total protein obtained from roseoflavin grown cells in addition contained RoFMN. RoFAD was not detected.
Subsequently, 40 different recombinant *E. coli* strains each overproducing another his_6-tagged *E. coli* flavoenzyme were obtained through the ASKA library (3). The flavoenzymes were synthesized in a PnuX overproducing *E. coli* strain in the presence of roseoflavin, purified by affinity chromatography and it was found that they contained RoFMN.
It was reported that some enzymes are inactive in their roseoflavin cofactor form e.g. D-amino acid oxidase from *Sus scrofa* (RoFAD) (EC 1.4.3.3). Exemplarily, AzoR an azobenzene reductase (EC 1.7.1.6) from *E. coli* naturally containing FMN was purified in its FMN and RoFMN form. Present results indicate a decrease in activity up to 90%. All in all, we could show that roseoflavin was converted to RoFMN *in vivo* and that this flavin analog was accepted as a cofactor by flavoenzymes of *E. Coli* which seems to result in a loss off activity.

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OTP048

Managing Zoonotic Diseases - Research Networking

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Zoonoses are infectious diseases which are transmitted from animals to humans and vice-versa. They are caused by different types of agents - bacteria, parasites, fungi, prions or viruses. Over 200 zoonoses have been described and the number is still increasing as new biomedical knowledge is acquired. Due to the rapid world population growth and other global reasons, the study of zoonoses becomes ever more important. Recent outbreaks of Influenza and SARS are such examples.
The National Platform for Zoonoses aims to develop a network of scientists to improve research on preparedness, prevention, detection, and control of zoonotic diseases. Our objective is to promote exchange of expertise on the national and international level and thus to accelerate research activities in the field of zoonoses. In addition, we pursue the wide horizontal cross-linking of human and veterinary medicine.
These objectives will be achieved by the following activities:

- Organization and realization of joint events which support interdisciplinary exchange and interaction.
- Promotion of national, European and international collaborations.

- Set-up of databases containing zoonoses-related experts, institutions, research projects, research funding programmes, samples, and cell lines.
- Providing independent information about zoonotic infectious diseases for the general public.
- Initiation and realization of innovative and interdisciplinary pilot projects with cross-sectional characters.
- Support and counselling for the design and implementation of zoonotic funding schemes.

Since 2009 more than 280 scientists joined the National Platform for Zoonoses. Three main professional groups are represented within the research platform: veterinarians (38%), infectiologists (33%), and physicians (23%). Regarding the diversity of zoonoses, our members are mainly located at universities & university hospitals (55%) as well as at federal research institutes (30%). Thereby the National Platform for Zoonoses ensures broad exchange of knowledge and experiences. The platforms' priority will be linkage of further researchers and research groups to expand intersectional research activities.

OTP049
Structure and functional studies of the *Wolinella succinogenes* STAS-Domain

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Anion transporters of to the Sulp family and the related SLC26 transporters have an N-terminal transmembrane domain that is connected via a linker region to the cytoplasmic c-terminal STAS domain (Sulfate Transporter Antagonist of anti-sigma factor). The name of this domain is due to a remote but significant sequence similarity with bacterial ASA (anti sigma factor antagonist) protein[2]. Mutation studies indicate that the STAS domain is critical for the transporters activity and plays a role in intra- and intermolecular interactions[3]. In human members of the SLC26 transporters, mutations in the transmembrane domain as well as in the STAS domain cause a number of inherited diseases[4]. The structure of E.Coli YchM (SLC26 anion transporter) in complex with Acyl-Carrier protein indicates that YchM has a function in Fatty Acid Metabolism[1].
We are working with the STAS domain of *Wolinella succinogenes* and *Aquifex aeolicus*. In order to crystallize the STAS domain of *Wolinella succinogenes* and *Aquifex aeolicus* different constructs were created by mutagenesis, a purification strategy was esthablished and the STAS domain was crystallized to solve its structure by X-Ray diffraction. The other focus of our work is to show the interactions between the STAS domain and putative binding partners.

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OTP050
In vivo Tat substrate-translocon interactions in *Escherichia coli*

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Folded proteins can be translocated across energy-transducing membranes of prokaryotes and plant plastids by the twin-arginine translocation (Tat) system. Tat-dependently translocated proteins (Tat substrates) possess N-terminal signal peptides that contain the eponymous twin-arginine motif, an amino acid pattern that usually includes two consecutive arginines and two consecutive hydrophobic residues, separated by one residue. In this study, we analyzed for the first time *in vivo* in
Escherichia coli the interactions of Tat signal peptides and mature domains of Tat substrates with the TatABC translocon subunits. Our data reveal the influence of an RR > KK exchange in the twin-arginine motif of translocon binding and indicate that the translocon interaction of mature Tat substrate domains strongly depends on specific parameters of the substrates. An integrated transport model will be presented that takes all interaction data into account.

OTP051
Structural and biochemical characterization of the formate channel FocA from *Salmonella typhimurium*

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Formate is a key substrate and regulatory molecule during anaerobic bacterial fermentation. Therefore the transport of formate across the cytoplasmic membrane must be finely regulated in response to the change of environmental parameters such as pH value or the availability of exogenous electron acceptors. The first protein identified to mediate this transport was FocA, encoded in the anaerobically induced *pfl* operon. Previous work suggests that FocA, a member of the formate and nitrite transporter family, may function as a channel rather than transporter. However, to date little is known about its regulatory mechanism. In this work we determined the crystal structure of FocA from *Salmonella typhimurium* at 2.8 A, which forms a pentameric assembly. Surprisingly, within the FocA pentamer three different monomer conformations were observed for the very first N-terminal helix preceding the first transmembrane segment. This resulted in three different states of the formate channel: open, intermediate and closed. With this finding, a working mechanism for the pH-dependent transport of FocA is proposed.

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OTP052
From 2-Oxoglutarate sensing to enzyme control by the *Synechococcus elongatus* PII signal transduction protein

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P_{II} signal transduction proteins have key functions in coordination of central metabolism by integrating signals from carbon, nitrogen and energy status of the cell. In the cyanobacterium *Synechococcus elongatus* PCC7942 P_{II} binds ATP and 2-oxoglutarate (2-OG) in a synergistic manner, with the ATP-binding sites also accepting ADP. Depending on its effector molecule binding status, P_{II} from this cyanobacterium and other oxygenic phototrophs complexes regulates the key enzyme of the cyclic ornitine pathway, *N*-acetyl-L-glutamate kinase (NAGK), to control arginine biosynthesis.
In wild type PII E85 forms a salt bridge with R233 of NAGK, and consequently E85-PII mutants loose the ability to interact with NAGK. We found PII variants (I86N and I86T) that are able to bind to a NAGK variant (R233A) that was previously shown to be unable to bind wild type PII protein. Analysis of interactions between these P_{II} variants and wild type NAGK as well as the NAGK R233A variant suggested that the I86N variant in the presence of ATP was a superactive NAGK binder, also indicating that P_{II}-E85/NAGK-R233 is not essential for the interaction of the two proteins. To reveal the structural basis of this property, the crystal structure of the PII I86N variant was solved at atomic resolution. Based on the data we propose a two-step model for the mechanism of P_{II}-NAGK complex formation: in an initiating step, a contact between R233 of NAGK and E85 of PII initiates the bending of the extended T-loop of P_{II}, followed by a second step, where a bended T-loop deeply inserts into the NAGK clefts to form the tight complex.
Crystal structures identify the binding site of 2-OG located in the vicinity between the subunit clefts and the base of the T-loop showing a novel conformation and explaining the negative effect of 2-OG on PII-NAGK interaction. Trimers with one or two 2-OG molecules shed light on the inter-subunit signalling mechanism by which P_{II} senses effectors in a wide range of concentrations.

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PSV001
Do Gram positives recycle their cell wall?

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The peptidoglycan, the stabilizing component of the bacterial cell wall, is not inert but is permanently degraded, remodelled, and re-synthesized during cell growth and differentiation. Although the release of a substantial amount of peptidoglycan turnover products (muropeptides) has been reported for many bacteria, their reutilization (cell wall recycling) has been studied, so far, only in the Gram-negative bacterium *Escherichia coli*. The Gram-positive cell wall differs from the Gram-negative cell envelope by the lack of an outer membrane, by the formation of a thick, multi-layered peptidoglycan that contrasts to the essentially single-layered peptidoglycan of Gram-negative bacteria, and by the presence of long anionic polymers called teichoic acids that are covalently attached to the peptidoglycan (wall teichoic acids). Therefore, cell wall turnover in Gram-positive bacteria has to proceed different from the Gram-negative pathway. Whether the cell wall turnover products in Gram-positives are also recycled and under which conditions this may occur is currently unclear. We identified pathways that are used for the recovery of N-acetyl-glucosamine (GlcNAc)-N-acetyl-muramic acid (MurNAc)-peptides (muropeptides) derived from the cell wall in *Bacillus subtilis* and *Clostridium acetobutylicum*. Interestingly, mutations within this pathways result in lytic phenotypes. We explored the conditions for autolysis, cell wall shedding and recovery in these Gram-positive bacteria and characterized the enzymes of these pathways.

PSV002
A RubisCO-like Protein links SAM-Metabolism with Isoprenoid Biosynthesis

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D-Ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) is one of the most abundant enzymes in the biosphere and catalyzes the key reaction of the Calvin cycle, the major process of CO_2-fixation on earth. To date, three different types of RubisCO have been identified that all serve as true carboxylases in plants, bacteria, and archaea, respectively [1].
Recent sequencing projects identified close RubisCO-homologues (RubisCO-like proteins) in a number of bacterial and archaeal genomes, such as *Bacillus subtilis*, *Pseudomonas putida, Mesorhizobium loti*, *Chlorobaculum tepidum, Archaeoglobus fulgidus*, or *Rhodospirillum rubrum*. In contrast to true RubisCOs, these RubisCO-like proteins (RLPs) miss residues essential for the carboxylation reaction and consequently lack the ability to fix CO_2. However, genomic context and active site residue differences suggest that all these RLPs serve different physiological functions.
We recently assigned a function to the RubisCO-like protein of *Rhodospirillum rubrum*, studying its mechanistic diversity *in vitro*. This RLP can use methylthioribulose-1-phosphate as substrate to catalyze two subsequent enolization reactions [2]. Further investigation on the physiological significance of this new reaction *in vivo* was carried out using a combined approach of RNA sequencing (RNAseq), knockout metabolomics, cell extract NMR, and functional enzymology.
Our results led to the identification of a completely novel bacterial strategy to salvage methylthioadenosine, a dead end product of S-adenosyl methionine (SAM) in spermidine and biotin biosynthesis. This strategy involves the release of methanethiol (CH_3SH) from the carbon skeleton, whereas the rest of the molecule is transformed into deoxyxylulose-5-phosphate (DXP), an essential intermediate in isoprenoid biosynthesis.
In summary, the RubisCO-like protein of *R. rubrum* provides a novel biosynthetic route to isoprenoids by linking two key processes of purple non-sulfur bacteria, polyamine and carotenoid biosynthesis, in an efficient and elegant manner. These findings will add another piece to our understanding of the evolutionary and functional relationship between RubisCO and RubisCO-like proteins.

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PSV003
Flagellar motor tuning - a novel hybrid motor in *Shewanella oneidensis* MR-1

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The flagellar motor consists of two major structures: the rotor, which is the rotating component and the stator, which provides a fixed component in the membrane. The stator complexes are thought to surround the rotor, however, the stator ring system is surprisingly dynamic. It has recently been shown that stator complexes are constantly exchanged with a membrane located pool of precomplexes which are activated upon incorporation into the motor. Our physiological and localization studies on *Shewanella oneidensis* MR-1 revealed that two different sets of stators, annotated as PomAB (sodium ion-dependent) and MotAB (proton-dependent) differentially support the flagellar rotation. Our current working model suggests that PomAB and MotAB are present as precomplexes in the cell membrane and compete for incorporation into the stator ring system. High sodium ion concentrations strongly favour incorporation of PomAB stator complexes, whereas low sodium ion concentrations decrease the presence of PomAB stator complexes. Instead the proton-driven MotAB stator complexes are recruited. Our data strongly suggest that under low sodium ion concentrations the flagellar motor is simultaneously driven by PomAB and MotAB stator complexes. We therefore propose that the single polar flagellum of *S. oneidensis* MR-1 is powered by a hybrid motor which concurrently uses sodium ions and protons. Interestingly, our *in silico* analysis of 400 organisms with a single flagella system revealed that 134 organisms harbor multiple stator complexes. Thus, adaptation to different environmental conditions might be conferred by stator swapping.
So far, the natural occurrence of a hybrid motor has never been demonstrated. However, our data provide strong indications that *S. oneidensis* MR-1 harbors this novel kind of a hybrid motor to adapt to environmental changes. In addition, we propose that stator swapping to modify motor functions is widespread among bacteria.

PSP001
Nutrient depending volatile emission of *Serratia odorifera* 4Rx13

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The Gram-negative rhizobacterium *Serratia odorifera* 4Rx13 emits a wealth of volatiles. Such volatiles possess different effects on neighboring organisms plants, fungi, protozoa [1]. Within the volatile mixture *S. odorifera* emits a major compound, with a structure new to science. (Octamethylbicyclo(3.2.1)octadiene, `Sodorifen´) [2,3]. The underlying biosynthesis of this compound is completely unknown. The unusual mass spectrum is accompanied by several isomers which indicate along with $C^{13}$ labelled acetate experiments a novel pathway of `sodorifen´. Two strategies are presently pursued to unravel the biosynthesis and regulation of this new compound, i) genetic analysis and ii) physiological analysis. The latter includes tests on various media such as complex media, +/- glucose, or synthetic media +/- amino acids or +/- variety of carbon sources. Highest 'sodorifen' production was observed on complex medium or on synthetic medium with the addition of three amino acids. Furthermore, experiments with C^{13} labelled methionine advert that only one of the eight methyl groups originates from a methyltransferase reaction. The genetic analysis became possible after sequencing the full genome of *S. odorifera* (NCBI Project ID 42253). Currently a knock out system will be established to allow the test of candidate genes involved in the biosynthesis of `sodorifen´.
Acknowledgement: We thank our collaborators W. Francke, S. von Reuß (University of Hamburg, D), G. Gottschalk, R. Daniel, A. Thürmer, J. Voss, R. Lehmann (University of Göttingen, D) and E.Crespo, S. Cristescu, F.v Harren (Nijmegen, NL).

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PSP002
Production of the antibiotic Gramicidin S in *Aneurinibacillus migulanus*: Phenotype specificity and intracellular peptide accumulation in granules

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The cyclic decapeptide Gramicidin S (GS) is a potent antimicrobial agent [1,2]. Its production for clinical use requires the maintenance of biosynthetically active strains. Unfortunately, the producer strains ATCC 9999^T=DSM 2895^T, DSM 5759 and DSM 5668, which are available today in culture collections, do not produce GS with good yield. We could attribute this problem to dissociation of the producing rough (R) colony phenotypes into non-producing smooth (S) colony phenotypes [3], and we found conditions for reversible dissociation of S back into R to recover the industrially valuable properties. Since GS is accumulated in the cells its yield depends on both the biomass and biosynthetic activity of the cells, which is regulated by medium composition. We found that GS accumulation has a feedback correlation with cell respiration, as monitored by an alamarBlue® assay during the fermentation process. The amount of GS in the cells can be readily estimated by pH-selective fluorescent staining with 5(6)-carboxy-fluorescein-hydroxysuccinimide, which is selective for the ornithine residues in GS. The bacteria can accumulate up to 250 mg of the membrane-active antibiotic GS per gram of dry cell weight, without disturbing their own cells. By comparing fluorescent and electron microscopy images of producing and non-producing phenotypes, we found that the peptide is accumulated in electron-dense granules. The granules are localized in vacuoles close to multilamellar stacks of membraneous structures. Studies of the isolated granules by ^{31}P-NMR and MALDI showed the presence of phosphate and GS. The mass increased in units of 154 and 168 Da, indicating that GS is bound to phosphate containing compounds such as butyryl and propionyl phosphate. We thus suggest that GS plays an active role in the formation and stability of these polyphosphate granules.

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PSP003
Ferric siderophore uptake and intracellular iron release in *Bacillus* species from a structure-functional view

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Iron availability is one major constraint of microbial life. In various habitats, microbes use siderophores for high affinity iron acquisition. In bacteria, iron-loaded siderophores are imported into the cytosol, where the iron has to be efficiently released to become metabolically available. We have solved the crystal structures of the ferric bacillibactin uptake component FeuA in complex with the native endogenous ligand ferric bacillibactin, the native exogenous ligand ferric enterobactin, and the synthetic aryl-based analog ferric mecam which may serve as a competitive uptake inhibitor. The ferric triscatecholate ligands are bound by electrostatic interactions formed with positively charged residues in the protein binding pocket. The dissociation constants determined by fluorescence titration are in the nanomolar to low micromolar range. Further, the binding induces lambda configuration of the ligand stereochemistries as monitored by CD spectroscopy. Intracellular iron release was studied with different classes of siderophores requiring different types of release mechanisms. While the triscatecholate-trilactone siderophores bacillibactin and enterobactin were found to be hydrolyzed in *B. subtilis* by the BesA esterase prefering the iron-charged siderophores as substrates, iron release in the related species *B. halodurans* was found to depend mainly on the ferric siderophore reductase FchR. This cytosolic reductase efficiently reduces both ferric dicitrate and several ferric hydroxamates via electron transfer through its iron-sulfur cofactor, demonstrating a redox-controlled iron release mechanism for ferric siderophores with moderately low redox potentials.

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PSP004
The glycogen branching enzyme GlgB is essential for glycogen accumulation in *Corynebacterium glutamicum*

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Glycogen serves in various bacteria as a long-term carbon storage compound and is therefore accumulated when nutrients become limiting. However, in the Gram-positive bacteria *Corynebacterium glutamicum* and *Mycobacterium smegmatis* glycogen is only transiently accumulated as carbon capacitor during the early exponential growth phase [1,2]. Glycogen is generally synthesized by the consecutive action of ADP-glucose pyrophosphorylase (GlgC), glycogen synthase (GlgA) and glycogen branching enzyme (GlgB). The *glgC* and *glgA* gene products of *C. glutamicum* were shown to be necessary for the glycogen accumulation in this organism during cultivation with glucose [3,4]. Due to the similarity to *glgB* genes in other organisms, cg1381 has been annotated as *C. glutamicum glgB* gene, however, its gene product has not been characterized and its role for the transient glycogen accumulation has not been investigated yet. We here show, that the cg1381 gene product of *C. glutamicum* indeed catalyses the formation of α-1,6-glycosidic bonds in polysaccharides and therefore has been correctly designated as glycogen branching enzyme. RT-PCR experiments revealed the transcriptional organisation of *glgB* in an operon with *glgE* (probably encoding a maltosytransferase). Promoter activity assays with the *glgE* promoter region revealed a carbon source-dependent regulation of the *glgEB* operon. Furthermore, characterisation of growth and of glycogen content in the *glgB*-mutant strain *C. glutamicum* ImglgB showed that the glycogen branching enzyme GlgB is necessary for glycogen formation in *C. glutamicum*. Taken together these results suggest that an interplay of the enzymes GlgC, GlgA and GlgB is not essential for growth, but is required for synthesis of the transient carbon capacitor glycogen in *C. glutamicum*.

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PSP005
Studies on the carbon metabolism of *Gluconobacter oxydans* 621H

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Gluconobacter oxydans is a strictly aerobic Gram-negative bacterium that is able to incompletely oxidize sugars, sugar alcohols and polyols regioselectively by membrane-bound enzymes. As it is used e.g. for the production of vitamin C, ketogluconates or dihydroxyacetone, it plays an important role in industrial biotechnology. In 2005 the genome sequence of *G. oxydans* 621H was published and revealed characteristic traits concerning sugar metabolism. As the gene encoding phosphofructokinase is missing, the intracellular sugar metabolism cannot proceed via the Embden-Meyerhof-Parnas pathway, but only by the pentose phosphate pathway or the Entner-Doudoroff pathway. In order to study the importance of these two pathways, two deletion mutants were constructed. One lacked the *gnd* gene for 6-phosphogluconate dehydrogenase and thus a functional pentose phosphate pathway. The other mutant lacked the genes *edd* and *eda* encoding 6-phosphogluconate dehydratase and 2-keto-3-deoxy-6-phosphogluconate aldolase and thus a functional Entner-Doudoroff pathway. The characterization of these mutants will be presented, which indicate that the pentose phosphate pathway is of major importance for sugar metabolism in *G. oxydans*.

PSP006
Investigation of PEP-PTS homologous proteins in *Ralstonia eutropha* H16

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Ralstonia eutropha H16 is a facultative chemolithoautotrophic, H_2-oxidizing β-Proteobacterium. The genome consists of two chromosomes and the megaplasmid pHG1 and its nucleotide sequence was published in 2006 [1]. The genome sequence was investigated to identify by *in silico* analysis components of the phosphoenolpyruvate-carbohydrate phosphotransferase system (PEP-PTS), an important method of sugar uptake in many bacteria. Seven gene loci were found to encode for putative PEP-PTS proteins. Besides the *N*-acetylglucosamine-specific PEP-PTS (*nagFE*), a complete PEP-dependent phosphoryl transfer chain is lacking in strain H16. Based on these findings, we generated single and multiple deletion mutants defective in the PEP-PTS genes and gene regions known to be responsible for fructose transport (*frcACB*) to investigate their influence on carbon source utilization, growth behavior and PHB accumulation. In many cases no effect on carbohydrate uptake was observed. As supposed, the H16 Δ*frcACB* and H16 Δ*nagFEC* mutants exhibited no growth when cultivated on fructose and *N*-acetylglucosamine, respectively. In addition to the altered utilization of carbon sources, different phenotypes and modified PHB contents were observed in many mutants. The *fruA*, *ptsH* and *ptsI* single, double and triple mutants stored much less PHB than the wild type and caused reduced PHB synthesis in mutants lacking the H16_A2203, H16_A0384, *frcACB* or *nagFEC* genes. Mutant strain H16 ΔH16_A0384 accumulated 11.5% (wt/wt) more PHB in the cells than the wild type when grown on gluconate and suppressed partially the negative effect of the *fruAptsHI* mutant on PHB synthesis. In contrast, deletion of gene H16_A2203 resulted in no significant difference to the wild type regarding growth and storage behavior. Based on our experimental data we confirmed that the PEP-PTS homologous proteins present in *R. eutropha* H16 are not exclusively involved in the complex sugar transport system but also in cellular regulatory functions.

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PSP007
Two stators contribute to the motility of *Shewanella putrefaciens* CN-32

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Many bacteria are motile by rotating flagella, which generally consist of three major parts: filament, hook, and basal body. The latter includes stator and rotor elements which create torque to drive the flagellum. The rotation is energized by gradients of either protons or sodium-ions across the membrane. The preference of the stators for the driving ions specifies the two major subtypes of flagellar motors. Genome analysis revealed that in several bacteria the number of encoded stator complexes exceeds the number of motor systems. In contrast, *Shewanella putrefaciens* CN-32 harbors two complete flagella gene clusters encoding a putative polar flagella system and a putative lateral flagella system along with two sets of stator elements: the putative sodium-driven PomAB and the putative proton-dependent MotAB complex. By tagging the stator components MotB and PomB with GFP we demonstrated that PomB predominantly localizes at the pole of the cell whereas MotB-GFP has a lateral and polar localization pattern. The deletion of the respective stator genes revealed that each stator element is sufficient to maintain motility. Uncoupling of either the sodium-gradient by the addition of phenamil or collapsing the proton motive force with the protonophor CCCP resulted in a reduced but not abolished motility of the wild type cells. A study using a fusion of GFP to the promoter of the stators indicate a substrate-dependent regulation of the stator elements.
Since most *S. putrefaciens* CN-32 cells possess only a single polar flagellum under planktonic conditions, we propose that both stators might be simultaneously incorporated and function in a single motor system.

PSP008
Multiple β-ketothiolases of *Ralstonia eutropha* H16

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β-Ketothiolases catalyze the first step of poly(3HB) synthesis in bacteria by condensing two molecules of acetyl-CoA to acetoacetyl-CoA. Analyses of the genome sequence of *Ralstonia eutropha* H16 revealed 15 isoenzymes of PhaA in this bacterium. In this study, we generated knockout mutants of various *phaA* homologues to investigate their role and contributions to poly(3HB) metabolism and to suppress biosynthesis of 3HB-CoA for obtaining enhanced molar 3-mercaptopriopionate (3MP) contents in poly(3HB-*co*-3MP) copolymers. Additionally, to examine the role of single homologue, each gene was cloned for heterologous expression in *E.coli*, protein purification and enzyme characterization. *In silico* sequence analysis of PhaA homologues and transcriptome data recommended the homologues *phaA*, *bktB*, H16_A1713/H16_B1771, H16_A1528, H16_B1369, H16_B0381 and H16_A0170 for further analysis. Single and multiple deletion mutants were generated to investigate the influence of these β-ketothiolases on growth and polymer accumulation. The deletion of single genes resulted in no significant differences to the wild type during cultivation on gluconate or gluconate plus 3MP. Deletion of *phaA* plus *bktB* (= H16Δ2 mutant) resulted in approximately 30% less polymer accumulation than in the wild type. Deletion of H16_A1713/H16_B1771, H16_A1528, H16_B0381 and H16_B1369 in addition to *phaA* and *bktB* gave no differences in comparison to the H16Δ2 mutant. In contrast, deletion of H16_A0170 additionally to *phaA* and *bktB* yielded a mutant which accumulated about 30% poly(3HB) (wt/wt, of CDW). We could demonstrate that PhaA, BktB and H16_A0170 are majorly involved in poly(3HB) synthesis in *R. eutropha* H16. We were not able to suppress poly(3HB) biosynthesis completely, but the copolymer compositions could be altered significantly to a lower percentage of 3HB (from 85 to 52 mol%) and a higher percentage of 3MP (from 15 to 48 mol%), respectively.

PSP009
Penicillin Binding Protein 4b of *Escherichia coli* is not a D,D-carboxypeptidase but rather an *N*-acetylmuramyl-L-alanine amidase involved in cell wall recycling

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Penicillin-binding proteins (PBPs) are characterized by their affinity for penicillin and constitute a group of enzymes required for the biosynthesis and modification of the bacterial cell wall. They either catalyze the cross-linking of peptidoglycan (D,D-amino acid transpeptidation) or have D,D-peptidase activity. PBP4b of *E. coli* has been reported to bind penicillin but possesses only very low D,D-carboxypeptidase activity (Vega & Ayala, 2006, Arch. Microbiol. 185: 23-27). Here we report that this protein is rather an *N*-acetylmuramyl-L-alanine amidase (D,L-peptidase). It cleaves the D-lactyl-L-alanine bond of *N*-acetylmuramic acid (MurNAc)-peptides including muramyl dipeptide (MDP). The PBP4b-encoding gene *yfeW* is located in a putative operon together with the genes encoding the MurNAc-specific transporter MurP and the MurNAc etherase MurQ. Therefore a role in MurNAc-peptide recovery is proposed for PBP4b. Interestingly, the enzyme does not accept anhydro-MurNAc-peptides or muropeptides (*N*-acetylglucosamine(GlcNAc)-MurNAc-peptides), indicating a critical role of the MurNAc residue for substrate specificity. Reinvestigation of substrate requirement and a biochemical characterization of PBP4b was enabled by the development of novel highly sensitive coupled assay that bases on the radioactive phosphorylation of MurNAc or anhydro-MurNAc.

PSP010
Will not be presented!

PSP011

Growth rate-dependent physiology of *Aromatoleum aromaticum* EbN1 in anaerobic, benzoate-limited chemostats

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The growth rate-dependent physiological and proteomic response of *Aromatoleum aromaticum* EbN1 was analyzed in anaerobic chemostats under benzoate-limited conditions. Constrained by a defined and constant supply of the limiting nutrient at a specific rate (dilution rate), the bacterial population in the chemostat approached a steady state characterized by a specific growth rate and stable growth parameters (optical density, cell number, nitrate and nitrite concentration). Stabilization of growth parameters was observed after 3 to 5 residence times (calculated as the inverse of the dilution rate).
To analyze global changes in response to different growth rates, cells were harvested from continuous cultures during steady state (12 +/- 0.9 residence times) at low (0.036 h^{-1}), medium (0.108 h^{-1}) or high (0.180 h^{-1}) growth rates, and from batch cultures during growth at the maximum specific growth rate (0.20 h^{-1}). For each growth condition four biological replicates were comparatively analyzed by two-dimensional difference gel electrophoresis (2D DIGE). This revealed dynamic, growth rate-dependent changes in the protein abundance of more than 160 proteins including also proteins involved in benzoate catabolism. Compared to benzoate-limited growth at a high growth rate, the most dramatic changes were observed at a low growth rate, e.g. up-regulation of several periplasmic binding proteins involved in nutrient uptake and in proteins related to other aromatic catabolic pathways. In addition, growth rate-dependent changes in the membrane phospholipid composition and polyhydroxybutyrate (PHB) content were also observed for each of the four growth conditions.

PSP012

Analysis of antibiotic tolerance in *Staphylococcus aureus* - towards the characterization of *S. aureus* persister cells

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Bacterial cultures contain subpopulations of dormant cells, so called persisters, able to survive antibiotic treatment without acquiring heritable resistance. Persisters are not mutants, but reversible phenotypic variants of normally growing cells. We aimed to study mechanisms governing persister formation and their physiologic, cellular, and genetic properties in *S. aureus*.
Different planktonically grown *S. aureus* strains were treated in log or stationary growth phase with various antibiotics. Strains included SA113, the small colony variants (SCVs) *hemB* and *menD*, as well as HG001, HG002, and HG003. Antibiotics applied were daptomycin, tobramycin, ciprofloxacin, rifampin, and penicillin in a range of 1-100-fold MIC.
Time-dependent CFU analyses revealed widely minimal killing of stationary-phase cells, almost irrespective of the strain or the kind and concentration of antibiotic. Hence, the persister state may be the predominant *S. aureus* phenotype in stationary-phase. Two striking exceptions were observed: I) Treatment of SA113 with 100-fold MIC of daptomycin eradicated about 99.98 % of cells within 1 h, whereas the remaining population appeared less vulnerable over time. The biphasic temporal killing kinetics are highly indicative of persister cells. II) Upon treatment with 100-fold MIC tobramycin, the *menD* culture displayed a similar, albeit less pronounced effect.
In exponentially growing cultures daptomycin killed SA113 cells completely within 4 h at 10-fold MIC or 1 h at 100-fold MIC, while SCV killing was retarded. HG001-003 strains were efficiently killed after 1 h at 100-fold MIC of daptomycin. Intriguingly, tobramycin treatment appeared to eradicate SA113 wt less efficiently as SCV strains at both 10- and 100-fold MIC. Killing curves indicated a large fraction of SA113 persisters 1 h after tobramycin treatment, lasting for 5-7 h. Tobramycin treatment at 10-fold MIC of HG001-003 resulted in SCV-like-cells upon cultivation on solid media.
Thus, growth phase, strain background, and genotype appear to be important factors in the formation of *S. aureus* persister cells. We suggest that *S. aureus* tolerance to antibiotics in stationary-phase is strongly associated with elevated levels of persisters.

PSP013

The membrane protein MusI is indispensable for maltose uptake in *Corynebacterium glutamicum* by the ABC-transport system $MusEFGK_2$

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The disaccharide maltose is efficiently used by the Gram-positive *Corynebacterium glutamicum* as substrate for growth and amino acid production. Furthermore maltose can be used as an additive in L-valine fermentations to increase the overall productivity of *C. glutamicum* strains [1]. Maltose is metabolized in *C. glutamicum* by a pathway requiring maltodextrin and glucose formation by the 4-α-glucanotransferase MalQ with maltose as substrate, glucose phosphorylation by the glucose kinases Glk and PPgk and maltodextrin degradation via the reactions of maltodextrin phosphorylase and α-phosphoglucomutase [2, 3]. Maltose uptake is accomplished by an ABC transport system encoded by *musK* (cg2708), *musE* (cg2705), *musF* (cg2704), and *musG* (cg2703).
We here analysed the transcriptional organisation of the *mus* genes using Northern Blots and RT-PCRs: Whereas *musK* and *musE* are transcribed monocistronically in *C. glutamicum*, *musF* and *musG* are part of an operon, which also includes the orf cg2701 (*musI*). The gene *musI* encodes a putative membrane protein, which shares no homologies to so far characterised proteins. Characterisation of growth and of ^{14}C-maltose uptake in the *musI*-mutant strain *C. glutamicum* IMcg2701 showed that maltose utilisation and uptake were abolished. Plasmid encoded expression of *musI* and of *musI-strep* (encodes a N-terminal Streptavidin tagged version of MusI) fully complemented *C. glutamicum* IMcg2701. In Western blot experiments the tagged MusI protein was detected exclusively in the membrane fraction of *C. glutamicum*.
From these results we conclude, that the *musI* encoded protein encodes a novel essential component of the maltose ABC-transporter of *C. glutamicum*, which should be therefore designated $MusEFGK_2I$.

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PSP014

Characterization of a novel subtilisin-like serine protease of *Pseudomonas aeruginosa*

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P. aeruginosa is ubiquitously distributed, living in wet or humid surroundings ranging from soil to human and produces a huge variety of extracellular proteins including several proteases. Some of these proteases like Elastase and Protease IV are well characterized but others exist of which nothing is known so far. Proteases in general are highly relevant for technical enzyme applications. Subtilases for example are typical detergent proteases and are defined as serine proteases belonging to the peptidase_S8 family. These subtilases are encoded as preproenzymes carrying a signal peptide which drives their translocation through the cytoplasmic membrane and a propeptide acting as a folding mediator required to give the protease its final native conformation.
By homology we have identified the open reading frame *PA1242* in the genome sequence of *P. aeruginosa* PAO1 encoding a so far hypothetical protein as a putative member of the subtilisin-like serine protease family S8.

The gene product of *PA1242* (*sprP*) contains a predicted signal sequence and a peptidase S8 domain. However, it contains a non-canonical catalytic triad composed of histidine, asparagine and serine. Sequence analysis revealed the presence of an additional element in the domain organization of the protease. SprP carries beside its signal peptide and the S8 domain a domain of unknown function (DUF) between both elements. After identification SprP was cloned, expressed in *E. coli* and the protease activity was measured with protease substrates like casein and Suc-AAPF-*p*NA. Proteases have also an impact on different physiological processes like protein processing and activation, secretion of other proteins and pathogenicity of the host bacterium. A *P. aeruginosa sprP*-negative mutant was constructed and different phenotypes were tested to elucidate the physiological role of SprP. We were able to illustrate an eminent role of SprP by characterization of different phenotypes. Deletion of *sprP* causes the loss of motility, an increased biofilm formation and the accumulation of cell aggregates during growth.

PSP015
The lipase specific chaperone LipH is required for proper inner membrane translocation of Lipase A in *Pseudomonas aeruginosa*

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Folding of lipase A from *P. aeruginosa* essentially requires *in vivo* and *in vitro* the action of the steric chaperone LipH. Such lipase specific foldases (Lif) consist of an amino terminal membrane anchor followed by a variable 40 aa domain and the large carboxy terminal folding domain. *In vitro* refolding experiments revealed that only the folding domain of LipH is needed to fold lipases into their enzymatically active conformation. The 3D structure of such a folding domain, of the closely related lif protein from *Burkholderia glumae,* was solved in complex with its cognate lipase. In this structure the variable region could not be modeled and was therefore suggested to be very flexible or unstructured. A physiological function of this domain is unknown at present.
We constructed a LipH variant, in which the variable domain was deleted. As consequence the N-terminal membrane anchor was directly attached to the folding domain of LipH. Upon expression of this modified LipH together with its cognate lipase LipA in the homologous host *P. aeruginosa* a complete loss of secretion was detected. Not only lipase LipA was no longer secreted but also other extracellular Sec substrates proteins such as ElastaseB and ExotoxinA failed to reach the culture supernatant, whereas TAT substrates like phospholipase where perfectly secreted.
Expression of the lipase together with the truncated foldase in *P. aeruginosa* leads to a blockage of the Sec apparatus and thus suggests a probable function of the variable domain for interaction of the protein with the Sec-apparatus thereby probably being involved in the release of lipase from the Sec machinery.

PSP016
Biosynthesis and occurence of open chain tetrapyrroles in cryptophytes

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Phycobiliproteins are light-harvesting proteins, which occur in cyanobacteria, red algae and cryptophytes in addition to chlorophyll containing antenna complexes. They allow the organisms to efficiently absorb light in regions of the visible spectrum that are poorly covered by chlorophylls. Cryptophytes are unicellular, eukaryotic algae and widespread in marine and limnic waters. Their phycobiliproteins consist of an (αα‘ββ) heterotetrameric apo-protein covalently associated with characteristic open chain tetrapyrroles, which act as light absorbing chromophores. Cryptophytes employ the six different chromophores phycocyanobilin (PCB), phycoerythrobilin (PEB), 15,16 dihydrobiliverdin (15,16-DHBV), mesobiliverdin (MBV), bilin 584 and bilin 618 for light-harvesting.
The chromophore composition of the novel phycobiliproteins PC577 and PC630 from the cryptophytes *Hemiselmis pacifica* and *Chroomonas sp.* is still unknown. Purification of those phycobiliproteins and subsequent analysis of isolated chromopeptides using High Performance Liquid Chromatography (HPLC) and UV-Vis spectroscopy identified several candidate chromophores. While the PC630 α and α‘ subunits seem to be associated with biliverdin IXα, the chromophore of the PC577 α and α‘ subunit is still unknown. In contrast, PEB is most likely attached to the β subunits of both proteins. Continuative HPLC and NMR experiments will be done to elucidate the correct chromophore composition, which will give further insights into the evolutionary history of cryptophytes.
Not only the chromophore composition of several phycobiliproteins in cryptophytes is unknown but also the biosynthetic pathway of the open chain tetrapyrroles. Therefore the cryptophyte *Guillardia theta* in which the phycobiliprotein PE545 is associated with the chromophores 15,16-DHBV and PEB will serve as a model organism for the elucidation of the biosynthetic pathway. Extensive bioinformatic analyses and amino acid sequence alignments identified a putative heme oxygenase and a PebB-like bilin reductase in *G. theta*. Currently, the enzymatic activities of these putative bilin biosynthesis enzymes is investigated and compared to known activities of cyanobacteria and higher plants.

PSP017
Bacterial cytochrome c peroxidase BCCP of *Shewanella oneidensis* Structure and physiological role under dissimilatory iron reducing conditions

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Bacterial diheme c-type cytochrome peroxidases (CcpAs) catalyze the periplasmic reduction of hydrogen peroxide to water. The γ-proteobacterium *S. oneidensis* produces the peroxidase BCCP under dissimilatory iron reducing conditions. We wanted to understand the function of this protein in the organism as well as its putative connection to the electron transport chain to ferric iron. BCCP was isolated after heterologous expression and tested for its peroxidase activity as well as for its structural conformation as analyzed by X-ray crystallography. BCCP exhibited in vitro peroxidase activity and had a structure typical for diheme peroxidases. It was produced in almost equal amounts under anaerobic as well as microaerophilic conditions. With 50 mM ferric citrate and 50 µM oxygen in the growth medium, BCCP expression results in a strong selective advantage for the cell as was detected in competitive growth experiments between wild type and ΔccpA mutant cells that lack the entire *ccpA* gene due to a markerless deletion. This was expected since we observed a large fraction of the available oxygen being converted into hydrogen peroxide. Hydrogen peroxide production occurred during the entire time course of the growth experiment and was apparently not coupled to a specific growth phase. We were unable to reduce BCCP directly with either CymA, MtrA or FccA but isolated the small monoheme ScyA as an electron transport mediator between CymA and BCCP. As we also were unable to reduce ScyA with other periplasmic cytochromes CymA, ScyA and BCCP seem to build a specific electron transport chain to hydrogen peroxide. Consequently, the so far believed lack of specificity in interprotein electron transport between *c*-type cytochromes has to be questioned.

PSP018
Detoxification of propionyl-CoA in *Candida albicans*: Implications for a modified beta-oxidation pathway

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Propionyl-CoA is a common metabolite deriving from amino acid degradation or breakdown of odd-chain fatty acids. All cells need to avoid an accumulation of propionyl-CoA, since this CoA-ester can interfere with various enzymatic reactions of primary carbon metabolism. Mammals and several bacteria use the so-called methylmalonyl-CoA pathway for detoxification and metabolism of propionyl-CoA, leading to the citric acid cycle intermediate succinyl-CoA. Contrarily, some bacteria and most fungi utilize the methylcitrate cycle for propionyl-CoA degradation. In the latter pathway propionyl-CoA is alpha-oxidized and yields pyruvate. Interestingly, *Candida albicans* neither contains genes encoding enzymes of the methylmalonyl-CoA nor of the methylcitrate cycle, but is able to grow on propionate, odd-chain fatty acids and proteins as carbon sources. Thus, an

alternative pathway for propionyl-CoA degradation must exist. To elucidate the responsible pathway we performed several proteomic and microarray studies. Interestingly, all experiments implied that propionyl-CoA is degraded via beta-oxidation of fatty acids, although is has been assumed that the dehydrogenation of propionyl-CoA to acryloyl-CoA is thermodynamically unfavored. However, in agreement with the assumption of beta-oxidation, a *fox*2 mutant, encoding for a 3-hydroxyacyl-CoA epimerase, required for fatty acid beta-oxidation, was unable to use propionate as sole carbon and energy source. Surprisingly, growth tests showed that the *fox2* mutant is still able to use 3-hydroxypropionate as sole carbon source. Thus, it appears likely that 3-hydroxypropionate is an intermediate of a modified beta oxidation for propionyl-CoA degradation and the final product most likely consists of acetyl-CoA. To further confirm this assumption, we are currently generating mutant strains of the postulated branch of the beta oxidation and apply NMR analyzes on *C. albicans* wild type and mutant cells grown on ^{13}C-labeled propionate. Results will show, whether intermediates of a modified beta-oxidation of propionyl-CoA accumulate in the respective mutants.

PSP019
Resting spores of *Streptomyces coelicolor* harbour an active respiratory nitrate reductase

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Streptomyces coelicolor is an obligate aerobic soil bacterium that belongs to the high-GC Gram-positive actinobacteria. A characteristic of this group is a complex life cycle with stages that include vegetative hyphae, hydrophobic aerial hyphae and production of exospores. During spore formation specific structural proteins, enzymes and storage compounds are synthesized and incorporated into the final spore compartment. These various cellular components ensure that metabolism of these resting spores is maintained at a low-level to retain viability over long periods and at the same time allows them to survive a barrage of environmental insults. Long-term survival requires that essential metabolic pathways to cope with anaerobic conditions are also present. The ability to respire with nitrate is one means by which this can be achieved. The genome of *S. coelicolor* has three *narGHJI* operons, each encoding a respiratory nitrate reductase (Nar) [1], which is membrane-associated with the active site facing the cytoplasm. Previous studies have demonstrated that in spores and exponentially growing mycelium Nar-dependent nitrate reduction occurs [2].
In this study we investigated which Nar is active in spores. Freshly harvested spores of *S. coelicolor* wild type M145 could reduce nitrate at a significant rate without addition of an exogenous electron donor. Moreover, this activity was also detectable in crude extracts of spores and could be visualized by direct staining after native PAGE. Analysis of defined knockout mutants demonstrated that this activity was due to Nar1. Using a discontinuous assay to measure nitrite production by spores we could show that Nar1 was only capable of nitrate reduction under anaerobic conditions. Since Nar1 activity was measurable in crude extracts of spores that were incubated both anaerobically and aerobically this finding suggests that spores regulate either nitrate transport or Nar1 activity in response to oxygen. Notably, studies using protein synthesis inhibitors revealed that Nar1 is always present and active in resting spores.

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PSP020
Diversity in bacterial degradation of the steroid compound cholate

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Steroids are ubiquitous natural compounds with diverse functions in eukaryotic organisms. They enter the environment mainly via excretion by and decay of animals and plants. In bacteria, steroids occur only as rare exceptions but the ability of transforming and degrading steroids is widespread among bacteria. The only well-described pathway for aerobic degradation of steroid compounds is the so-called 9,10-seco pathway [1]. In this study, the organismic and biochemical diversity of steroid-degrading bacteria was assessed by quantitative enrichments of steroid-degrading bacteria with littoral sediments of Lake Constance and the bile salt cholate as a model substance.
Fifteen different strains of cholate-degrading bacteria were isolated from high dilutions of littoral sediments. Two strains were characterized further. According to growth experiments and HPLC-analysis the first strain, *Zoogloea* sp. strain 1, degraded cholate via the 9,10-seco pathway as indicated by the formation of the characteristic degradation intermediates DHADD (7,12-dihydroxy-1,4-androstadiene-3,17-dione) and THSATD (3,7,12-trihydroxy-9,10-secoandrosta-1,3,5(10)triene-9,17-dione). During cholate degradation by the second strain, *Dietzia* sp. strain 2, the characteristic intermediates of the 9,10-seco-pathway were not detected. Instead, two new compounds were detected by HPLC-analysis that differed from the UV-spectra of steroid compounds occurring in the 9,10-seco-pathway. Strain 2 could also not grow with the characteristic intermediates of cholate degradation, which were isolated from cultures of the cholate-degrading bacterium *Pseudomonas* sp. strain Chol1 [2, 3]. In addition, the presence of these compounds inhibited cholate degradation by strain 2. These results clearly showed that strain 2 harbours a different pathway for cholate degradation, which has not been described so far, indicating that the biochemical diversity of aerobic steroid degradation in bacteria has been underestimated.

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PSP021
A novel high-affinity hydrogenase in *Ralstonia eutropha*

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Within the global hydrogen cycle, soil deposition is the most important natural process responsible for removal of H_2 from the atmosphere. However, the mechanism by which H_2 is taken up remained elusive. Recently, a high-affinity hydrogenase has been indentified in spore-forming Actinomycetes of the genus *Streptomyces*, which is able to oxidize H_2 at atmospheric levels. It has been suggested that this class of [NiFe]-hydrogenases is responsible for the H_2 uptake in soils [1].
Interestingly, the genes coding for this high-affinity hydrogenase are also present in the genome of the beta-proteobacterium *Ralstonia eutropha* [2]. The two structural genes, encoded the hydrogenase small and large subunits, are part of a conserved operon structure, which also contains a complete set of hydrogenase maturation genes and a number of conserved unknown genes. On the basis of its high similarity to the hydrogenases from spore-forming actinomycetes, the protein was designated actinomycetes hydrogenase (AH).
Recently, we could show that *Ralstonia eutropha* cells containing solely the AH are capable in H_2 uptake as determined by gas chromatography. For a detailed investigation of the biochemical properties of the AH, a strain was constructed in which the weak native promoter of the AH operon was exchanged by the strong promoter of the membrane-bound hydrogenase genes from *Ralstonia eutropha*. AH-mediated H_2-oxidizing activity in soluble protein extracts was shown by activity staining in native gels using NBT as an artificial electron acceptor. The AH was active also in the production of HD and D_2 from D_2O as shown by H/D-exchange experiments. We are currently constructing an AH derivative carrying an affinity tag for facile purification and subsequent electrochemical and spectroscopic characterization. Furthermore, we are conducting experiments in order to determine the regulatory background of AH gene expression and the role of this interesting enzyme in *R. eutropha*. One attractive hypothesis is that the AH may contribute to the survival of the cells under starvation conditions by using the atmospheric trace concentrations of H_2.

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PSP022

Genome analysis and heterologous expression of acetate-activating enzymes in the anammox bacterium *Kuenenia stuttgartiensis*

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Bacteria capable of anaerobic ammonium oxidation (anammox) derive their energy for growth from the conversion of ammonium and nitrite into dinitrogen gas, thereby constituting a significant sink for fixed nitrogen under anoxic conditions. Cellular carbon is hypothesized to be fixed via the acetyl-CoA pathway, suggesting a chemolithoautotrophic lifestyle. However, it was shown that anammox bacteria have a more versatile metabolism than previously assumed: several genera have been shown to use organic compounds i.e. acetate as electron donors to reduce nitrate and nitrite to dinitrogen gas via ammonium. Acetate is an environmentally relevant organic acid that has to be activated to acetyl-CoA prior to its utilization in metabolism. One of the key enzymes catalyzing the direct formation of acetyl-CoA from acetate is AMP-forming acetyl-CoA synthetase (ACS). In prokaryotes it is known to operate in an assimilatory route during growth on low acetate concentrations.
The present study focuses on the functional expression of the most highly expressed acetate-activating enzyme of *K. stuttgartiensis*, a putative *acs* gene. An *ackA-pta-acs* triple mutant of *E. coli* was complemented with the *K. stuttgartiensis acs* gene resulting in recovery of growth on acetate. The purified enzyme showed activity towards several short chain organic acids with the highest conversion rates for acetate. The specific activity with propionate and formate was reduced by 1.2 and 1.5-fold respectively; whereas butyrate and isobutyrate were converted at even lower rates. The broad substrate specificity might be established by a substitution in one of four conserved residues in the acetate-binding pocket that determines specificity of the acyl-substrate as has been shown previously.
Here we could demonstrate that acetate could be activated by an *acs*-like protein of *K. stuttgartiensis.* This is a first indication about the mechanism of acetate utilization in anammox, although the incorporation of acetate-derived carbon into cellular biomass could not be detected so far.

PSP023

The CoxD protein, a novel AAA+ ATPase involved in metal cluster assembly: hydrolysis of nucleotide-triphosphates and oligomerization

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The CoxD protein from the aerobic CO-utilizing, chemolithoautotrophic α-proteobacterium *Oligotropha carboxidovorans* is involved in the posttranslational biosynthesis of [$CuSMoO_2$] active site of CO dehydrogenase [1]. CoxD is predicted as a MoxR-like AAA+ ATPase chaperone related to the hexameric, ring-shaped BchI component of Mg^{2+}-chelatases [1,2]. Because it was not possible to purify homologous CoxD in an active state from cytoplasmic membranes its role as an AAA+ ATPase was mainly confined to the knowledge of its primary sequence. Here we show the recombinant production of functional CoxD protein from inclusion bodies produced in *E. coli* and present direct evidence which establishes CoxD as an AAA+ ATPase.
Recombinant CoxD protein was expressed in inclusion bodies at a level of 38 % of the total cell protein and was purified to 95 % homogeneity. The CoxD inclusion bodies were solubilized employing elevated concentrations of urea, and CoxD was refolded by pulsed ultradilution (~50-fold). Uv-vis and circular dichroism spectroscopy indicated that refolded CoxD is stably soluble and contains secondary structural elements. Refolded CoxD protein was shown to hydrolyze ATP in a Mg^{2+} depending reaction yielding inorganic phosphate (P_i) and ADP in equimolar amounts. V_{max} of MgATP hydrolysis was 8.86 nmol P_i min^{-1} mg^{-1} with a K_M of 0.58 mM MgATP. Hydrolysis of MgATP was hampered by MgATPγS but not affected by MgGTP. Sucrose density gradient centrifugation suggested that CoxD oligomerizes as a hexamer, and direct evidence for the oligomerization of CoxD was obtained from electron microscopy of negatively stained (uranyl acetate) samples. With the BchI subunit of Mg-chelatase as template, a 3D structure prediction of CoxD was generated.

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PSP024

Denitrification is linked to magnetite biomineralization in *Magnetosprillum gryphiswaldense*

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Magnetospirillum gryphiswaldense is an aquatic microorganism, which can synthesize intracellular magnetic particles referred to as bacterial magnetic particles or magnetosomes. *M. gryphiswaldense* is also capable of dissimilatory nitrate reduction. The magnetite synthesis is only induced when the oxygen concentration is below a threshold value [1], and it has been suggested that NirS protein had a novel function, Fe (II): nitrite oxidoreductase *in vitro* [2]. However, the relationship between denitrification and magnetite biomineralization is poorly understood.
Metabolic reconstruction from *M. gryphiswaldense* genome data revealed a complete pathway of denitrification, including genes for nitrate reductase (*nap*), nitrite reductase (*nirS*), nitric oxide reductase (*norCB*) and nitrous oxide reductase (*nosZ*).
A *Δnap* deletion mutant had no obvious effect on growth and magnetosome formation. A *ΔnirS* mutant in aerobic culture showed a similar growth rate as wild type. However, *ΔnirS* was clearly impacted on growth and magnetism under micro- and anaerobic conditions in the present of nitrate. Smaller, misshapen and misaligned magnetite crystals were formed in *ΔnirS* mutant. In addition NirS protein was upregulated by nitrate and downregulated by nitrite. *ΔnorCB* could not grow under micro- and anaerobic conditions, but had a lower magnetism and poor growth when higher oxygen was supplied. *ΔnosZ* did not affect magnetosome formation, but only showed a lower growth under anaerobic conditions, which might be resulted from less energy supply. Our data indicate the denitrification genes have effects on growth and magnetosome formation in *M. gryphiswaldense*. The effects of denitrification, in particular nirS, are consistent with former suggestion. NirS protein might participate in magnetosome formation during denitrification by oxidation of ferrous to ferric formation of mixed-valence Fe_3O_4 under anaerobic conditions.

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PSP025

Biosynthesis of (Bacterio)chlorophylls: ATP-Dependent Transient Subunit Interaction and Electron Transfer of Dark Operative Protochlorophyllide Oxidoreductase

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Dark operative protochlorophyllide oxidoreductase (DPOR) catalyzes the two electron reduction of protochlorophyllide *a* to form chlorophyllide *a*, the last common precursor of chlorophyll *a* and bacteriochlorophyll *a* biosynthesis. Although DPOR shares significant amino acid sequence homologies to nitrogenase only the initial catalytic steps resemble nitrogenase catalysis. During ATP-dependent DPOR catalysis the homodimeric $ChlL_2$ subunit carrying a [4Fe-4S] cluster, transfers electrons to the corresponding heterotetrameric subunit $(ChlN/ChlB)_2$ which also possesses a redox active [4Fe-4S] cluster. To investigate the transient interaction of both subcomplexes and the resulting electron transfer reactions, the ternary DPOR enzyme holocomplex comprising subunits ChlN, ChlB and ChlL was trapped as an octameric $(ChlN/ChlB)_2(ChlL_2)_2$ complex after incubation with the non hydrolyzable ATP analogs adenosine-5´(γ-thio)-triphosphate, adenosine-5´(βγ-imido)-triphosphate or MgADP in combination with AlF_4^-. Additionally, a mutant $ChlL_2$ protein, with a deleted $Leucin^{153}$ in the switch-II region also allowed for the formation of a stable octameric complex. Electron paramagnetic resonance spectroscopy of ternary DPOR complexes revealed a reduced [4Fe-4S] cluster located on $ChlL_2$, indicating that complete ATP hydrolysis is a prerequisite for

intersubunit electron transfer. Circular dichroism spectroscopic experiments indicated nucleotide-dependent conformational changes for $ChlL_2$ after ATP binding. A nucleotide-dependent switch mechanism triggering ternary complex formation and electron transfer was concluded. The crystal structure of the $(ChlN/ChlB)_2$ complex revealed three cysteine residues and a highly unusual aspartate residue for the coordination of the redox active [4Fe-4S] cluster of this catalytic subcomplex.

PSP026

Serratia odorifera emits a complex bouquet of volatiles

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Only very recently it was realized that bacteria, including aboveground and belowground living species, are able to emit enourmous spectra of volatiles. The physiological and ecological functions of compound mixtures and/or individual compounds are presently far from being understood. To determine the complete volatile spectrum of the rhizobacterium *Serratia odorifera* 4Rx13 a combination of different techniques including coupled gas chromatography/mass spectrometry (GC/MS), proton-transfer-reaction mass spectrometry (PTR-MS), laser photoacoustic spectroscopy, mid-infrared laser based spectroscopy and different analytical chemistry methods were applied [1]. More than 100 compounds were emitted from *S. odorifera* 4Rx13 comprising one of the most comprehensive bacterial volatile profiles known to date. Two main components methanethiol and ´sodorifen`, a novel bicyclic multiple methylated octadien, were accompanied by dimethyl disulfide (DMDS), dimethyl trisulfide (DMTS), 2-phenylethanol, several terpenoids and methanol. In addition to organic volatiles ammonia was released, while ethylene, nitric oxid (NO) and hydrogen cyanide (HCN) could not be detected. Experiments showed that the composition of the bouquet did not alter during the growth of *S. odorifera*. The highest emission was detected at the beginning of the stationary phase.
We are presently investigating which role these volatiles play in organismic interactions (e.g. communication, defence, attraction).

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PSP027

The phototrophic bacterium *Chloroflexus aurantiacus* forms acetate from acetyl-CoA via an „archaeal" ADP-forming acetyl-CoA synthetase

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In prokaryotes, the mechanism of acetate formation from acetyl-CoA and the concomitant synthesis of ATP from ADP and phosphate appear to be domain specific. In archaea, this reaction is catalyzed by an unusual Acetyl-CoA Synthetase (ADP-forming) (ACD), (acetyl-CoA + ADP + P ↔ acetate + ATP + CoA) (1) whereas bacteria utilize the classical two-enzyme mechanism involving phosphotransacetylase (PTA) and acetate kinase (AK). Here we studied the mechanism of acetate formation in the bacterium *Chloroflexus aurantiacus*, which excrete significant amounts of acetate during phototrophic growth on glucose. In acetate-forming cells, activities of PTA and AK could not be detected; however, the cells contained inducible activity of an ACD. The ACD was purified and the encoding gene identified via MALDI-TOF analysis. The *acd* gene was expressed in *E. coli* and the recombinant enzyme biochemically characterized. The enzyme is a homotetrameric protein composed of 70 kDa-subunits. Substrate-specificities for acetyl-CoA/acetate and other acyl-CoA esters/acids were determined, defining the *Chloroflexus* enzyme as ACD-isoenzyme I. This isoenzyme has been reported to be the predominant ACD in sugar fermentation of archaea. It is concluded that the bacterium *C. aurantiacus* utilizes an ACD, i.e. the „archaeal mechanism" for conversion of acetyl-CoA to acetate. This is the first report of a functional acetate forming ACD in the domain of bacteria.

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PSP028

Requirement of the proteins CoxE and CoxF for the assembly of the $[CuSMoO_2]$ cluster in the active site of CO dehydrogenase

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CO dehydrogenase of the chemolithoautotrophic α-proteobacterium *Oligotropha carboxidovorans* OM5 is a structurally characterized molybdenum containing iron-sulfur flavoenzyme which catalyzes the oxidation of CO ($CO + H_2O \rightarrow CO_2 + 2\ e^- + 2\ H^+$) [1]. The $[CuSMoO_2]$ cluster in its active site is subject to posttranslational assembly. The proteins CoxD, CoxE, and CoxF are assumed to form a complex, which introduces S and Cu^+ into $[MoO_3]$ using apo-CO dehydrogenase as a scaffold. The three Cox-proteins resemble BchI, BchD and BchH of Mg-chelatase which catalyses the introduction of Mg^{2+} into protoporphyrin IX [2]. CoxD is a novel AAA+ ATPase which is required for the sulfuration of $[MoO_3]$ [2].
CoxE is a Von Willebrand Factor A (VWA) protein with a VWA domain and a MIDAS motif. BchD (which is analogous to CoxE) serves as a platform for the assembly of the Mg-chelatase complex [4]. CoxF is predicted as a histidine acid phosphatase with a VWA binding motif and the copper binding motif MCxxHxxM [5].
To get information on the functions of CoxE and CoxF, the corresponding genes have been inactivated by insertion of a kanamycin resistance cassette which led to the mutants *O. carboxidovorans* OM5 E::km and F::km, respectively. Both mutants were unable to utilize CO under chemolithoautotrophic conditions, but they could be cultivated with H_2 plus CO_2 in the presence of CO to induce the transcription of *cox* genes. The mutant in *coxE* formed a fully assembled, but completely inactive apo-CO dehydrogenase, whereas the mutant in *coxF* was leaky to some extent because its apo-CO dehydrogenase showed roughly 1% of the holo-enzyme activity. The CO-oxidizing activity in both apo-CO dehydrogenases could be restored through reconstitution with the $[Cu^+(thiourea)_3]$ complex, which suggests the presence of a $[MoO_2S]$ site. However, this applied only to a fraction of the entire apo-CO dehydrogenase population, which might be explained by chemical modifications at the Mo-SH; this aspect is subject to current research. The presence of $[MoO_2S]$ in the apo-CO dehydrogenases of the two mutants is further corroborated by EPR spectroscopy which showed Mo(V) resting signals. Based on this data a model on the assembly of the $[CuSMoO_2]$ cluster is proposed.

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PSP029

A novel (*S*)-citramalyl-CoA/(*R*)-3-hydroxy-3-methylglutaryl-CoA lyase in Archaea, Bacteria and Eukarya

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The genomes of many actino- and proteobacteria as well as of haloarchaea and animals possess homologues of a gene encoding citrate lyase β-subunit, although a gene for the α-subunit of this protein is absent. Examples of such organisms are *Pseudomonas aeruginosa*, *Mycobacterium tuberculosis*, *Haloarcula marismortui* as well as *Homo sapiens*. The corresponding genes from these organisms were cloned, overexpressed in *Escherichia coli*. The encoded enzymes were identified as bifunctional (*S*)-citramalyl-CoA/ (*R*)-3-hydroxy-3-methylglutaryl-CoA lyases catalyzing the following reactions:
(*S*)-citramalyl-CoA → acetyl-CoA + pyruvate
(*R*)-3-hydroxy-3-methylglutaryl-CoA → acetyl-CoA + acetoacetate
Furthermore, we showed that in *M. tuberculosis* and *H. marismortui* this enzyme is involved in a modified leucine degradation pathway. In the classical pathway, leucine is first converted to 3-methylglutaconyl-CoA, which is further hydrated to (*S*)-3-hydroxy-3-methylglutaryl-CoA and then cleaved to acetyl-CoA and acetoacetate. In the novel modified pathway, 3-methylglutaconyl-CoA hydratation is catalyzed by an (*R*)-specific enoyl-CoA hydratase and leads to (*R*)-stereoisomer of 3-hydroxy-3-methylglutaryl-CoA. As in the classical pathway, this compound is further split into acetyl-CoA and acetoacetate in the CitE catalyzed reaction. In contrast to haloarchaea and mycobacteria, in *P. aeruginosa* this enzyme functions in vivo as (*S*)-citramalyl-CoA lyase in itaconate catabolism.

Correspondingly, *P. aeruginosa* mutants defect in *citE* were not capable to grow on itaconate. In mammals, (*S*)-citramalyl-CoA/ (*R*)-3-hydroxy-3-methylglutaryl-CoA lyase is probably involved in itaconate degradation as well. The source of itaconate and the role of this pathway in the mitochondrial metabolism remains to be shown.

PSP030
Gene regulation of *Geobacter metallireducens* under different growing conditions

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Aromatic hydrocarbons concentration in groundwater are often exceeding the electron acceptor availability of oxygen dissolved in groundwater, which results in a change from oxic to anoxic conditions. Contaminant degradation has, therefore, to proceed anaerobically. However, it is totally unknown how gene regulation functions under in situ conditions. On chemostat experiments showed that under carbon limiting conditions all catabolic pathways are expressed and different carbon sources are utilized simultaneously.
We are interested in how genes are regulated in the model organism *Geobacter metallireducens* under in situ conditions. We cultivated G. metallireducens with different carbon sources (phenol, toluene, benzyl alcohol, benzoate, acetate) and under excess and limiting carbon sources (batch culture and retentostat). The expression levels of different catabolic genes were quantified by RT-qPCR and transcriptome approaches. Our work will contribute to elucidate what microbes are really doing in the environment.

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RGV001
The role of c-di-GMP in phototactic motility of *Synechocystis* sp. PCC 6803 cells

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The cyanobacterium *Synechocystis* sp. PCC 6803 exhibits flagellar-independent „twitching motility" that allows bacteria to move over moist surfaces using type IV pili. Mutants that lost type IV pili are non-motile. In order to use optimal light qualities and quantities for photosynthesis, they are able of directed movement along a light gradient. Regulation of phototactic motility is complex and involves many different gene products, including amongst others different photoreceptors, the RNA chaperone Hfq and adenylate cyclases.Here we demonstrate a biological function of the Cph2 photoreceptor in motility. Wild-type *Synechocystis* cells fail to move towards blue light, whereas *Dcph2* mutant cells show blue-light induced motility. Accordingly, Cph2 is responsible for inhibiting cyanobacterial phototaxis towards blue light. Apart from possessing two distinct photosensory modules, *Synechocystis* Cph2 differs from most other phytochromes by harbouring two GGDEF and one EAL domains as effector regions instead of histidine kinase domains. GGDEF and EAL domains were found to be involved in the turnover of c-di-GMP, a novel second messenger molecule involved in motility and sessility behaviour in bacteria. Signalling proteins with GGDEF domains synthesize c-di-GMP from two GTPs. Cleavage of c-di-GMP is carried out by EAL domains that exhibit phosphodiesterase activity. We show here that overexpression of the C-terminal GAF domain together with the associated GGDEF domain leads to inhibition of motility, suggesting that light induced changes of the c-di-GMP level in *Synechocystis* cells regulate phototactic responses. In addition, we performed experiments demonstrating that expression of Cph2 in *E. coli* leads to changes in flagellar-based motility of these cells.

RGV002
Protein exchange dynamics and chemotaxis cluster stability in *Escherichia coli*

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Chemotaxis enables bacteria to quickly find optimum growth conditions. Sensing attractants or repellents is based on a simple two-component signal transduction system. The chemotaxis system of *Escherichia coli* is thoroughly studied and allows cells to move towards attractants and away from repellents. Effectors are sensed by transmembrane receptors, which are organized in clusters. The cluster core is composed of receptors, the histidine kinase CheA and the adaptor protein CheW. All other chemotaxis proteins, like response regulator CheY and its phosphatase CheZ, as well as the adaptation proteins CheR and CheB localize either to receptors or to CheA. Despite minimal complexity, this system demonstrates amazing performance that remains partly unaccounted for, despite decades of intensive research.
We established fluorescence recovery after photobleaching (FRAP) to systematically analyze the turnover of all chemotaxis proteins at bacterial receptor clusters *in vivo*, and thereby filled one of the last gaps in quantitative understanding of the chemotaxis pathway. We could separate several classes of chemotaxis proteins, that can be assigned to their characteristic signaling function [1]. Moreover, we recently extended FRAP analyses to investigate effects of temperature and pathway activity on cluster stability. Contrary to biochemical observations, temperature did not affect cluster stability *in vivo.* However, in accordance with biochemical studies we observed that active clusters were indeed more stable, implying an additional level of regulation in chemotaxis.

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RGV003
Helicobacter pylori as a new model organism for riboregulation in bacteria lacking the RNA chaperone Hfq

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Question: Genome sequencing of *Helicobacter pylori* has revealed the potential proteins and genetic diversity of this prevalent human pathogen, yet little is known about its transcriptional organization and non-coding RNA output. The microaerophilic, Gram-negative ε-proteobacterium was even regarded as an organism without riboregulation as it lacks the RNA chaperone Hfq, a key player in small RNA (sRNA)-mediated regulation in many bacteria. However, also *Helicobacter* has to cope with diverse stresses, e.g. pH fluctuations or changes in nutrient availability, during infection and colonization of the human stomach. Therefore, we reasoned that it might also use sRNAs as an additional layer for regulation of gene expression during stress or virulence.
Methods: Massively parallel cDNA sequencing (RNA-seq) has been revolutionizing the analysis of transcriptomes from both eukaryotes and prokaryotes. Recently, we have developed a novel differential approach (dRNA-seq) selective for the 5' end of primary transcripts that allowed us to present a global map of *H. pylori* transcriptional start sites (TSS) and its operon structure [1]. We discovered hundreds of TSS within operons, and opposite to annotated genes, suggesting that the complexity of gene expression from the small *H. pylori* genome is increased by uncoupling of polycistrons and by genome-wide antisense transcription. Furthermore, we also discovered around 60 small RNAs including the ε-subdivision counterpart of the regulatory 6S RNA and associated pRNAs, and potential regulators of *cis*- and *trans*-encoded target mRNAs. Now we aim at a functional characterization of abundant sRNAs and antisense RNAs along with their potential role in *Helicobacter* virulence as well as the identification of associated RNA-binding proteins and new regulatory mechanisms. For example, microarray-based analyses of global whole-transcriptome changes of sRNA deletion or overexpression mutants will facilitate to identify direct mRNA targets. A first example of a classical trans-acting sRNA which represses one of the chemotaxis receptors in *Helicobacter* will be presented.
Conclusion: Based on the transcriptome dataset, we are now using *H. pylori* as a new model organism for sRNA-mediated regulation in bacteria without

the common Hfq protein. The identification of diverse sRNA candidates indicates that riboregulation constitutes an important layer of gene regulation in *Helicobacter*. Research in *H. pylori* will also help to shed light on sRNA-mediated regulation in other ε-proteobacteria, including widespread and emerging pathogens such as *Campylobacter*.

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RGV004
Regulation of Ammonium Uptake and Complex Formation between Amt and GlnK Proteins

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Ammonium transport (Amt) proteins are a family of integral membrane proteins that specifically transport NH_3/NH_4^+ across biological membranes. Although high resolution structures are known for *E. coli* AmtB [1], *A. fulgidus* Amt-1 [2], *N. europaea* Rh50 [3] and the human RhCG [4], a number of controversies persist around several aspects of the transport mechanism [5]. In order to gain a better understanding of Amt function and regulation, we use the hyperthermophilic euryarchaeon *Archaeoglobus fulgidus* as a working model. Its genome organization shows three *amt* genes, each directly linked to a *glnK* gene within one operon. GlnKs are trimeric cytoplasmic proteins that belong to the P_{II} family and have a key function in the regulation of nitrogen assimilation in the cell. They can bind, and thus directly sense, effector molecules such as adenosine diphosphate, adenosine triphosphate and 2-oxoglutarate. The integration of these signals (energy and carbon cellular status, respectively) by GlnK proteins can result in a complex formation with Amt in the membrane and consequent blockage of NH_3/NH_4^+ uptake [6]. We have characterized all three *A. fulgidus* GlnK proteins in their interaction mode with effector molecules by means of X-ray crystallography and isothermal titration calorimetry [7] and investigate Amt-GlnK complex formation events. These findings provide new insights into the regulation of ammonium uptake and nitrogen assimilation in *A. fulgidus* in particular, and in archaea in general.

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RGV005
The role of the cytoplasmic PAS domain of the *Escherichia coli* histidine kinase DcuS in signal transduction

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Bacteria contain membrane integral sensors for the response to changing environmental conditions. Many of the sensors are two-component systems consisting of a sensor histidine kinase and a response regulator that triggers the cellular response [1]. DcuS, the C_4-dicarboxylate sensor of *E. coli* is a membrane integral histidine kinase [2]. DcuS is a multidomain protein consisting of a sensory periplasmic PAS_P (Per-Arnt-Sim) domain, two transmembrane helices, a cytoplasmic PAS_C and the C-terminal kinase domain.
PAS domains are ubiquitous signalling modules found in all kingdoms of life. They can detect many different stimuli including light, oxygen, redox potential and various small molecules and also modulate protein-protein interactions. PAS domains are characterised by a conserved α/β-fold. Many PAS domains with sensory function have been identified but a large number of PAS_C domains contain no apparent cofactor and their function is unknown [3].
A combination of mutation, protein-protein interaction and solid-state NMR experiments [4] were used to study the structure and function of a membrane embedded construct of DcuS and of the PAS_C domain. The experiments show that PAS_C has no sensory function and is responsible for signal transduction from PAS_P to the C-terminal histidine kinase.

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RGV006
LaeA control of velvet family regulatory proteins for light-dependent development and fungal cell-type specificity

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VeA is the founding member of the velvet superfamily of fungal regulatory proteins. This protein is involved in light response and coordinates sexual reproduction and secondary metabolism in *Aspergillus nidulans*. In the dark, VeA bridges VelB and LaeA to form the VelB-VeA-LaeA (velvet) complex. The VeA-like protein VelB is another developmental regulator, and LaeA has been known as global regulator of secondary metabolism. In this study, we show that VelB forms a second light-regulated developmental complex together with VosA, another member of the velvet family, which represses asexual development. LaeA plays a key role not only in secondary metabolism but also in directing formation of the VelB-VosA and VelB-VeA-LaeA complexes. LaeA controls VeA modification and protein levels and possesses additional developmental functions. The *laeA* null mutant results in constitutive sexual differentiation, indicating that LaeA plays a pivotal role in inhibiting sexual development in response to light. Moreover, the absence of LaeA results in the formation of significantly smaller fruiting bodies. This is due to the lack of a specific globose cell type (Hülle cells), which nurse the young fruiting body during development. This suggests that LaeA controls Hülle cells. In summary, LaeA plays a dynamic role in fungal morphological and chemical development, and controls expression, interactions and modification of the velvet regulators.

RGP001
Fluorescence-based monitoring of the nitrogen status in *Corynebacterium glutamicum*

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AmtR, a member of the TetR protein family, is the master regulator of nitrogen control in *Corynebacterium glutamicum* [1]. This repressor, which acts as a dimer, regulates transcription of at least 38 genes when ammonium, the preferred nitrogen source of *C. glutamicum*, becomes limiting [2, 3]. Upon ammonium starvation, AmtR-controlled genes are transcribed. Until now, detection of nitrogen starvation in C. glutamicum was only possible by *in vitro* assays such as RNA hybridization experiments and DNA microarrays. The aim of this study was to establish an *in vivo* nitrogen monitoring system that allows a more rapid detection of nitrogen limitation. Therefore, promoter regions of AmtR-controlled genes were cloned upstream of a plasmid-encoded gfpuv gene using the pEPR1 plasmid [4]. Fluorescence spectroscopy as well as fluorescence microscopy showed that the strictly AmtR-dependent promoters $amtA_P$, $amtB_P$ and $gltB_P$ are well suited for a fluorescence-based reporter system: whereas under good ammonium supply no fluorescence was observed, cells that were starved for ammonium showed high fluorescence signals. Moreover, this method also revealed that alternative nitrogen sources than ammonium differentially affect AmtR-controlled gene expression.

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RGP002
Bistability in *myo*-inositol utilization by *Salmonella enterica* serovar Typhimurium

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The capability of *Salmonella enterica* serovar Typhimurium to utilize *myo*-inositol (MI) is determined by the genomic island GEI4417/4436 carrying the *iol* genes. These encode enzymes, transporters and the repressor IolR. This autoregulated protein binds to four *iol* promoters and is released upon binding of DKP, a metabolite of MI degradation . In contrast to all gram-negative and gram-positive bacteria investigated so far, *S. enterica* serovar Typhimurium strain 14028 growing on MI as sole carbon source is characterized by a remarkable long lag phase of 40-60 hours. On solid medium containing MI as sole carbon source, this human pathogen exhibits a bistable phenotype characterized by a dissection into large colonies and a slow-growing bacterial background. This heterogeneity is reversible and not caused by mutation. It is not observed in the absence of the *iol* gene repressor IolR, nor in the presence of at least 0.55% CO_2. Upon analysis of promoter-*gfp* fusions, bistability could be linked to the activity of the *iolE* promoter (P_{iolE}) that is not controlled by IolR. On the single cell level, fluorescence microscopy and flow cytometry analysis revealed a gradual switch of P_{iolE} from the „off" to the „on" status during the late lag phase and the transition to the log phase. Adding of ethoxyzolamide, an inhibitor of carboanhydrases, elongated the lag phase in the presence of bicarbonate. The positive feedback loop *via* repressor release and positive induction by bicarbonate/CO_2 might allow strain 14028 to adapt to rapidly changing environments. This is a novel example of bistability in substrate degradation, and, to our knowledge, the first example of gene regulation by bicarbonate/CO_2 in *Salmonella*.

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RGP003
Regulation of mitochondrial DNA inheritance and integrity by the *a2* mating-type locus genes *lga2* and *rga2* of *Ustilago maydis*

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The *Ustilago maydis a2* mating-type locus genes *lga2* and *rga2* play a role in controlling uniparental mitochondrial DNA (mtDNA) inheritance during the sexual cycle. In particular, *lga2* triggers selective loss of mtDNA of the *a1* partner, while *rga2* plays a role in protecting the *a2*-associated mtDNA from elimination. The mode of action of Lga2 and Rga2 is currently unclear, however, Lga2 likely acts by causing transient damage of unprotected mitochondria. This is exemplified by large-scale transcriptional deregulation as well as efficient mitophagy in cells conditionally overexpressing *lga2*. Here, mitophagy, albeit controlled by *atg8*, follows a different induction mechanism than under starvation conditions and involves the mitochondrial fission factor *dnm1*. Interference with mitochondrial fusion during mating is a major consequence of *lga2* and efficiently constrains recombination between parental mtDNAs. In this regard, we could provide evidence for mitochondrial intron-encoded homing endonuclease activity and an underlying role in promoting mtDNA recombination under conditions of biparental inheritance.

RGP004
Three distinct NssR-type regulators are involved in transcriptional control of *Wolinella succinogenes* gene clusters encoding reductases for nitrate, nitrite and nitrous oxide

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Epsilonproteobacteria form a globally ubiquitous group of ecologically significant organisms and comprise a diverse range of host-associated and free-living bacteria. Many of these reduce nitrate to nitrite followed by either nitrite ammonification or denitrification [1], but little is known about epsilonproteobacterial nitrosative stress defence, nitrogen compound sensing and the corresponding transcriptional regulation of respiratory enzymes.
The model Epsilonproteobacterium *Wolinella succinogenes* uses the Nap, Nrf and *c*Nos systems to reduce nitrate, nitrite or nitrous oxide (yielding either ammonium or dinitrogen) and all three enzyme systems are upregulated in the presence of nitrate or nitrous oxide. Typical NssR binding sequences are present upstream of the transcriptional start sites of the *nap*, *nrf* and *nos* gene clusters and three distinct NssR-type regulators belonging to the Crp-Fnr-Dnr superfamily of transcription regulators are encoded on the *W. succinogenes* genome. Corresponding gene deletion mutants were constructed and characterized with respect to anaerobic growth and induction of the terminal reductases NapA, NrfA and *c*NosZ by various nitrogen compounds.
The experimental data indicate that all three NssR-type regulators are specifically involved in respiratory nitrogen metabolism and/or nitrosative stress defence by activating *nap*, *nrf* and *nos* gene expression in response to either nitrate, nitrous oxide or nitric oxide-induced stress.

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RGP005
Characterization of the GlnR regulon in *Mycobacterium smegmatis*

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Based on sequence analyses and studies of a deletion mutant, the OmpR-type regulator GlnR was recently identified as the transcriptional regulator of nitrogen metabolism in *Mycobacterium smegmatis* [1, 2]. Transcriptional regulation of the two target genes *amtB* (ammonium transporter) and *glnA* (glutamine synthetase) by GlnR was already shown, as well as binding of the regulator protein to the corresponding promoter regions [2].
For further investigations, a global analysis method was chosen: gene expression under nitrogen starvation was compared between the *M. smegmatis* wild type and a *glnR* deletion mutant in a DNA microarray experiment. 123 new putative GlnR target genes, including genes for different ammonium transporters, glutamine synthetases, a nitrite reduction system, a methylamine oxidase, amidases, and purine and amino acid permeases, were identified. These results were confirmed for more than 30 genes in RNA hybridization experiments, where an expression of these genes depending on GlnR was observed. These data were validated for about 20 genes in a second, independent approach, performing quantitative RT PCR. Binding of purified GlnR to promoter sequences of 13 target genes or operons was also shown.
Growth experiments with the *M. smegmatis* wild type strain and the *glnR* deletion mutant were carried out using different new nitrogen sources indicated by the microarray data. Indeed, reduced or no growth of the *glnR* deletion mutant was observed for about 10 of the tested nitrogen sources.
All these data confirm the global role of GlnR as the main regulator of nitrogen metabolism and its great influence on the expression of genes involved in uptake and assimilation of various nitrogen sources.

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[2] Amon, J. et al (2008): Nitrogen control in *Mycobacterium smegmatis*: Nitrogen-dependent expression of ammonium transport and assimilation proteins depends on OmpR-type regulator GlnR. J. Bacteriol. 190(21): 7108-7116.

RGP006
Quorum-sensing control of tropodithietic acid biosynthesis in *Phaeobacter gallaeciensis*

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Production of the tropolone antibiotic tropodithietic acid (TDA) is a trait of some marine bacteria affiliated to different genera within the *Rhodobacterales* (*Alphaproteobacteria*) and includes the genome sequenced strain *Phaeobacter gallaeciensis* DSM 17395. The synthesis of TDA requires the expression of *tdaA-F*, as well as six additional genes (*cysI*, *malY*, *paaIJK*, and *tdaH*). The factors controlling *tda* gene expression in *P. gallaeciensis* are not known, but the TDA production correlates with the production of acyl-homoserine lactone (AHL) in a growth-phase-dependent manner. This indicates that TDA production could be controlled by AHL quorum sensing. The genome of *P. gallaeciensis* codes for a LuxR-LuxI type system with homology to the RaiR-RaiI quorum sensing system of *Rhizobium etli*. We constructed *P. gallaeciensis* mutants negative for the corresponding *raiI* and *raiR* homologous genes and determined the chemical structure of the signalling molecules to investigate the role of quorum sensing with regard to the TDA biosynthesis. The *raiI* gene product catalyzes the production of 3-hydroxy-decanoyl-HSL and is positively regulated by RaiR. In contrast to the wild-type *P. gallaeciensis*, the *raiR* and the *raiI* mutants do not produce TDA when grown in liquid Marine Broth 2216 medium. This indicates that the RaiR-RaiI quorum sensing system is required for TDA synthesis. Subsequently, we compared the transcription levels of *tda* genes in the wild-type and the *raiR* mutant by real-time PCR. The results demonstrated a clear decrease of the expression of all investigated *tda* genes in the *raiR* mutant, including *tdaA*, which codes for a potential regulatory protein. Thus, the RaiR-RaiI quorum sensing system obviously activates the *tda* gene expression and accordingly the biosynthesis of TDA in *P. gallaeciensis* in a cell density dependent manner.

RGP007
Activation of betaine carrier BetP from *Corynebacterium glutamicum* in intact cells and in proteoliposomes: a quantitative comparison

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In their natural habitats microorganisms are frequently exposed to hyperosmotic stress. Hyperosmotic stress induces water efflux from a cell, thus increasing the ionic strength of the cytoplasm and reducing the turgor. To counteract dehydration, cells elevate the osmolality of the cytoplasm by importing inorganic ions or compatible solutes. The best studied transporter for compatible solutes is the betaine permease (BetP) in the Gram-positive soil bacterium *Corynebacterium glutamicum*. BetP is a secondary active transport protein which imports its specific substrate glycine betaine in symport with two sodium ions from the external medium. Besides transport activity, BetP comprises the functions of an osmosensor and an osmoregulator. Purified BetP reconstituted in proteoliposomes can detect hyperosmotic stress and regulate its activity in dependence of the external osmolality. Studies in proteoliposomes and in *C. glutamicum* demonstrated that BetP is activated by an increased internal potassium concentration. However, increasing potassium concentration was found not to be the only stimulus sensed by BetP and not to be sufficient to stimulate the transporter to its maximal activity in *C. glutamicum*. *In vivo* studies showed that BetP can be partially stimulated by high external osmolality also at low internal potassium concentration. Only a combination of increasing potassium concentration and high external osmolality leads to full activation of BetP in cells. Consequently, the extent of BetP activity in lipid vesicles might be different than *in vivo*. To test this hypothesis the molecular activity of BetP in proteoliposmes was compared with the molecular activity (turnover) in *C. glutamicum* upon the application of different stimuli. To determine the molecular activity of BetP *in vivo* and *in vitro*, BetP was quantified in both *C. glutamicum* cells and in proteoliposomes. On the basis of this comparison, the impact of the different stimuli was analyzed in both systems.

RGP008
Gene conversion in archaea

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The halophilic archaeon *Haloferax volcanii* and the methanogenic archaeon *Methanococcus maripaludis* were recently shown to be polyploid [1, 2]. *H. volcanii* contains 18 genome copies during exponential growth and 10 genome copies in the stationary phase, *M. maripaludis* 30 to 55 chromosome copies with a maximum in the transition phase. This raises the question if the alleles on the multiple genome copies are homozygous and if yes, how the sequences are harmonized.
To address this question, a heterozygous *M. maripaludis* strain containing a wild type copy of the essential *selD* gene as well as an allele interrupted by a puromycin resistance cassette (*selD::PM^R*) was used [3]. Cultivation in the presence of different puromycin concentrations revealed that the number of alleles encoding the resistance depends on the puromycin concentration. In the absence of antibiotic, the initial ratio of about 25 *selD::PM^R* alleles to 2 *selD* wild type alleles reversed within 14 generations [2].
To study gene conversion in more detail, a *H. volcanii* model strain containing two different selectable alleles was constructed on the basis of a *trpA* deletion mutant [4]. Part of the *leuB* alleles were interrupted by a functional *trpA* copy (*leuB::trpA*) preserving some wild type *leuB* copies in the same cell. This strain is only prototroph for tryptophan as well as leucine as long as it keeps the two different genome copies. Southern blots showed that cultivation in the presence of only one amino acid leads to a loss of the „unnecessary" allele. The presence of both amino acids caused a gene conversion to the *leuB* allele which can be explained by the different amounts of DNA to be synthesized, 53 bp to convert *leuB::trpA* to *leuB* and 958 bp vice versa. Real time PCR quantification of genome copies [1] at different time points of the experiment revealed that also the velocity of gene conversion depends on DNA synthesis. The conversion from *leuB::trpA* to *leuB* took 4 days in the presence of tryptophan and 9 days in the presence of tryptophan and leucine. The conversion from *leuB* to *leuB::trpA* was not totally finished after 37 days, 1 of 25 initial *leuB* copies was left.
The experiments clearly demonstrate that the genome copies of polyploid archaeal species are permanentlyharmonized by gene conversion, favouring the evolutionary advantageous variant.

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RGP009
Polyploidy in Prokaryotes

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In addition to diploid species many polyploid species of eukaryotes exist. In contrast, prokaryotes are believed to be generally monoploid and to contain one copy of a circular chromosome. This assumption is mainly based on generalization of the results obtained with the model species *Bacillus subtilis* and *Escherichia coli* [1;2]. A literature survey revealed that the ploidy level has been determined for a very limited number of species. More than half of them have several copies of the chromosome, indicating that polyploidy might be more widespread in prokaryotes than anticipated.
To get a better overview of the distribution of ploidy levels, genome copy numbers were quantified in 11 bacterial and archaeal species of various groups. A recently developed PCR approach [3], originally applied to haloarchaea, was optimized for the characterization of bacteria. It was validated using slow-growing (t_D 103 min) and fast-growing (t_D 25 min) *E. coli* cultures. The copy numbers of the origin and terminus region were quantified and the results were in excellent agreement with published data [2].
The approach was applied to determine the ploidy levels of *Caulobacter crescentus* (α-proteobacterium) and *Wolinella succinogenes* (ε-proteobacterium), both of which are monoploid. In contrast, *Pseudomonas putida* (γ-proteobacterium) contains 20 genome copies and is thus polyploid. A survey of proteobacteria with experimentally-determined genome copy numbers revealed that monoploidy is not typical for proteobacteria [4].
The cyanobacteria *Synechococcus elongatus* and *Synechococcus sp.* were found to be polyploid with 4 genome copies, while *Synechocystis sp*. is highly polyploid with 58 genome copies. Of two gram-positive species *Corynebacterium glutamicum* is monoploid, while *Staphylococcus carnosus*

contains 6 genome copies in early exponential phase and 10 genome copies in exponential phase.
Methanosarcina acetivorans was found to be polyploid during fast growth (17 copies) and oligoploid during slow growth (3 copies). *Methanococcus maripaludis* has the highest ploidy level found for any archaea with 55 genome copies in exponential phase and 30 in stationary phase [5].
In summary, the results reveal that many polyploid species of archaea and bacteria exist and that monoploidy is exeptional, in contrast to the current belief.

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RGP010
Regulation of the *Escherichia coli* sensor histidine kinase DcuS by direct interaction with the C_4-dicarboxylate carriers DctA and DcuB

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Escherichia coli can use various C_4-dicarboxylates as carbon and energy sources for aerobic or anaerobic respiration. The two component system DcuSR activates the transcription of *dctA* (succinate import), *dcuB* (fumarate-succinate antiport), *fumB* (fumarase) and *frdABCD* (fumarate reductase) in the presence of C_4-dicarboxylates [1]. DcuSR consists of the membrane integral sensor kinase DcuS and the cytoplasmic response regulator DcuR.
Under anaerobic conditions the main transport proteins for C_4-dicarboxylates are DcuA, DcuB and DcuC (1). DctA is the main transport protein for C_4-dicarboxylates under aerobic conditions. It mediates the uptake of succinate and other C_4-dicarboxylates in symport with protons.
DctA and DcuB function as co-sensors of DcuS. Deletion of the carriers causes constitutive activation of DcuSR [2, 3]. Interaction of the integral membrane protein DcuS with DctA and DcuB was analysed *in vivo* with a bacterial two-hybrid system based on the *Bordetella pertussis* adenylate cyclase (BACTH) and by fluorescence resonance energy transfer (FRET). Direct interaction of DctA and DcuB with DcuS was detected. The interaction of DcuS with DctA is modulated by fumarate. DctA and DcuB contain specific sites which are essential for the interaction with DcuS.

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RGP011
Identification of cryptochrome-dependent signalling pathways in *Rhodobacter sphaeroides* - Genome wide analysis under blue light and singlet oxygen stress conditions

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Rhodobacter sphaeroides belongs to the alpha subdivision of proteobacteria. The bacterium is known for its high metabolic versatility, as it can, besides respiration, also perform anoxygenic photosynthesis. To prevent the formation of reactive oxygen species (ROS), the formation of the photosynthetic apparatus has to be tightly controlled. ROS are generated when light, oxygen and a photosensitizer (e.g. chlorophyll) are present simultaneously.
The blue light photoreceptor AppA belongs to the BLUF domain proteins and plays a major role in the regulation of photosynthetic apparatus formation. This protein shows dual sensing abilities, sensing both, light and oxygen.
Besides AppA other blue light photoreceptors were identified in *R. sphaeroides*, recently. Cryptochromes belong to a superfamily together with photolyases. Although both exhibit a high sequence homology, cryptochromes do not show the photolyase-dependent DNA repair activity. It is known that cryptochromes regulate different processes like the entrainment of the circadian clock in plants and animals. However, a biological function and a complete signalling pathway had not been shown for a prokaryotic cryptochrome, yet.
Earlier we were able to demonstrate that a cryptochrome in *R. sphaeroides* (CryB) shows an active, light-dependent photocycle, binds FAD as cofactor and is involved in the regulation of photosynthetic apparatus expression [1]. We could also identify an $RpoH_{II}$ promoter in front of *cryB* which brings its expression into a singlet oxygen ($^1O_2$) stress-dependent context. We now present a genome wide transcriptional analysis of *R. sphaeroides* using DNA microarrays. For this we compared *Rhodobacter* wildtype to the *cryB* deletion mutant under blue light illumination and under $^1O_2$ stress conditions. Furthermore, we were able to identify several putative interaction partners to CryB by a Yeast Two Hybrid system. Interestingly, pulldown experiments also revealed an interaction of CryB to AppA which could link the cryptochrome in the photosynthesis regulation system. As indicated by the DNA microarray data, a role of small RNAs in a CryB-dependent signalling pathway is also likely.

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RGP012
Role of the small RNA RSs2430 in the regulation of photosynthesis genes in *Rhodobacter sphaeroides*

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Small RNAs (sRNAs) play a regulatory role in the adaptation of various bacteria to changing environmental conditions. The identification of sRNAs, using RNA-seq based on 454 pyrosequencing, in the phototrophic bacterium *Rhodobacter sphaeroides* (1) was of major interest because of its high metabolic versatility. In particular, synthesis of the photosynthetic apparatus is regulated in an oxygen- and light-dependent manner. In a physiological screen the sRNA RSs2430 was also found to be influenced by the oxygen tension. Induction of RSs2430 depends on the PrrB/PrrA system, which is a major regulatory system for redox control of photosynthesis genes. Here we present how overexpression of RSs2430 influences the expression of photosynthesis genes in *Rhodobacter sphaeroides*. Northern blots showed that RSs2430 is processed, whereby different 3'ends are generated. The different 3'ends were identified by 3'RACE. Interestingly, only the processed RSs2430-fragments, not the primary transcript, were enriched in the overexpression strain. By using real time RT-PCR and microarray analyses we showed that overexpression of RSs2430 results in a decreased expression of photosynthesis genes.

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RGP013
Examination of a timing mechanism in *Rhodobacter sphaeroides*

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Timing mechanisms are known for over 250 years in eukaryotes. Until now amongst prokaryotes only cyanobacteria could be shown to possess a system to measure time. In *Synechococcus elongatus* a circadian clock builds upon an oscillator of three proteins, KaiA, KaiB and KaiC. A phosphorylation of KaiC in a circadian manner could be shown *in vitro* [1]. All three proteins are essential for clock function. Accordingly, most cyanobacteria possess at least one copy of each gene. An exception is the marine cyanobacterium *Prochlorococcus marinus*, which has suffered a stepwise deletion of the *kaiA* gene [2] but retains a 24 hour rhythm in DNA replication, which is strongly synchronized by alternation of day and night cycles. Surprisingly, the facultative phototrophic proteobacterium *Rhodobacter sphaeroides* possesses a cluster of *kaiBC* genes similar to *Prochlorococcus*. Therefore it has been hypothesized that *R. sphaeroides* may exhibit a rhythmic behavior in gene expression. Such a rhythm has been reported earlier via a luciferase reporter gene system [3]. We were able to show a rhythmic expression of photosynthesis genes for over 4 days in a continuously growing *R. sphaeroides* culture which had been entrained by a 12 hour light and dark

rhythm. Furthermore, an autokinase activity of the RspKaiC could be shown by an *in vitro* phosporylation assay. These data suggest the existence of a functional timing mechanism in purple photosynthetic bacteria. Future results may shed some light on the evolution of clock systems and circadian rhythms in bacteria.

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RGP014

The global regulator Hfq participates in the singlet oxygen stress response of *Rhodobacter sphaeroides*

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Rhodobacter sphaeroides is a facultative phototrophic alpha-proteobacterium which is intensively studied in regard to regulation of photosynthesis genes. Furthermore, it is an established model organism for studying the response to singlet oxygen ($^1O_2$), a highly reactive oxygen species, generated by illumination of the photosynthetic apparatus under oxic conditions. The regulatory response to $^1O_2$ encompasses the induction of several alternative sigma factors, which in turn induce several small RNAs (sRNAs). In a previous RNA-seq study based on 454 pyrosequencing, we have identified five sRNAs which were either induced or processed under $^1O_2$ stress [1, 2]. Their induction depends on the RpoE and $RpoH_{II}$ sigma factors, which are known to be the major regulators of the $^1O_2$ response. Accordingly, $^1O_2$ dependent regulatory networks, comprised of sigma factors and sRNAs, exist in *R. sphaeroides*.
The conserved RNA-chaperone Hfq is one of the key players in sRNA-mediated regulation in many bacteria and is required for the stability of many sRNAs as well as to facilitate the interaction between sRNAs and their target mRNAs. The phenotype of the *R. sphaeroides* 2.4.1Δ*hfq* strain comprises higher sensitivity towards $^1O_2$, reduced pigmentation, and minicell formation.
To get insights into the possible roles of Hfq in *R. sphaeroides* and the $^1O_2$ response and to identify the direct sRNA and mRNA binding partners of Hfq in this bacterium, we used a co-immunoprecipitation strategy combined with deep sequencing as previously described for *Salmonella* [3] and confirmed the Hfq-dependency of several known and also newly identified sRNAs by Northern blot analysis. Strikingly, >70% of the Hfq-associated sRNAs were $^1O_2$-affected. Among Hfq-associated mRNAs we found several mRNAs for cell division and ribosomal proteins. In addition, gel-based proteomics revealed an influence of Hfq on $RpoH_{II}$-dependent genes, amino acid transport/metabolism, and ATP synthase.
Overall, this study suggests Hfq to be a global regulator like in other bacteria and largely explains the pleiotropic phenotype of strain 2.4.1Δ*hfq*. The extensive work on sRNAs in *R. sphaeroides* will help to solve the question of how photosynthetic bacteria manage an effective $^1O_2$ stress response.

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RGP015

Response of the three-component system NreABC of *Staphylococcus carnosus* to oxygen and nitrate

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The NreBC two-component system is required for activation of nitrate respiration in *Staphylococcus carnosus* [1]. The sensor kinase NreB contains an O_2 sensitive $[4Fe\text{-}4S]^{2+}$ cluster which is converted by O_2 to a $[2Fe\text{-}2S]^{2+}$ cluster followed by complete degradation and formation of FeS-less apoNreB [2]. The accessibility of the four Cys residues of NreB to alkylating agents was used to differentiate Fe-S-containing NreB and Fe-S-less apoNreB *in vivo* [3]. In anaerobic bacteria most of the NreB exists as $[4Fe\text{-}4S]^{2+}$-NreB, whereas in aerobic bacteria apoNreB represents the major and physiological relevant form. The half-life of $[4Fe\text{-}4S]^{2+}$-NreB/apo-NreB conversion was 3 minutes after addition of air to anaerobic bacteria.
NreB and NreC are encoded in one operon together with the GAF-domain protein NreA. Deletion of NreA results in activation of nitrate respiration under aerobic conditions. The lipase gene *lip* from *S. hyicus* was fused to the *narG* promoter and used as a reporter gene to investigate mutations in NreA. NreA was required for normal function in O_2 and nitrate sensing, suggesting the presence of an NreABC three-component system.

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RGP016

Activity of the two-component regulatory system CiaRH in *Streptococcus pneumoniae* R6

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The two-component regulatory system CiaRH of *Streptococcus pneumoniae* affects a variety of processes such as competence development, autolysis, bacteriocin production, host colonization, and virulence. While the targets of the regulator CiaR are known, the role of phosphorylation in CiaR regulation has not been defined. To address this issue, the presumed phosphorylation site of CiaR, aspartic acid at position 51, was replaced by alanine. The mutant CiaRD51A protein was no longer able to activate CiaR-dependent promoters, strongly suggesting that the phosphorylated form of CiaR is active in regulation. However, depending on the growth medium, inactivation of the kinase gene *ciaH* resulted in a subtle increase of CiaR-dependent promoter activities or in a strong reduction. Therefore, CiaH may act as a kinase or phosphatase and CiaR is apparently able to obtain its phosphate independently of CiaH. On the other hand, promoter measurements in cells with an intact CiaRH system demonstrated a high, nearly constitutive, expression level of the CiaR regulon independent from the growth medium. Thus, in contrast to many other two-component regulatory systems, CiaRH has apparently evolved to maintain high levels of gene expression under a variety of conditions rather than responding strongly to a signal.

RGP017

Temporal and spatial changes in the localization and the composition of the RNA degrading exosome in *Sulfolobus solfataricus*

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Many macromolecular complexes and even RNA molecules previously thought to be distributed in the cytoplasm, were recently shown to have specific subcellular localization in prokaryotic cells (1). Recently we have shown that the archaeal exosome, an RNA degrading and RNA-tailing protein complex (2), is localized at the cell periphery in the hyperthermophilic and acidophilic archaeon *Sulfolobus solfataricus* (3). Further studies revealed that the localization of the exosome changes in different growth phases: while the vast majority of the exosome is insoluble (at the cell periphery, most probably at the membrane) during the exponential growth, more than the half of the exosome is soluble (in the cytoplasm) in the stationary phase. At the cell periphery, the exosome interacts with the archaeal DnaG, which seems to be responsible for the localization. DnaG is exchanged by another protein, annotated as a pre-mRNA splicing protein, in the cytoplasmic form of the exosome. Data on the analysis of the protein-protein interactions in the two forms of the exosome as well as on the impact of the composition on the function of the exosome will be shown and discussed.

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RGP018
Towards the composition of a regulatoryredox-network for photosynthetic gene expression in *Rhodospirillum rubrum*

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The anoxygenic photosynthetic bacterium *R. rubrum* serves as a model organism for redox-controlled gene expression. The expression of photosynthetic membranes (PM) is a fair indicator for the redox states of the cells. Even if *R. rubrum* is grown in the dark, high levels of PM can be reached, depending not only on the oxygen supply, but also on the composition of the culture medium.
The choice of carbon sources for example is of major importance: Succinate combined with fructose will reach the highest PM levels under semiaerobic conditions. The addition of glutathione to the culture broth results in significantly elevated PM-levels if an organic acid is used as carbon source. Glutathione can not be utilized as sole carbon source by *R. rubrum*. Supplementation with the amino acids contained in glutathione does not result in elevated PM-levels. The influence of light on PM-expression is versatile.
The control of PM expression in Rhodobacter species has been unravelled to some extent, so we could identify some major differences in comparison with *R.rubrum*.
1. In Rhodobacter species, the PM-elevating effect of fructose is absent
2. In *R. rubrum*, no homologue to the RegB/RegA two component redox sensing system from Rhodobacter could be identified by BLAST analysis
3. Active uptake of glutathione *R. rubrum* is possible, which then enhances PM expression. In Rhodobacter glutathione causes no PM-elevating effect if added to the culture broth.
In this work, we combine our results from different working approaches to define a redox-regulated network for *R. rubrum*. It includes data from a ligand-affinity chromatography with coenzyme Q_{10} as a ligand for the screening of redox-sensitive histidine kinases, as well as intracellular glutathione data and information from deletion mutants. Additionally, the necessary redoxpotential for the PpsR-switch was estimated and redox-sensitive staining of reduced thiols was applied.

RGP019
Mechanism and Function of non standard Circadian Clock Systems in Cyanobacteria

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Circadian rhythms, oscillations with approximately 24 h periods concerning many physiological activities, are found in most eukaryotes. Among prokaryotes, exclusively cyanobacteria are known to harbour an internal clock. In the model strain *Synechococcus elongatus* PCC 7942, the *kaiABC* gene cluster is essential for the generation of circadian rhythms. It has been shown by *in vitro* and *in vivo* experiments that the timing process itself is based on rhythmic phosphorylation of KaiC hexamers, whereas ATP hydrolysis catalyzed by KaiC accounts for the reaction that defines the 24-hour period of the clock. In addition to the *kaiABC* gene cluster, the genome of *Synechocystis* sp. PCC 6803 holds additional orphan *kai* genes located at different sites on the chromosome which functions have not been investigated. In contrast, different strains of the marine cyanobacterium *Prochlorococcus* are lacking the *kaiA* gene and components of the input and output pathways are missing or truncated. We aim to undeceive the phenomenon of multiple *kai* gene copies and of reduced *kai* operons in comparison to the well-studied protein clock of *Synechococcus*. The loss of *kaiA* in *Prochlorococcus* transforms the circadian clock mechanism into that of an hourglass. First analyses of *Synechocystis kai* knockout mutants indicate that the deletion of the *kaiABC* cluster results in reduced fitness compared to the wild type, while deletion of *kaiC2B2* is lethal. Further biochemical characterization of the purified *Synechocystis* Kai proteins will yield insights into Kai protein complex formation, as well as ATPase activity and phosphorylation cycles of the three different KaiC proteins from *Synechocystis*.

RGP020
Signal perception by the oxygen-sensing transcriptional regulator Fnr of *Bacillus subtilis*

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The *Bacillus subtilis* redox regulator Fnr controls genes of the anaerobic metabolism in response to low oxygen tension. Unlike its *E. coli* counterpart *B. subtilis* Fnr utilizes three cysteine residues and one unknown non-cysteine ligand for the formation of the oxygen sensing $[4Fe\text{-}4S]^{2+}$ cluster. Using site-directed mutagenesis of *fnr* a variety of mutant proteins were created and activity was tested *in vivo* using a *fnr* mutant complementation system with an Fnr-dependent *narG-lacZ* reporter gene fusion. Furthermore, recombinant anaerobically purified Fnr proteins were characterized by *in vitro* DNA binding studies and transcription assays. An unusual structure for the oxygen-sensing $[4Fe\text{-}4S]^{2+}$ cluster was detected by a combination of genetic experiments with UV/Vis and Mössbauer spectroscopy. Aspartate residue 141 was identified as fourth iron-sulphur cluster ligand beside three cysteine residues. Exchange of aspartate 141 to alanine abolished functional *in vivo* complementation of an *fnr* knock out strain by the mutagenized *fnr* gene and *in vitro* DNA binding of the recombinant regulator FnrD141A. In contrast, substitution of aspartate 141 with cysteine preserved $[4Fe\text{-}4S]^{2+}$ structure and regulator function.
J. Biol Chemistry *in press*

RGP021
Quantitative analysis of the *pmoA* expression level in type I and type II methanotrophs

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Methane is a well-known greenhouse gas and the atmospheric concentration has increased dramatically over the last 250 years. Wetlands are a major source of methane where it is produced by methanogens and diffuses to the atmosphere. Aerobic methanotrophs are active at the oxic-anoxic interface of these environments and mitigate the release of methane to the atmosphere. Methanotrophs are unique in their ability to utilize methane as their only carbon and energy source. On the basis of morphological differences like structure of intracytoplasmic membranes, physiological characteristics and phylogenetic placements, methanotrophs can be separated into two groups: type I and type II. Type I methanotrophs belong to the family *Methylococcaceae* within the γ-subdivision of Proteobacteria, whereas type II methanotrophs belong to the family *Methylocystaceae* in the α-Proteobacteria. The first step and key reaction of methane oxidation is the introduction of a hydroxyl group catalyzed by a methane monooxygenase (MMO) enzyme. The membrane bound form of this enzyme (pMMO) is present in almost all known methanotrophs and is found to be composed of three polypeptides: an α-subunit (PmoB), a β-subunit (PmoA) and a γ-subunit (PmoC). The genes encoding pMMO are encoding within the *pmoCAB* operon, which is regulated by a σ^{70} promoter. The *pmoA* gene is frequently used as a functional and phylogenetic marker for methanotrophs. A recent trend has been to use the relative abundance of *pmoA* mRNA recovered from environmental samples as a proxy for the relative activity of different methanotroph species. The objective of this study was to investigate how well *pmoA* transcript abundance correlates with the activity of various methanotroph species. Ten different methanotrophs, including both type I and II representatives, were grown in batch culture and the abundance of *pmoA* transcripts was determined by reverse transcription real-time PCR. Here we show the relationship between the absolute cell number and the *pmoA* transcript levels of various species of type I and II methanotrophs at different growth stages.

RGP022
Identification and Characterization of small RNAs in *Agrobacterium tumefaciens*

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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 (1).
Using a comparative bioinformatic approach (2) we identified diverse sRNAs in the plant pathogen *Agrobacterium tumefaciens*. Two tandem sRNAs control the expression of at least three ABC transporters among them the periplasmic binding protein of the GABA transporter. The molecular details of the sRNA-mRNA interaction will be presented.
By using a differential RNA sequencing (dRNA-seq) technology (3) we discovered many new sRNA candidates on all four *A. tumefaciens* replicons, the circular chromosome, the linear chromosome, the At-plasmid and the Ti-plasmid. At least one sRNA is highly induced under virulence conditions.

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[2] Axmann, I.M. et al (2005): Identification of cyanobacterial non-coding RNAs by comparative genome analysis. Genome Biol 6: R73.
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RGP023
FrlR, a novel transcription factor that strongly regulates the catabolic *frl*-operon in *B. subtilis* 168

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The Gram-positive model organism *Bacillus subtilis* metabolizes the carbon- and nitrogen source Amadori product that occurs in soil and long stored food. Amadori products are. CodY, a global transcription regulator in Gram-positive bacteria, was shown to regulate the promoter upstream of the *frlB* gene [2]. In this study, the transcriptional regulator named FrlR was investigated which is a GntR-type transcription factor and also represses the expression of *frlBONMD*[3]. Its gene is located downstream of the *frlBONMD* operon and is inversely orientated to them. Electrophoretic mobility shift assays revealed a total of three FrlR binding sites within the *frlBONMD-frlR* region. The regulator protein binds to the promoter P_{frlB}, the first intergenic region of the operon and the promoter P_{frlR}. From these regions a GntR binding motif 5´-$(N)_y$GT.N_2.TA.N_2.AC$(N)_y$-3´ was derived. However, the *frl*-operon is regulated by CodY and FrlR together because they bind at P_{frlB}, simultaneously. Remarkably, the intergenic region of *frlB* and *frlO* genes contains a 38 bp perfect palindrome in which the FrlR binding site is located. By this, FrlR causes repression of the downstream genes. Additionally, first experiments indicate a negative effect on the transcription of the downstream located genes by the palindrome itself.

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RGP024
pH-dependent expression of the *alsSD* Operon of *B. subtilis* and regulation by AlsR

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Bacillus subtilis forms Acetoin under anaerobic fermentative growth conditions. It requires acetolactate synthase and -decarbobxylase encoded by the *alsSD* operon. The *alsSD* expression is induced by addition of acetate to the growth medium, low pH and aerobic stationary phase. The regulator AlsR is essential for *alsS-lacZ* reportergene expression under all growth conditions tested. The AlsR regulator is a member of the LysR-type transcriptional regulators (LTTR) and composed of two domains: an N-terminal DNA binding domain with a winged HTH motif and a C-terminal regulatory domain. Most regulators of the LysR family are activated by binding of an inducer to the regulatory domain. For AlsR acetate or a reduced pH is postulated as inducing signal.
We measured *alsS-lacZ* expression under different pH conditions and in the presence of various organic acids to discriminate between reduced pH or accumulation of organic acids like acetate as inducing signal. In addition we performed *in vitro* DNA-binding studies with pH values from 5 to 9 to analyze AlsR binding.
In order to identify functional relevant amino acid residues of the effector domain we mutagenized the *alsR* gene and tested the *in vivo* activity of mutant AlsR proteins in an *in vivo* complementation system. Here, mutated *alsR* genes were integrated into the *amyE* locus of a *B. subtilis alsR* knock out mutant strain and were expressed under the control of the xylose-inducible *xylA* promoter. AlsR activity was monitored by ß-galactosidase activities deriving from the AlsR-dependent *alsS-lacZ* reporter gene fusion.

RGP025
A specialized FMN riboswitch confers roseoflavin resistance to *Streptomyces davawensis*

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The expression of bacterial genes involved in riboflavin production and transport are regulated by FMN riboswitches present in the 5'-untranslated regions of the corresponding mRNAs. The aptamer portion of the FMN riboswitches binds FMN (flavin mononucleotide, the phosphorylated derivative of riboflavin) and regulates gene expression in combination with an expression platform either by transcription termination or by preventing translation initiation. *Streptomyces davawensis* synthesizes the antibiotic roseoflavin, which is toxic to gram-positive but also to gram-negative bacteria if the compound is able to enter the cell. Roseoflavin is phosphorylated to roseoflavin mononucleotide (RoFMN) which subsequently is adenylated to roseoflavin adenine dinucleotide (RoFAD). RoFMN and RoFAD may inactivate flavoenzymes. In addition, bacterial FMN riboswitches were found to be targets for roseoflavin/RoFMN. *S. davawensis*, in contrast to *Bacillus subtilis* or *Streptomyces coelicolor*, is roseoflavin resistant. Our hypothesis was that *S. davawensis* contained a specialized FMN riboswitch, which is not affected by RoFMN. To test this, plasmids were constructed, which contained the FMN riboswitches from *B. subtilis*, *S. coelicolor* and *S. davawensis* directly downstream of the T7 promoter and upstream of the firefly luciferase reporter gene. The plasmids were used for an *in vitro* transcription/translation reaction (TK/TL) in the presence of FMN or RoFMN. RoFMN, which was not commercially available, was produced by human flavokinase. A strong reduction of the luciferase reporter activity was found in the TK/TL in the presence of FMN, which suggests that less of the reporter enzyme was produced. Apparently, the FMN riboswitches of the three bacteria responded similarly to FMN. Upon addition of RoFMN in the TK/TL, the luciferase activity was reduced in the assays containing the FMN riboswitches from the roseoflavin sensitive organisms *B. subtilis* and *S. coelicolor*. In the corresponding TK/TL containing the *S. davawensis* FMN riboswitch, however, the luciferase activity was not reduced in the presence of RoFMN. Based on the known ability of the flavokinase/FAD synthetase from *S davawensis* to convert roseoflavin into RoFMN, we conclude that the FMN riboswitch from this bacterium is specialized to not respond to RoFMN. Subsequent *in vivo* studies are necessary to confirm this finding.

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RGP026
Regulation of translation in halophilic archaea

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Translation is a very important step for the expression of genetic information into the phenotypes of cells or organisms. Regulation of translation typically occurs during initiation because this step is rate-limiting. Three different mechanisms for translation initiation were shown to operate in haloarchaea. About 2/3 of the transcripts are leaderless. Surprisingly most leadered transcripts are devoid of a Shine Dalgarno (SD) motif and it was shown that

a novel initiation mechanism operates on these transcripts [1]. At very low frequency transcripts with SD motifs also exist, therefore three different initiation mechanisms operate in haloarchaea simultaneously [2]. To analyze these mechanisms transcripts containing three different initiation sites in front of the *dhfr* reporter gene were generated and it was verified that all three initiation sites operate *in vivo*. This enables the characterization of the differential usage of the mechanisms under various conditions, i.e. in different media with various C-sources, N-sources and salt concentrations or at different temperatures. Quantification of the protein levels by Western blots and the transcript levels by Northern blots allowed determination of translational efficiencies. We could reveal that the initiation mechanisms are used differentially under specific conditions. These results show that *H. volcanii* applies the three different initiation mechanisms for conditional regulation of translational efficiencies and thus uses translational regulons to adapt to changing environmental conditions.

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RGP027
Light-dependent gene induction in *Aspergillus nidulans* requires release of the repressor LreA and binding of the activator FphA

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Light serves as an important environmental signal to regulate development and metabolism in many fungi and has been studied to some detail in *Neurospora crassa* and *Aspergillus nidulans*. *A. nidulans* develops mainly asexually in the light and sexually in the dark. The red-light sensor phytochrome (FphA) and the WC-1 homologue blue-light receptor LreA have been shown to mediate the light response in A. nidulans [1]. There is evidence that both proteins form a light regulator complex (LRC). LreB (WC-2) and VeA are probably also components of this complex [2].
Using Chromatin-Immunoprecipitation (ChIP) and quantitative Real Time PCR we show that HA-tagged FphA and LreA bind to the promoters of the *A. nidulans* homologues of *N. crassa con-10* (*conJ*) and *ccg-1* (*ccgA*). In *A. nidulans conJ* and *ccgA* are both induced during development but are also strongly upregulated in hyphae after short exposure to light.
Surprisingly we found LreA bound to the *conJ* and *ccgA* promoter only in the dark probably acting as a repressor. In contrast, FphA is recruited to the promoters after short illumination and seems to function as activator of transcription. These results suggest that the LRC is not a tight protein complex but rather transient and that light induction depends on derepression followed by induction through FphA.

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[2] Purschwitz, J. et al (2008): Mol. Genet. Genomics 18(4):255-9.

RGP028
Effect of primary metabolism on secondary metabolite production in *Aspergillus terreus*

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Genome sequencing has shown that *Aspergillus terreus* has the potential to produce a great variety of different natural products. Although several metabolites have been identified, it can be assumed that the potential to produce secondary metabolites is much larger than currently known. Several strategies have been developed to discover new metabolites produced by filamentous fungi. Besides the use of epigenetic modifiers or co-cultivation experiments, targeted overexpression of putative transcription factors provides a promising tool to activate silent gene clusters. Here, we investigated the expression of the only complete PKS-NRPS hybrid gene present in the genome of *A. terreus*. Since overexpression of a putative transcriptional activator adjacent to the PKS-NRPS gene did not activate gene transcription, we constructed a *lacZ* reporter strain to screen for naturally inducing conditions. Results revealed that expression was activated in the presence of several amino acids and enhanced by alkaline pH. However, glucose mediated carbon catabolite repression remained as the dominating inhibiting factor. When the adjacent transcription factor, which failed to induce PKS-NRPS expression in initial experiments, was expressed under naturally non-inducing, but also non-repressing conditions, activation of the PKS-NRPS gene was observed. Thus, factors involved in regulation of primary metabolism can override activating effects from cluster specific transcription factors. Finally, product identification revealed that the gene cluster is responsible for producing metabolites of the fruit rot toxin family.

RGP029
Analysis of DNA binding by Qdr1 and Qdr2, two transcriptional regulators of quinaldine degradation by *Arthrobacter nitroguajacolicus* Rü61a

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Arthrobacter nitroguajacolicus Rü61a is able to utilize quinaldine as source of carbon and energy. The genes that enable *A. nitroguajacolicus* to convert quinaldine to anthranilate are clustered in two „upper pathway" operons which are localized on the 113 kbp linear plasmid pAL1. A third operon located downstream of the „upper pathway" operons codes for anthranilate conversion via CoA-thioester intermediates [1].
Qdr1 and Qdr2, two PaaX-like DNA binding proteins encoded by pAL1, are involved in the regulation of the utilization of quinaldine. The canonical PaaX repressors use phenylacetyl-CoA as effector and are known to transcriptionally regulate the phenylacetate catabolon of *E. coli* [2] and *Pseudomonas putida* [3]. Electrophoretic mobility shift assays with recombinant Qdr1 and Qdr2 showed that both regulators bind specifically to the promoter regions of all three operons, and revealed that the dissociation of Qdr-DNA complexes is induced by anthraniloyl-CoA.
The transcriptional start points of *qdr1* and *qdr2* were identified by 5´RACE (rapid amplification of 5´cDNA ends) analysis. The deduced promoter regions of *qdr1* and *qdr2* bear a strong resemblance to the -10 and -35 region of the σ^{32} promoter sequence of *E. coli*. The interaction of each regulator with these promoters is currently being studied by gel shift analysis. The DNA sequences recognized by Qdr1 and Qdr2 will be identified by DNase I footprinting analysis.

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RGP030
A novel *Pseudomonas putida* bioreporter strain for the detection of alkylquinolone-type quorum sensing signal molecules

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The opportunistic pathogen *Pseudomonas aeruginosa* regulates its virulence via a complex quorum sensing (QS) network which incorporates both *N*-acylhomoserine lactone and 2-alkyl-4(1*H*)-quinolone (AQ) signal molecules. The >50 different AQs produced by *P. aeruginosa* differ mainly in the degree of saturation and length of the alkyl chain as well as in the presence or absence of a hydroxyl substituent at the C3-position [1]. Among these AQs, 2-heptyl-3-hydroxy-4(1*H*)-quinolone (the *Pseudomonas* quinolone signal, PQS) and 2-heptyl-4(1*H*)-quinolone (HHQ) were identified as autoinducers in QS. HHQ as well as PQS act as the effectors of the LysR-type transcriptional regulator PqsR [2, 3].
This study focuses on the validation of a *lacZ*-based *Pseudomonas putida* bioreporter strain that enables the detection of AQ signal molecules at low concentrations (nM to µM). *P. putida* KT2440 was transformed with a reporter plasmid that confers constitutive expression of the *pqsR* gene, and contains a transcriptional fusion of the PqsR-responsive *pqsA* promoter to the reporter gene *lacZ*. Therefore, β-galactosidase activity is a function of the PqsR-stimulated transcription under the control of the *pqsA* promoter. The presence of HHQ or PQS (1 µM) increases the β-galactosidase activity of the bioreporter three- to four-fold compared to the activity mediated by PqsR in the absence of an effector. The bioreporter may be used to screen AQ analogues for their ability to act as HHQ/PQS agonists or antagonists, and to identify genes which encode PQS or HHQ converting enzymes.

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RGP031

The ratio of autoinducers determines Quorum Sensing regulated phenotypes in *Vibrio harveyi*

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Quorum Sensing (QS) plays an important role in regulating gene expression in bacterial populations. This intercellular communication through low-molecular diffusible molecules, called autoinducers (AIs), enables single cells to coordinate their behaviour within a population. *Vibrio harveyi* produces three AIs which are recognized by three hybrid sensor kinases. Information is transduced via phosphorelay to LuxU and subsequently to LuxO. At low AI concentration the intracellular concentration of phospho-LuxO is high which in turn induces transcription of four regulatory sRNAs. These sRNAs destabilize upon interaction with Hfq the mRNA of LuxR. LuxR induces or represses QS-dependent genes.
The extracellular concentration of the three AIs of *V. harveyi*, HAI-1, CAI-1 and AI-2, was monitored in a growing culture over time. According to the distribution of AIs and QS regulated phenotypes three stages could be distinguished. In the early exponential growth phase only AI-2 was detectable, and bioluminescence was induced (stage 1). In the late exponential growth phase both HAI-1 and AI-2 reached their maximal values, and bioluminescence further increased (stage 2). In the stationary growth phase HAI-1 and AI-2 were adjusted to equal concentrations, and CAI-1 was detectable (stage 3). These stages are consistent with *in vitro* phosphorylation data. The influence of AIs on the *in vitro* reconstructed signaling cascade consisting of all three hybrid sensor kinases, LuxP and LuxU was tested. The presence of AI-2 inhibited LuxU phosphorylation by 61%, while the additional presence of HAI-1 revealed an inhibition of LuxU phosphorylation by 92%. When all three AIs were present, phosphorylation of LuxU was completely prevented. The data suggest cooperative behaviour of the QS receptors that allows a very sensitive response to various ratios of external AIs.

RGP032

Analyzing the extent of FtsH-dependent proteolysis by substrate trapping

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Proteolysis is a wide-spread mechanism to ensure the sensitive balance of regulatory and metabolic proteins at certain conditions. In *Escherichia coli*, five ATP-dependent proteases are responsible for the specific degradation of proteins. Among these, FtsH is the only membrane-bound and essential protease. Besides the quality control of membrane proteins and SsrA-tagged proteins, the most important role of FtsH is the degradation of regulatory proteins in the cytosol. For example, FtsH is involved in the heat shock response by proteolysis of the heat shock sigma factor RpoH. The essential function of FtsH is the control of LPS biosynthesis by degradation of the LpxC and KdtA enzymes [1, 2].
Compared to other proteases, the numbers of identified FtsH-substrates is limited. To find new substrates, a comparative substrate trapping approach was used. An FtsH-trap version carrying a mutation in the proteolytic center (FtsH_H417Y) was constructed and expressed in *E. coli*. Substrates are predicted to be unfolded and translocated into the proteolytic chamber of the FtsH-trap protein. Protease-substrate complexes were co-purified, separated by 2D PAGE and subjected to mass spectrometry. We identified 12 putative substrates of FtsH, among them the known substrate LpxC, validating this method as a powerful tool to identify new protease substrates. The list of putative substrates of FtsH includes proteins with a variety of cellular functions. For example, the phage shock protein PspA, the anti sigma factor of RpoD (Rsd), the key enzyme of histidine biosynthesis (HisG) or the uncharacterized and putative protein YfgM co-purified with FtsH_H417Y. First degradation experiments revealed a growth phase-dependent proteolysis of YfgM. Using this experimental setup, we set out to broaden the understanding of the physiological role of FtsH-dependent proteolysis.

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RGP033

Analysis of new $P_{xyl/tet}$ promoters for Tet-ON and Tet-OFF regulation in *Staphylococcus aureus*

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Inducible gene expression systems are useful tools for investigating gene-function relationships. The tetracycline-dependent gene expression system (*tet* system) is based on the regulator TetR which binds to its cognate DNA sequence *tetO* that is embedded in one or more copies within the promoter region. $P_{xyl/tet}$ is the most prominent promoter for *tet* target gene control in *Staphylococcus aureus*. In Tet-ON architectures, expression of a target gene is repressed by TetR and induced upon addition of an effector such as anhydrotetracycline (ATc) which causes TetR detachment from *tetO*. By contrast, reverse TetR variants only bind to *tetO* upon interaction with ATc. Tet-OFF systems employing reverse TetR enable rapid silencing of a target gene by adding this compound.
Transcriptional control of the nuclease 1 gene (*nuc1*) of *S. aureus* SA113 in a pRMC2 vector system (Corrigan and Foster, 2008) under control of TetR has shown leakiness under non-induced conditions. Thus a second *tetO* site was inserted, creating the vector pRAB11. Semi quantitative evaluation of nuclease activity on DNA-containing media indicated enhanced repression capabilities of pRAB11. Using *lacZ* as reporter gene downstream of the $P_{xyl/tet}$ promoter, β-galactosidase measurements verified that the pRAB11 vector system enables tighter repression, however at the cost of slightly lower expression levels compared to pRMC2.
Since $P_{xyl/tet}$ is a very strong promoter in *S. aureus*, a promoter pool was generated by randomly mutating up to six conserved positions of the -35 and -10 regions of $P_{xyl/tet}$ to gain different expression levels of target genes in the pRAB11 vector system. 16 different $P_{xyl/tet}$ variants, exhibiting one to three nucleotide exchanges, were characterised in β-galactosidase assays. All of them displayed weaker transcriptional potency. Three of them indicated intermediate expression levels in the induced state accompanied with tighter repression compared to the wildtype $P_{xyl/tet}$ promoter. This makes pRAB11 and its promoter derivatives suitable vector systems for *tet* regulation in staphylococci, tailored to the specific transcriptional requirements of target genes of choice.

RGP034

The two-component system YehU/YehT of *Escherichia coli* - further insights into its transcriptional regulation.

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Two-component systems (TCS) are the predominant signal transduction systems in prokaryotes and consist of at least two components: a membrane-integrated histidine kinase (HK) which senses a stimulus and transduces it in a cellular signal by autophosphorylation, and a response regulator (RR) with DNA-binding activity. Whereas most TCS in *Escherichia coli* are well characterized, little is known about the YehU/YehT system. The membrane-integrated HK YehU has a GAF-domain, and the highly conserved input domain is structurally similar to the input domain of LytS, a potential sensor for murein subunits in Gram-positive bacteria. YehT posseses a CheY-like receiver domain and a LytTR DNA-binding domain. The structure of AgrA, a RR with a LytTR DNA-binding domain, represents a novel DNA-binding type.
When information on the environmental signal is lacking, overproduction of RRs provides an alternative approach to identify target genes. Thus, the RRs YehT and KdpE, respectively, were overproduced in *E. coli,* and a comparative transcriptome analysis revealed several target genes. Transcriptional analysis via Northern Blot hybridization using different strains and derivatives of YehT and electromobility shift assays confirmed that only *yjiY* is under direct transcriptional control of YehU/YehT. The YehT-binding site was further narrowed by DNase I footprinting.
An emerging theme in the field of TCS signaling is the discovery of auxiliary factors. *In vivo* protein-protein interaction studies unraveled the auxiliary protein YehS that interacts with YehU and YehT. On the other hand, bioinformatic tools link YehU/YehT with the YpdA/YpdB two-component system. Therefore, we hypothesize that the YehU/YehT/YehS system is embedded in a signaling network together with the YpdA/YpdB HK/RR system.

RGP035
Kinase-Phosphatase Switch of *Shewanella oneidensis* MR-1 ArcS is mediated by interplay of a sensory PAS-domain and a regulatory receiver domain

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Shewanella oneidensis MR-1 is well known for its respiratory versatility. An enormous amount of alternative electron acceptors are utilized under anaerobic conditions. Among gamma proteobacteria, the anoxic redox control (arc) system is mediating the response to changes in environmental oxygen levels. In *E. coli,* the response regulator ArcA regulates gene expression upon signal perception from its cognate sensor kinase ArcB. Conversely we have shown by phenotypic mutant characterization, transcriptomic analysis, and protein-protein interaction that in *Shewanella*, ArcA, HptA, and ArcS constitute an atypical Arc-System. Phylogenetic analyses suggest that HptA might be a relict of ArcB. In contrast, the sensor kinase ArcS is substantially different with respect to overall sequence homology and domain organization. Compared to ArcB, the sensory as well as the catalytic part of ArcS are extended by a PAS domain and a receiver domain, respectively. *In vitro* and *in vivo* studies with ArcS substitution mutants reveal distinct roles for the two receiver domains. While one receiver is mediating the phosphorelay to ArcA, the second receiver controls kinase activity presumably through interplay with the sensory PAS domain. Thus, we speculate that ArcS has adopted the role of ArcB after loss of the original sensor kinase as a consequence of regulatory and sensory adaptation to a redox-stratified environment.

RGP036
YhdA (CsrD) acts on curli fimbriae expression in *Escherichia coli* via the Rcs phosphorelay and the small RNA RprA

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The switch from the motile-planktonic to the adhesive state and therefore to bacterial biofilm formation is stimulated by the second messenger c-di-GMP. Synthesis and degradation of this messenger is controlled by diguanylate cyclases (DGC, with GGDEF domains) and phosphodiesterases (PDE, with EAL domains), respectively. *Escherichia coli* possesses 29 GGDEF/EAL genes, which most likely encode 12 DGCs, 13 PDEs as well as four proteins with degenerate GGDEF/EAL motifs and alternative functions (Hengge, 2009; Sommerfeldt et al., 2009).
YhdA (also known as CsrD) is a degenerate GGDEF/EAL protein known to affect the turnover of the small RNAs CsrB and CsrC, which, via the CsrA protein, modulate motility, glycogen formation and other cellular functions (Suzuki et al., 2006). We showed that YhdA also has a positive effect on CsgD/curli formation and motility (Sommerfeldt et al., 2009). Our new data indicate that this effect is independent of the Csr system. We demonstrate that part of the reducing effect on curli expression of a *yhdA::kan* insertion is due to increased expression of MreB, an actin homolog encoded by the gene directly downstream of *yhdA*; nevertheless also a non-polar *yhdA::cat* insertion, i.e. also the absence of YhdA per se, reduces CsgD/curli expression. Down-regulation of curli formation as a consequence of a lack of YhdA and/or increased MreB expression (which can also be mimicked by expressing MreB from a plasmid) is mediated by the Rcs phosphorelay pathway, which triggers increased expression of the small RNA RprA. Knocking out *rprA* suppresses the effect of *yhdA* mutations and/or increased expression of MreB. This is consistent with recent data showing *csgD* mRNA to be a direct target of RprA (Mika et al, in preparation). Furthermore, we observed that a few other small RNAs are present at higher or lower levels in a *yhdA* mutant, suggesting that YhdA may have a more pleiotropic function by affecting additional targets besides the small RNAs CsrB, CsrC and, indirectly, RprA.

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RGP037
NADPH oxidases (Nox) as source of endogenous reactive oxygen species (ROS) - a proteomic analysis

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Oxidative stress and redox regulation play a key role in development and hyphal growth in *Aspergillus nidulans*. The ROS-signalling network controls apical growth and development of *A. nidulans*. Within this network NADPH oxidases are important members due to their function as source for endogenous ROS which have signalling function.
Here we describe recent results concerning mutants of NADPH oxidase of *A. nidulans* designated Nox. It is known that deletion of *noxA/ noxR* causes distinct phenotypes in growth, sexual and asexual development [1]. Therefore, proteome analyses of wild-type *A. nidulans* and *noxA/ noxR* deletion strains were performed to identify key proteins associated with these mutations and therefore with an altered ROS-level in the cell. We compared wild-type *A. nidulans* and *noxA/ noxR* deletion strains with the help of 2-D gel proteome analysis to identify proteins with higher or lower abundance in the cellular extracts. Protein spots were identified by MALDI-TOF-MS/MS and classified by their cellular function. This gave us an overview about the global effect of endogenous ROS on the *A. nidulans* proteome. Furthermore, we established the first 2-D reference map for wild-type *A. nidulans* which possesses 435 spots representing 364 proteins.

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RGP038
ncRNA Syr1 is a possible regulator of Alb3 in the cyanobacterium *Synechocystis* sp. PCC6803

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Non-coding RNAs (ncRNA) are known as novel regulators of gene expression in different bacteria, including cyanobacteria. Using a tiling microarray about 60 ncRNAs and 73 asRNAs were identified in the model cyanobacterium *Synechocystis* sp. PCC 6803 [1], 28 of which were verified by further methods.
One of these ncRNAs Syr1 is a 135 nt long ncRNA located in the 206 nt long intergenic spacer between the *fabF* and *hoxH* genes. It is not co-transcribed with *fabF* as judged by Northern blot.
Our aim is to investigate the function of Syr1 in the regulatory network of *Synechocystis* sp.PCC 6803. Bioinformatic analyses revealed a possible interaction with the 5' region of *alb3* gene (*slr1471*). The putative binding site of Syr1 ncRNA overlaps the ribosome binding site of *alb3* mRNA, possibly destabilizing mRNA or preventing its translation. Alb3 is a homologue of the YidC/OxaI/Alb3 protein family, which play an essential role in the insertion of a wide range of membrane proteins in bacteria and mitochondria, respectively. In thylakoids chloroplasts the homologue of this protein family Albino3 (Alb3) facilitates the insertion of a specialized subset of proteins, involved in photosynthesis [2].
We over-expressed the ncRNA Syr1 under control of inducible by the copper depletion promoter P_{petJ} on the self-replicating pVZ321 vector in *Synechocystis*. Over-expression led to a bleaching phenotype and retarded growth of the mutant culture. Using antibody against the Alb3 protein, we demonstrated the reduced amount of Alb3 in an Syr1 over-expressing strain. The content of phycobilisomes was also strongly reduced as shown by SDS-PAGE and pigment measurements. This phenotype affirms the previous bioinformatic prediction.
We concluded that the ncRNA Syr1 plays a role in regulation of the photosynthetic apparatus in *Synechocystis* through expression control of the Alb3 protein.

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RGP039

Comparison of different *Escherichia coli* K-12 laboratory strains with respect to growth characteristics and biofilm formation

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We systematically compared phenotypic traits of four commonly used *Escherichia coli* K-12 strains: the nonmotile MC4100 (which carries an *flhDC* frame shift mutation) (1), the motile W3110 and two MG1655 strains with different motility (affected by the presence or absence of IS elements upstream of *flhD*) (2).
The four strains grow differently in complex medium. They also show differences in cell length. RpoS is stationary phase-induced in all, but W3110 was found to exhibit higher RpoS levels. Colonies of W3110, but not of the other strains, can form complex patterns of wrinkles and rings under some conditions. Formation of these structures requires motility, RpoS as well as regulatory and structural genes for adhesive curli fimbriae, but not cellulose formation, which could not be detected in any of the four strains. Curli fimbriae expression is different in the four strains. MC4100 induced *csgB::lacZ* earlier during entry into stationary phase, since it does not express the RpoS antagonist FliZ, which is under FlhDC control and determines the timing of *csgB::lacZ* expression in the highly motile strains W3110 and MG1665. The low-motility variant of MG1665 showed normal timing but reduced levels of *csgB::lacZ* expression. Interestingly, neither RpoS nor curli formation are required for W3110 and MG1655 to form biofilms in the standard crystal violet assay in polystyrole microtiter plates (MC4100 does not form a biofilm at all). This confirms that *E. coli* can form different types of biofilms dependent on different regulatory genes and structural components.
Overall, our work shows that the commonly used laboratory strains of *E. coli* K-12 differ in characteristics like motility, stress gene expression and complex biofilm formation. Overall, W3110 may be the strain with the most optimal combination of these complex traits. Moreover, our data suggest that care should be taken in generalizing results obtained with only one of these four strains.

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RGP040

Induction dynamics of quorum sensing in *Pseudomonas putida* colonies under flow conditions

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Bacterial communication via release and sensing of signal molecules (autoinducer, AI) has been mainly investigated in batch cultures. Here usually coordinated, synchronous response of the whole population is induced in a cell density dependent manner (quorum sensing, QS). However, most bacteria live heterogeneously distributed in aggregates or biofilms attached to surfaces. Under these conditions, functionality of the signalling system is less well understood and more difficult to approach experimentally. We thus use a combined experimental/mathematical modelling strategy to investigate the induction dynamics of the PpuI/R QS system in *Pseudomonas putida* IsoF. Induction of AI controlled expression of a *gfp* gene was followed with high spatio-temporal (single cell or colony level) resolution. The influence of flow respectively addition of external AI was examined. Main results were: Mass transfer (flow) delays the induction behaviour, probably by removal of AIs. A compartmentation of yet unkown origin occurs, limiting the influence of AI from outside the colony. ÁI regulation promoted intra- as well as intercolonial heterogeneity. Summarized, there were fundamental differences between the AI functionality in cell aggregates and planktonic batch cultures, which have been analysed before [1]. These differences have consequences for the ecological functionality of autoinducers.

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RGP041

Characterization of mutations in the sensor kinase gene of the two-component system CiaRH in clinical *Streptococcus pneumoniae* strains

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The two-component regulatory system CiaRH (competence induction and altered cefotaxime susceptibility) of *Streptococcus pneumoniae* affects a variety of processes such as β-lactam resistance, competence development, autolysis, bacteriocin production, and virulence.
The response regulator CiaR directly controls the expression of 24 genes organized in 15 transcriptional units. Besides 19 protein coding genes, CiaR controls the expression of 5 small non-coding RNAs.
Mutations in the histidine kinase gene *ciaH* have been identified in spontaneous β-lactam resistant mutants of *Streptococcus pneumoniae* R6, a non-pathogenic laboratory strain. Gene expression analyses of the promoters directly controlled by CiaR revealed that the CiaR response regulator is more active in strains with altered kinase genes. Furthermore the mutations in these strains lead to decreased susceptibility to β-lactam antibiotics, prevent development of spontaneous genetic competence, and lead to a delayed autolysis during stationary growth.
In contrast to the detailed characterization of the CiaRH system in the laboratory strain R6, CiaRH-mediated regulation has hardly been studied in clinical isolates of *S. pneumoniae*. The *ciaH* mutations appeared even to be restricted to mutants isolated in the laboratory. However, new *ciaH* alleles are present in *S. pneumoniae* genome sequences that became recently available. To test their functions, they were introduced into *S. pneumoniae* R6 and CiaR-mediated regulation was analyzed. The results of these experiments clearly showed that most of these new *ciaH* alleles indeed activated the CiaR regulon. Therefore, mutations in *ciaH* apparently contribute to advanced fitness under antibiotic conditions not only in laboratory but also in nature.

RGP042

The phytochrome regulon of *Pseudomonas aeruginosa*

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Phytochromes are red/far-red light sensitive photoreceptores. First they were discovered in plants, but later on they have also been detected in fungi, cyanobacteria and other prokaryotes. In plants phytochromes control a wide variety of developmental processes, however, in prokaryotes the functions are widely unknown. Most bacterial phytochromes contain a histdine-kinase domain suggesting that signal transduction occurs via a two-component regulatory system. *Pseudomonas aeruginosa* is one of the first heterotrophic bacteria in which a phytochrome has been identified. With the two genes *bphO* and *bphP P. aeruginosa* owns the two necessary components to assemble a red-light photoreceptor system: *bphO* codes for the heme oxygenease to generate the chromophore biliverdin IXα and *bphP,* encoding the apo-phytochrome. So far, no corresponding phytochrome response regulator has been identified yet.
bphO and *bphP* form a bicistronic operon whose expression is controlled by the alternative sigma factor RpoS. New analyses provide a hint for an additional regulation of *bphP.* To investigate the function of *bphO* and *bphP* chromosomal knock-out mutants were constructed and analysed. However, no significant phenotypical difference between the mutants and wild type were observed. A combination of expression profile experiments and proteome analyses revealed a link to a *bphP*-mediated stress response. The most downregulated gene PA4739 (*osmY)* is used in a genetic screen to identify the corresponding response regulator of BphP to gain further insight into the function of the phytochrome in *P. aeruginosa* and the components of its regulon.

RGP043
Influence of Temperature on expression and stability of the RovA/SlyA regulator family

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Transcriptional regulation of genes under a specific set of conditions is a way in which bacteria adapt to a variety of environmental conditions. The regulatory proteins SlyA of *Salmonella typhimurium* and RovA of *Yersinia pseudotuberculosis* belong to the MarR regulator family and control several physiological processes relevant for virulence and survival. The RovA/SlyA proteins are very closely related in sequence. The helix-turn-helix DNA binding sites of *Salmonella* SlyA and the *Yersinia* RovA protein are almost identical but they control largely different gene sets, reflecting both regulation of species-specific targets and transcriptional rewiring of shared genes. SlyA of *Salmonella* was shown to interact with the stringent control signal molecule ppGpp, which enhanced its DNA-binding activity. In contrast, RovA acts as an intrinsic thermometer that undergoes structural alterations in response to a temperature shift from 25°C to 37°C. At 37°C RovA is rapidly degraded by the Lon protease. In contrast, protein degradation assays carried out at 25°C and 37°C demonstrated that SlyA of *Salmonella* is stable at both temperatures. To further investigate the thermosensor in RovA, amino acid exchanges using SlyA as a template were introduced into a P*lac*-driven RovA expression system. Using this strategy we were able to identify certain amino acids which render RovA resistant to temperature-induced degradation at 37°C. Furthermore, we found that in *Y. pseudotubercuolsis*, in stationary phase a factor is secreted into the growth medium. This factor completely stabilises RovA at 37°C. Current experiments are directed to identify this RovA-stabilising factor.

RGP044
The novel PAS4-LuxR solo Plu2018/Plu2019 of the insect pathogen *Photorhabdus luminescens* detects a eukaryotic signaling molecule

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In nature, bacteria live in close association with other bacteria and eukaryotes which means that they constantly need to monitor and communicate with other organisms. The best understood chemical language in proteobacteria is the communication via N-acylhomoserine lactones (AHLs), often produced as an endogenous signal and called quorum sensing. The typical proteobacterial quorum sensing system consists of an AHL synthase belonging to the LuxI-family and a cognate LuxR-family AHL sensor/regulator. Many proteobacteria possess further LuxR-family proteins with no cognate LuxI synthase. Initial investigations of those so called LuxR solos revealed that these regulators have diverse roles in bacteria interspecies and interkingdom communication. The insect pathogenic bacterium *Photorhabdus luminescens* possesses the uncommonly high number of 39 LuxR solos, 35 of them have a novel PAS4 signal binding domain. These PAS4-LuxR solos are speculated to detect yet unknown eukaryotic signaling molecules. Most of the corresponding genes of the PAS4-LuxR solos are located within two large gene clusters on the *P. luminsescens* chromosome. Here, we inactivated these large *PAS4-luxR* gene clusters and performed proteome analyses with the mutants in comparison to the wild-type with filtered insect homogenate as putative inducer. This allowed the identification of potential target genes of these regulators and, on the basis of this knowledge, the generation of corresponding reporter gene strains. We could show that the expression of several reporter genes was inducible with insect homogenate in the wild-type, but not in the mutant lacking PAS4-LuxR solos Plu2018/Plu2019. This clearly showed that these novel PAS4-LuxR solos are involved in interkingdom signaling in *P. luminescens*. Stability experiments with the insect homogenate revealed that the signaling molecule sensed by Plu2018/Plu2019 could be a hormone-like substance.

RGP045
Characterisation of *furA* expression in *Mycobacterium avium* spp. *paratuberculosis*

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Mycobacterium avium spp. *paratuberculosis* (MAP) is the etiological agent of paratuberculosis (Johne´s disease), a chronic, incurable, granulomatous enteritis in ruminants. Furthermore a contribution of MAP to human Crohn´s disease is discussed.
In the host MAP has to overcome the iron starvation by expressing ferric uptake systems via iron depending regulators. The ferric uptake regulator A (FurA), a homolog to the ferric uptake regulator (Fur) family, is an important regulator of iron homeostasis in many bacteria including mycobacteria. Only little is known about iron dependent regulation in MAP. The iron dependent regulator (IdeR) belonging to the diphtheria toxin regulator (DtxR) family has shown to be responsible for iron mediated gene regulation. IdeR is essential for the expression of a cohort of genes encoding proteins for iron uptake and storage. However, the function and regulation of FurA in MAP is still unknown.
By analysing the MAP DSMZ44135 genome the position of the *furA* gene was detected close to the *katG* gene encoding a catalase-peroxidase KatG. Both genes were expressed on a polycistronic RNA. The *furA-katG* region is highly conserved among the order actinomycetales and it was shown to be induced and expressed under oxidative stress and iron starvation. Additionally it has been demonstrated, that FurA auto-regulates its own expression in *Mycobacterium tuberculosis*. In the present study, we cultured MAP in the presence of dipyridyl, an iron chelating agent. These experiments revealed that in MAP *furA* mRNA expression is not inducible by iron starvation, while IdeR dependent genes were up-regulated. These data suggest that MAP *fur*A is not auto-regulatory or dependent on other divalent cations.
Genetic manipulation of MAP is hampered by its slow growth and clump formation. Therefore, in order to analyse the role of FurA more in detail, we applied the specialized transduction method for *furA* deletion. For transduction, we use the pHAE87 phage, a temperature-sensitive derivate of the TM4 mycobacteriophage and constructed a new Phage (pHAE151) to exchange the *furA* gene with a hygromycin resistance gene.

RGP046
An RpoS-dependent small RNA controls OmpD protein synthesis in *Salmonella*

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Question: Small non-coding RNAs (sRNA) are a steadily growing class of post-transcriptional regulators frequently involved in bacterial stress responses. While the transcription of two stationary phase-specific sRNAs, RybB and MicA, was reported to be tightly controlled by the alternative sigma factor, σ_E, no sRNA has yet been assigned to the regulon of the major stress sigma factor σ_S (RpoS).
Methods: In a genome-wide transposon screen we discovered the alternative sigma factor S as the direct transcriptional regulator of the conserved sRNA, SdsR. Over-expression of the sRNA readily inhibited the expression of the abundant outer membrane protein OmpD.
Results: We identified a highly conserved sRNA, SdsR, which accumulates in high amounts in stationary phase and is transcriptionally dependent on RpoS. In *Salmonella*, SdsR represses the expression of the porin OmpD through direct base-pairing. Similar to an additional regulatory RNA, MicC, SdsR binds within the coding sequence of $_{ompD}$ mRNA and down-regulation requires both the presence of the RNA chaperone Hfq as well as RNaseE.
Conclusions: In this study we report the characterization of a non-coding RNA, SdsR, as the first sRNA directly controlled by the alternative sigma factor σ_S. Over-expression of SdsR in *Salmonella* reduced the expression of the OmpD protein. SdsR-mediated repression of *ompD* requires binding in the coding sequence suggesting a mechanism independent of inhibition of translation initiation.

RGP047

Investigation of the relationship between the sigma factor PvdS and the lipase of *Pseudomonas aeruginosa*

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The Gram-negative bacterium *P. aeruginosa* with its large genome size and its ability to adapt to various environmental conditions is an interesting model organism to study microbial regulation. In addition to prominent virulence factors like Exotoxin A and several proteases, *P. aeruginosa* produces and secretes the virulence associated lipase LipA via the Type II secretion pathway. Lipases in general catalyse the hydrolysis of the ester bond in triacyl-glycerol lipids between glycerol and the fatty acid chains. The physiological function of the lipase LipA is still unknown, but a probably more complex role than simple nutrition of the cell has been suggested.
Another protein involved in the pathogenicity of *P. aeruginosa* is the transcriptional regulator PvdS. The major function of this sigma factor is regulation of the synthesis of the siderophor pyoverdine by nonribosomal peptidsynthetases under certain physiological stress conditions. PvdS-regulated genes typically show a specific consensus-motif - the so called IS-Box - for binding of PvdS to the target gene.
Although the lipase-gene does not contain the IS-Box motif, we observed in a *pvdS*-deficient *P. aeruginosa* strain that the deletion of *pvdS* leads to a significant lower lipase activity in the cell supernatant. This phenomenon is not caused by a defect in the secretion machinery, because we were able to reconstitute the lipase activity by expression of plasmid-borne lipase LipA. Transcript analysis revealed that PvdS influences *lipA* expression on the transcriptional level. Interestingly, lipase and pyoverdine production could not only be restored by complementation with *pvdS,* but also by a cosmid clone from a *P. aeruginosa* genomic DNA library containing three putative regulators. Current investigations will characterize the relationship of these regulators with the function of PvdS and the lipolytic system in more detail.

RGP048

Subpopulation specific transcriptome analysis of cytometrically sorted *Streptococcus mutans* cells: Analysis of CSP mediated intra-population diversity

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Competence stimulating peptide (CSP) mediated competence development in *Streptococcus mutans* is a transient and biphasic process, since only a subpopulation induces expression of ComX in the presence of CSP and activation of the DNA uptake machinery in this fraction shuts down ~3-4 hours post induction.
Here we combine, to our knowledge, for the first time in bacteria flow cytometric sorting of cells and subpopulation specific transcriptome analysis of both the competent and non-competent fraction of CSP treated *S. mutans* cells. Sorting was guided by a ComX-GFP reporter and the transcriptome analysis demonstrated the successful combination of both methods because a strong enrichment of transcripts for *comX* and its downstream genes was achieved. Three two component systems were expressed in the competent fraction, among them ComDE. Moreover, the recently identified regulator system ComR/S was expressed exclusively in the competent fraction. By contrast, expression of bacteriocin related genes was at the same level in all cells. GFP reporter strains for ComE and mutacin V confirmed this expression pattern on the single cell level. Fluorescence microscopy revealed that some ComX expressing cells committed autolysis in an early stage of competence initiation. In viable ComX expressing cells uptake of DNA could be shown on the single cell level.
This study demonstrates that all cells in the population respond to CSP through activation of bacteriocin related genes but that two subpopulations segregate, one becoming competent and another one that lyses, resulting in intra population diversity of the clonal culture.

RGP049

Phytochromes of *Agrobacterium tumefaciens*

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Agrobacterium tumefaciens has two phytochromes termed Agp1 and Agp2 which serve as model proteins for studies on photoconversion and light modulation of histidine kinase activity. We found that Agp1 might act as a thermosensor. At neutral pH, the chromophore of Agp2 in the so called Pr form is largely deprotonated, in contrast to canonical phytochromes. Thus, both phytochromes have exceptional features. The biological role of Agp1 and Agp2 will be discussed.

SBP001

Gradual insight into *Corynebacterium glutamicum's* central metabolism for the increase of L-lysine production

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Corynebacterium glutamicum is used for the large production of amino acids like L-glutamate, L-valine or L-lysine, the latter made in a scale of $8x10^5$ annual metric tons. We applied a stoichiometric model and identified citrate synthase (CS) as most promising target to increase L-lysine production. We therefore replaced the two promoters which we identified in front of the CS gene *gltA* of a lysine producer by nine promoters of decreasing strength. The resulting set of strains was subsequently analysed with respect to CS activity, growth, and L-lysine yield. The decrease of CS-activity below 30% led to an increase in L-lysine yield accompanied by a decrease in growth rate. A reduced CS-activity of 6% produced an increase in L-lysine yield from 0.17 g/g to 0.32 g/g. As a further step the global consequences at the transcriptome, metabolome, and fluxome level were monitored within the strain series. Reduced CS activity results in increased expression of genes controlled by RamA and RamB, and increased cytosolic concentrations of aspartate and aspartate-derived amino acids. The fluxome study revealed that reduced CS-activity surprisingly has only a marginal influence on CS flux itself, but increases the internal concentration of oxaloacetate and acetyl-CoA, thus showing the enormeous flexibility of *C. glutamicum's* central metabolism.
This systemic approach opens an exiting new view on the system *C. glutamicum* as an excellent producer of bulk compounds.and sheds new light on the validity of stoichiometric models applied to the living cell.

SBP002

Partial cyclisation of the pentose phosphate pathway for cofactor regeneration in *Escherichia coli*

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Reductive whole-cell biotransformation has become an important method in organic chemical synthesis, e.g. for the production of chiral intermediates used in the synthesis of pharmaceuticals. Recombinant nicotinamide adenine dinucleotide phosphate (NADP(H)) cofactor regeneration systems are highly important for these processes, because NADP(H) serves as reductant in many of the redox reactions involved. Prominent products whose synthesis by biotransformation requires NAD(P)H are chiral alcohols as building blocks for the synthesis of statins, compound that function as inhibitors of cholesterol synthesis.
With *Escherichia coli* different approaches for cofactor regeneration have been applied, e. g. using a one-enzyme-coupled system, like glucose dehydrogenase which oxidizes one mol glucose for regeneration of one mol NAD(P)H [1; 2]. Cyclization of the pentose phosphate pathway (PPP) theoretically affords generation of 12 mol reduction equivalents per mol glucose. A shift of glucose catabolism from glycolysis to the PPP can be brought about by reduction of phosphofructokinase activity. Therefore, a phosphofructokinase (Δ*pfkA/B*) deficient mutant should be useful for reductive whole-cell biotransformation processes.
In the present work, the reduction of methylacetoacetate (MAA) to (*R*)-methyl 3-hydroxybutanoate (MHB) with recombinant *E. coli* cells containing an *R*-specific alcohol dehydrogenase from *Lactobacillus brevis*

[3] was investigated. The specific biotransformation activity and the glucose consumption of the wild type *E. coli* BL21(DE3) was compared to its Δ*pfkA* mutant, which showed a 90% reduced phosphofructokinase activity. The specific biotransformation activity of both strains was similar, but in the Δ*pfkA* deletion mutant the NADPH concentration was increased and the glucose consumption required for conversion of 50 mM MAA to MHB was decreased by 50%.
It has previously been shown that the overexpression of the *zwf* gene encoding glucose 6-phosphate dehydrogenase resulted in higher productivities in several biotransformation processes [4]. Besides overexpression of *zwf* and *gnd* (encoding 6-phosphogluconate dehydrogenase), we also aimed at the elucidation of the optimum expression levels of the other PPP genes.

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[4] Lim, S. J. et al (2002): J Biosci Bioeng, 93(6): 543-9.

SBP003
Differences in signalling by directly and indirectly binding ligands in bacterial chemotaxis

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In chemotaxis of *Escherichia coli* and other bacteria, extracellular stimuli are perceived by transmembrane receptors that bind their ligands either directly, or indirectly through periplasmic-binding proteins (BPs). As BPs are also involved in ligand uptake, they provide a link between chemotaxis and nutrient utilization by cells.However, signalling by indirectly binding ligands remains much less understood than signalling by directly binding ligands. Here, we compared intracellular responses mediated by both types of ligands and developed a new mathematical model for signalling by indirectly binding ligands. We show that indirect binding allows cells to better control sensitivity to specific ligands in response to their nutrient environment and to coordinate chemotaxis with ligand transport, but at the cost of the dynamic range being much narrower than for directly binding ligands. We further demonstrate that signal integration by the chemosensory complexes does not depend on the type of ligand. Overall, our data suggest that the distinction between signalling by directly and indirectly binding ligands is more physiologically important than the traditional distinction between high- and low-abundance receptors.

SBP004
Heterologous gene expression in clonal populations: IPTG as inducer for *lac* operon expression and influence of transacetylase activity

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Question: The history of *lac* operon investigation started already 50 years ago and lasts until today in the field of systems biology. Research is focused on the question why bacteria use specific network designs to react to external stimuli. The *Escherichia coli lac* operon contains a positive feedback loop. Inducer is taken up by the lactose permease which in turn leads to the production of more permease molecules resulting in an increased capacity to take up inducer. This feedback regulation can cause heterologous gene expression in clonal populations. By using GFP as reporter it was previously shown that TMG can induce a bimodal induction behavior[1]. Because IPTG is the most preferred inducer in heterologous gene expression we were interested in the question whether IPTG can also cause differential gene expression.
Methods: We compared ß-galactosidase activities after TMG and IPTG induction in the wildtype and a *lacY* deletion strain and constructed *Plac-gfp* reporter strains to observe single cell behavior in the wildtype and in different mutant backgrounds.
Results: By comparing TMG and IPTG induction in the wildtype and a *lacY* deletion mutant we could show that both inducers are substrates of the lactose permease which is the prerequisite for the feedback loop. We constructed a *Plac-gfp* reporter strain and observed bimodal induction with TMG and also with IPTG. The comparison revealed that bimodal induction by using IPTG occurs at an approximately tenfold lower concentration than by using TMG. Furthermore, an influence of the lactose transacetylase on the induction could be demonstrated by transferring the *Plac-gfp* fusion into a *lacA* mutant background.
Conclusions: The data show that although IPTG can pass the cell membran independently of the lactose permease the feedback loop is sustained at low inducer concentrations leading to differential gene expression in clonal populations. This have to be considered in heterologous gene expression when the *lac* promoter is used as a tunable control element. Additionally, we provide a comprehensive comparison between the two gratuitous inducer in isogenic strains and can show that even the lactose transacetylase have to be considered in mathematic modelling.

[1] Ozbudak et al (2004): Nature 427, 737-740.

SBP005
Modelling the phosphotransferase system of *Pseudomonas putida* proposes an unexpected distribution of the involved phosphate fluxes

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The environmental bacterium *Pseudomonas putida* is known for its metabolic versatility and stress resistance. This requires various layers of control to coordinate the expression of specific genes to the overall physiology of the cell. One prevalent physiological sensor to this end is the phosphotransferase system (PTS). Apart from the classical phosphoenolpyruvate:carbohydrate PTS, many prokaryotes harbour also a PTS branch that is not involved in carbohydrate traffic, but participates in regulation of some metabolic processes in a fashion dependent on the phosphorylation state of the PTS proteins [1]. The genome of *Pseudomonas putida* KT2440 encodes only 5 recognizable PTS proteins building the PTS^{Fru}, a sugar PTS responsible for fructose uptake, and PTS^{Ntr}, the nitrogen PTS, which lacks any connection to sugar traffic but exerts various regulatory roles. Both branches cross talk under specific metabolic conditions by phosphate exchange. A mathematical model was set up to describe the available data of the state of phosphorylation of PtsN, one of the PTS^{Ntr} proteins, in different environmental conditions and different strain variants. Furthermore, data from flux balance analysis was used to determine some of the kinetic parameters of the involved reactions. Interestingly, modelling the system proposed that during growth on the PTS substrate fructose, about 80% of the required phosphoryl groups for fructose uptake *via* the PTS^{Fru} are provided by the PTS^{Ntr}. This result is rather unexpected and gives rise to new questions on the biological relevance of the cross talk between the two systems and its implementation in the overall metabolism.

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SBP006
Systems biotechnology towards superior production of recombinant proteins in *Aspergillus niger*

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The filamentous fungus *Aspergillus niger* is an important biocatalyst for industrial production of enzymes as well as organic acids or antibiotics. In submerged cultivation, *A. niger* exhibits a complex morphology which typically has a strong influence on production performance. In this regard, comprehensive approaches, combining systems-wide analysis and optimization at the cellular level with process-driven engineering of the bioreactor environment, seem most useful in order to achieve superior production processes and we applied to protein producing *A. niger*. The optimization included the use of microparticles, added to the culture, which allowed to precisely control the morphological shape of *A. niger* [1, 2] and increase enzyme production in different recombinant *A. niger* strains. This strategy was combined with model-based medium design and development of efficient feeding strategies. As result, production of the high-value enzyme fructofuranosidase, an important biocatalyst for neo-sugars in food

or pharmaceutical industry, could be increased to 2800 U/mL, more than tenfold as compared to previous processes [3]. In current work, ^{13}C-based metabolic flux analysis was used to quantify the underlying carbon core metabolism of *A. niger* under different production conditions including the comparison of different mutants as well as morphological forms. This promises valuable insights towards the further development of *A. niger* as a cell factory for recombinant proteins.

[1] Driouch, H. et al (2010): Morphology engineering of *Aspergillus niger* for improved enzyme production. Biotechnol Bioeng; 105:1058-68.
[2] Driouch H. et al (2010): Optimized bioprocess for production of fructofuranosidase by recombinant *Aspergillus niger*. Appl Microbiol Biotechnol 87:2011-24.
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SIV001
A Screen to identify fungal and plant signals during arbuscule formation in AM symbiosis

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The great success of arbuscular mycorrhiza (AM) symbiosis relies on the mutualistic exchange of nutrients between the vast majority of all land plants and the Glomeromycotan fungi. Most important for this interchange is the arbuscule as main organ of nutrition exchange. This fungal structure is build inside the root cortex cells via invagination of the plasma membrane followed by an intense dichotomous branching of the fungal hyphae. The formation of the arbuscule requires a complex exchange of signals between the two partners. The plant's cellular program must be adjusted to allow the entry of the hyphae and to initiate the rearrangement necessary for the accommodation of the fungus in the cell.
The Phosphate Transporter 4 of *Medicago truncatula* (MtPT4) is located in the periarbuscular membrane of the cortex cells, delivering the phosphate provided by the fungus to the plant [1]. The presence of this mycorrhiza specific expressed protein has been shown to be essential for proper arbuscule development indicating a role of MtPT4 during this process [2].
In order to identify fungal and plant proteins involved in the control of the arbuscule formation we developed a screen to search for factors that are able to drive the expression of MtPT4 as a reporter gene for the onset of arbuscule formation.

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SIV002
An effector protein from the symbiotic fungus *G. intraradices* suppresses plant early defense responses

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For many microbial pathogens the delivery of effector proteins represents a crucial mechanism to manipulate their host and to enable colonization. We show that the symbiotic mycorrhizal fungus *G. intraradices* secretes the small, tandem repeat protein SP7 that targets the pathogenesis-related transcription factor ERF19 at the nucleus of its host plants. *ERF19* is highly induced in roots infected with the fungal pathogen *Colletotrichum trifolii* but only transiently upregulated during early mycorrhizal colonization. The level of *C. trifolii*-mediated induction of *ERF19* can be reduced by the constitutive expression of SP7 *in planta.* Additionally these roots exhibit a higher mycorrhization status than control root lines after 5 and 12 days post inoculation with *G. intraradices.* Furthermore, expression of SP7 in the rice blast fungus *M. oryzae* attenuates root decay symptoms. Arbuscular mycorrhizal spore extracts highly induce *ERF19* as well as pathogenesis-related proteins 10a/b expression in control roots, while this is softened in SP7 expressing lines. Our results suggest that SP7 is an effector that contributes to establish/maintain the biotrophic status of arbuscular mycorrhizal fungi in roots by counteracting the early plant immune response.

SIV003
Drugs from bugs that kill bugs: Biosynthesis and function of natural products from entompathogenic bacteria

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Photorhabdus and *Xenorhabdus* live in symbiosis with *Heterorhabditis* and *Steinernema* nematodes, respectively. The bacteria-nematode complex is highly entomopathogenic and is used in organic farming to kill different insect pests. Moreover, the difference between symbiosis (towards the nematode) and pathogenesis (towards the insects) can be studied using these bacteria and we have started to look in detail into the role of bacterial secondary metabolites, which might play a role in both processes. In the last few years we could identify (i) novel secondary metabolites, (ii) their corresponding biosynthesis gene clusters, and (iii) could also propose functions to some of these compounds within the complex life cycle of bacteria, nematodes, and insects. Examples are the isopropylstilbenes, unusual urea derivatives, a family of new linear peptides, the xenocoumacin antibiotics, simple amides and indole derivatives as well as anthraquinones. Additionally, the formation of small molecules specific for different stages of the complex bacterial life style as well as the activation of the respective biosynthesis gene clusters under lab conditions will be presented and their function will be discussed.

SIV004
Host selection shapes microbial community structure in cockroach guts

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Termites harbor a diverse microbial community within their intestinal tracts that allows them to digest wood and other lignocellulosic diets. While the composition of such assemblages is easily established, the influence of the host on community structure and the role of gut microorganisms in host development remain uncertain. Greater insights into such reciprocal interactions could be obtained from gnotobiotic models, but due to their elaborate social system termites cannot be raised under germ-free conditions. As an alternative, we developed a gnotobiotic model of the cockroach Shelfordella lateralis. These cockroaches are non-social, can be raised under germ-free conditions, and are closely related to termites. We inoculated gnotobiotic cockroaches with a full complement of termite gut microbiota and determined the resulting bacterial community structure by 454 pyrosequencing of 16S rRNA genes. While certain members of the termite gut microbiota successfully colonized the cockroach gut, other lineages were completely lost. This demonstrates the importance of host selection in shaping the structure of the intestinal microbial community. In addition, we found that in the absence of a gut microbiota, host development was severely impaired but maturation was achieved after the animals were conventionalized with cockroach gut microbiota. We expect that our model will also provide further insights into the digestive symbiosis.

SIV005
Immune response of the ant *Camponotus floridanus* against pathogens and its obligate mutualistic endosymbiont

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Insects rely on innate defense mechanisms to combat infections. Whereas the immune system of *Drosophila melanogaster* is well characterized [1], much less is known in other insects, and in particular in social insects [2]. Thus, we focused on the characterization of the innate immune system of the carpenter ant *Camponotus floridanus*. This ant species harbours the obligate endosymbiont *Blochmannia floridanus* in bacteriocytes [3] and is therefore also an interesting model organism to study the interactions between hosts, endosymbionts and pathogens.
As a first step towards characterizing the ant´s immune response, we used suppression subtractive hybridization (SSH) to identify genes that are

transcriptionally induced in response to injection of dead bacteria into the haemocoel. Differentially expressed ESTs encode proteins that share significant sequence similarities with proteins from other insects known to be involved in immune reactions. Among these were factors involved in pathogen recognition, signal transduction, antimicrobial activity, or general stress response. A quantitative analysis of immune gene expression revealed different expression kinetics of individual factors and also characteristic expression profiles after injection of different bacterial species, including the endosymbiont *B. floridanus*. Furthermore some immune genes displayed interesting expression patterns according to developmental stage and tissue.
A detailed characterization of the mRNA and gene sequence of one AMP, a hymenoptaecin, revealed a special repeat structure which resembles the multipeptide precursor structure from the *Apis mellifera* apideaecin [4].

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SIV006
The gut symbionts of *Niphargus* amphipods

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Gut symbionts are widespread among metazoans and play a crucial role in evolution and ecology. They enable their hosts to develop new diets, further allowing them to invade ecosystems with novel nutrient conditions. The Frasassi caves in central Italy contain sulfide-rich water bodies, and the food chain in this ecosystem is fully sustained by chemoautotrophic, sulfur-oxidizing bacteria. The amphipod *Niphargus*, which is endemic to subterranean environments, is found in large numbers in the Frasassi caves. Three distinct species of *Niphargus* occur within Frasassi, and they have invaded the cave ecosystem independently within the last one million years. Whereas one of the species (*Niphargus montanarius*) lives in a habitat where sulfide is non-detectable, the other two species (*Niphargus ictus* and *Niphargus frasassianus*) are found in waters containing more than 500 micromoles of sulfide. In this study, the resident gut communities of the three Frasassi-dwelling *Niphargus* species were analyzed using 16S rDNA sequencing and DNA fingerprinting methods (Denaturing Gradient Gel Electrophoresis; DGGE and Automated Ribosomal Intergenic Spacer Analysis; ARISA). Our preliminary analyses suggest that all three species contain host-specific gut communities. *N. ictus* and *N. frasassianus* gut communities are more similar to each other than they are to the gut microbiota of *N. montanarius*. Both *N. ictus* and *N. frasassianus* guts harbor bacteria of the clade *Mollicutes* that closely resemble gut symbionts of a deep-sea hydrothermal vent shrimp, another crustacean living in a sulfide-rich environment. Our results suggest that gut symbioses suitable for invasion of both marine and freshwater sulfide-rich ecosystems could have developed by convergent evolution.

SIV007
Symbiont response of deep-sea hydrothermal vent mussels to energy source removal

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Hydrothermal vent mussels from the Mid-Atlantic Ridge live in dual symbioses with sulfur- and methane-oxidizing bacteria. The symbionts gain their energy from hydrogen sulfide and methane contained in the diffuse fluids emitted from the vents. A decrease in vent fluid emissions over time or space are known to lead to reductions in symbiont abundance, activity and productivity, but little is yet known about how these processes occur. In this study, we investigated the symbiont response of the vent mussel *Bathymodiolus puteoserpentis* to the sudden cessation of sulfide and methane supply by displacing mussels from active venting to a site with no hydrothermal influence.
The advantage of in situ displacement studies over aquaria experiments is that artifacts caused by depressurization are avoided.We examined the abundance of symbionts in mussels displaced for up to 10 days using fluorescence *in situ* hybridization (FISH) with confocal laser scanning microscopy (CLSM). The resulting 3D images were analysed with digital image analyses and deconvolution software for three dimensional distribution patterns and abundances of the symbiotic cells. Results showed that after only one day of displacement, symbiotic metabolic activity was strongly reduced as an immediate response to the lack of sulfide and methane, but there was little decrease in symbiont abundance. These morphological analyses were compared to quantitative PCR (qPCR) analyses using phylogenetic marker genes for the host (18S rRNA) and the symbionts (16S rRNA) as well as a single copy gene (*recA*). FISH and qPCR analyses were comparable at the beginning of the displacement experiment but differed markedly after several days of displacement. We are currently investigating if a decrease in symbiont polyploidy might explain this.

SIV008
Nitrogen fluxes in the Mediterranean sponge *Aplysina aerophoba* and its symbiotic microbial consortia

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Marine Sponges (*Porifera*) are known to harbor enormous amounts of microorganisms with numbers exceeding 10^{10} per g sponge belonging to at least 30 different bacterial phyla including several candidate phyla and both archaeal lineages. During the last decades huge sequencing efforts were applied to elucidate the microbial diversity associated with these animals, whereas the functional interactions between sponges and their potential symbionts mostly remain to be investigated. Using physiological incubation experiments with living sponges we explored the processes of nitrification, their variation during an annual cycle and specific inhibition. Additionally, we could identify *Proteobacteria* and *Crenarchaea* as the possible microbes involved in these processes by molecular and phylogenetic analysis. In a current project we are analyzing the diversity of microorganisms capable of nitrogen fixation by amplifying genes encoding the iron containing nitrogenase reductase (*nifH*), a key enzyme of nitrogen fixation. Comparison of DNA and RNA derived sequences as well as Denaturing-Gradient-Gel-Electrophoresis (DGGE) analysis shows differences between diversity and activity of the involved microbes. High diversity of *nifH* genes related to genes of diverse bacterial phyla was shown. Surprisingly the factual active nitrogen fixing bacteria seem to be affiliated to *Betaproteobacteria* only. Finally this research will aid to elaborate the picture of different metabolic pathways of symbionts living in sponges.

SIV009
Interactions between the ciliate *Stentor amethystinus*, green algae and prokaryotes in Lake Stechlin

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Stentor amethystinus usually lives in the benthos of freshwater ecosystems. In Lake Stechlin it also occurs in the pelagic zone and seasonally can substantially contribute to pelagic primary production (up to 60%). *S. amethystinus* cells are 250-500 microns long and have a spherical macronucleus (20-30 microns long) with many (>20) micronuclei. Ciliates (e.g. *Paramecium aurelia*) can harbour endosymbiotic bacteria in their nucleus (Müller 1856), which is also the case for *S. amethystinus* . While being heterotrophic, *S. amethystinus* often associates itself with green algae to form a symbiosis with algae. Interestingly, *S. amethystinus* is associated with *Chlorella* in North America, but the Lake Stechlin population contains *Micractenium* species. The alga-ciliate system can be also inhabited by cyanobacteria and heterotrophic bacteria, thus forming a complex symbiotic community. Since we are able to grow the cyanobacteria endosymbiont as well as the major bacterial endosymbiont in pure or enrichment cultures we assume that these endosymbiosis are still *in statu nascendi*. In this presentation, we will highlight specific interactions between the host and its symbionts and point to their functional consequences.

SIV010
Regulation of nutrient transporter genes in the extraradical mycelium of the arbuscular mycorrhizal fungus *Glomus intraradices*

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The establishment of an arbuscular mycorrhiza symbiosis between a fungal and a plant partner is mainly driven by a bidirectional exchange of nutrients. While the plant supplies the fungus with carbohydrates the fungus provides access to soil derived nutrients which are unavailable for the plant. The uptake and transport of soil nutrients to the plant occurs via an extraradical mycelium and its nutrient transporter systems, some of them are already characterized by *in vitro* studies. Examples are the high affinity phosphate (PT) and ammonium (AMT1) transporters from *Glomus intraradices*. Their gene expression is regulated in dependence of P_i (*GintPT*) or of ammonium and nitrate (*GintAMT1*) concentrations of the surrounding medium as shown in experiments with root organ cultures [1, 2].
In order to analyse *GintPT*, and *GintAMT1* expression under more natural conditions, a greenhouse pot culture experiment with two treatments of N-fertilization in combination with three P_i concentrations was set up. The pots contained compartments filled with a mixture of soil and glass beads for harvesting the extraradical mycelium [3]. Results show a regulation of the transporter gene expression only depending on the N fertilizations of this experiment. RNA accumulation of *GintAMT1* was increased under low nitrogen concentrations. In contrast, *GintPT* expression was induced at high amounts of nitrogen. No effect was found for the P_i-fertilization, but analysis of the plants phosphorus (P) concentrations at the day of harvest showed that all plants in the trial suffered from P deficiency. Besides *GintAMT1*, another N transporter gene responsible for the uptake of nitrate (*GintNT1*) was analysed. This transporter is not characterized by *in vitro* studies so far. The *GintNT1* expression pattern observed in this experiment was the same as for *GintAMT1*.
If the regulation of the transporter gene expression is directly a consequence of the soil nutrient concentrations, of the nutritional status of the symbiosis partners or of both has to be analysed in further experiments. Especially the results for *GintPT* expression suggest that nutrient concentrations in the plant shoot play a dominant role in the regulation of transporters in the extraradical hyphae of the fungal partner.

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SIP001
Improvement of yield, harvesting time and polysaccharide-protein complex content of *Agaricus blazei* Murrill with beneficial microbes

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It is widely known that mushrooms contain active organic ingredients that are associated with the maintenance of human health and the healing of diseases.[1] Pharmacological studies have shown that *Agaricus blazei* Murrill contains several bioactive substances (e.g. polysaccharides) that function as antioxidants,[2] antimutagenics,[3] and anticancer agents.[4] Furthermore, these substances have been reported to reduce blood sugar, blood pressure, cholesterol,[5] and prevent osteoporosis.[6] Therefore, it is not surprising that *A. blazei* has drawn the attention of food scientists and biotechnologists. The production of *A. blazei* requires extensive casing to allow fruitification of mushrooms. In light of the growth-promoting effects of beneficial microbes (BM) in agriculture, an extensive BM screen was conducted from the base of natural growing *A. blazei* stipe in attempt to increase the total yield and to reduce the harvesting time. A total of 42 different bacteria isolates were identified through 16S rDNA sequencing and with 15 isolates conferring mycelium growth-inducing abilities. Amongst, inoculation of *Arthrobacter* sp. K4-10C, *Exiguobacterium aurantiacum*, *Microbacterium humi* sp. nov. or *Advenella incenata* strains in the casing soil resulted in significant increases in *A. blazei* total fresh yield at 64%, 64%, 54% and 46%, respectively. In addition, inoculation of *Arthrobacter* sp. K4-10C or *Exiguobacterium aurantiacum* resulted in a significant increase in the polysaccharide-protein complex content. Interestingly, inoculation of *Exiguobacterium aurantiacum* reduced the harvesting time for 14 days as compared to the control without microbe inoculation. In conclusion, the identification of beneficial microbes for the culturing of *A. blazei* resulted in a reduced harvesting time, a significantly increased total fresh yield, and an increase polysaccharide-protein complex content show promise of being economically viable for applications within the commercial mushroom industry.

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SIP002
Chemical crosstalk between *Streptomyces sp.* Ach 505 of the rhizosphere and plant pathogenic fungus *Heterobasidion*

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The mycorrhiza helper bacterium *Streptomyces* sp. AcH 505 supports the mycorrhization of *Picea abies* (Norway spruce) with *Amanita muscaria* (fly agaric) by excretion of auxofuran, a growth promoting compound [1, 2]. Besides auxofuran, *S.* sp. AcH 505 produces WS-5995 B, an antibiotic with antagonistic activity against the root pathogenic fungus *Heterobasidion* which is the causal organism of 'annosum root rot'. *Heterobasidion* produces fomannoxin, a secondary metabolite with phytotoxic, fungicidal and bactericidal activity [3]. *S.* sp. AcH 505 acts antagonistic against eleven of twelve investigated *Heterobasidion* isolates. Only strain *H. abietinum* 331 is resistant and not affected, neither by *S.* sp. AcH 505 itself nor by the antifungal antibiotic WS-5995 B [4].
Co-culture of *S.* sp. AcH 505 with resistant *H. abietinum* 331 in liquid medium results in increased production of a novel compound, 331HaNZ, by the fungus. Its production is neither induced by auxofuran nor by WS-5995 B. Another compound 5-formylsalicylic acid (5-FSA) is also produced by *H. abietinum* 331 but it appears earlier than 331HaNZ during cultivation. Both compounds were isolated from liquid medium and the structures were elucidated. Both compounds are structural analogues to salicylic acid (SA) from plants which induces the systemic acquired resistance (SAR) against plant pathogens. SA induces the PR genes (pathogenesis related genes) which then generate the SAR. Investigations on the biological activity against the model organism *Arabidopsis thaliana* by northern blot were made.

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SIP003
A surface hydrophobin in ectomycorrhiza interaction

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Hydrophobins are small secreted proteins with a broad range of functions like in processes of growth and development of filamentous fungi, e.g. formation of aerial structures. Mutual symbiosis like ectomycorrhiza lead to differential gene expression. Up to 50% of fungal mRNAs is regulated

during development of the symbiotic interaction (1). Hydrophobins have been shown to play an important role in this interaction. At the same time, the hydrophobin TtHyd1 is specifically expressed in the Hartig'net in a compatible interaction of *Tricholoma* with pine (2). To investigate its function, heterologous expression in *Schizophyllum commune* was performed, where we know 13 hydrophobins of this class from the genome sequence. So far, a databank was generated and used to search for motifs, new hydrophobins and a phylogenetic tree was calculated based on hydrophobin protein and mRNA sequences. For the characterization of hydrophobins from *Tricholoma vaccinum*, an overexpression will be the future goal to investigate in which stage of the symbiotic interaction hydrophobins are produced and what kind of role they play with respect to function of the symbiotic tissue.

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SIP004
Kunitz-type protease inhibitors are involved in arbuscule development in mycorrhizal symbiosis

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The arbuscular mycorrhiza (AM) is the most widespread symbiosis and it is formed between plants and fungi of the Glomeromycota Phylum. To establish the plant-fungal interface, fungal hyphae invade the host root through the epidermal layer and continue growing toward the cortex, where they form highly branched structures - called arbuscules. This involves invagination of the plant plasma membrane around developing arbuscules to form the mutual interaction zone. Arbuscule development is a dynamic process. After several days of maturity arbuscules collapse and die, whereas an invaded plant cell can host successive arbuscules. TC106 encodes a secreted *Medicago truncatula* (barrel medic) protease inhibitor (Kunitz-type) specifically induced upon contact with AM fungi. Deregulation of TC106 expression level mediated by RNAi silencing and constitutive overexpression revealed abberrant mycorrhizal phenotypes showing disproportionally high numbers of crippled arbuscules. A non-directed yeast-two-hybrid screen identified a secreted cysteine protease as a potential interaction partner of TC106. Furthermore, direct yeast-two-hybrid interaction tests showed an interaction of TC100 - also encoding a secreted Kunitz protease inhibitor - with a recently described mycorrhiza specific induced subtilase. Proteolytic cleavage of peptide bonds is crucial to navigate development and regulatory processes. In this study, all investigated proteins contain a secretion signal peptide. This would suggest the plant-fungal interface as the potential locus of protein-protein interaction. We hypothezise that distinct members of the Kunitz protease inhibitor family are key players in the plant controlled part of arbuscule development. The protease inhibitors would fine-tune proteolytic activity required for arbuscule turnover to prepare the invaded plant cell for the next generation of arbuscules.

SIP005
Molecular basis of symbiosis in phototrophic consortia

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The phototrophic consortium *Chlorochromatium aggregatum* is a morphologically defined multicellular assemblage consisting of a central motile chemotrophic Betaproteobacterium that is associated with ~20 cells of the green sulfur bacterial epibiont *Chlorobium chlorochromatii*. The epibionts are connected with each other and the central bacterium through hair-like ultrastructures. The attachment site of the epibiont to the central bacterium is characterized by the absence of chlorosomes and a single contact layer. The central bacterium is flagellated and extends periplasmic tubules to the outer membrane of the epibionts. This highly structured association is culturable making it a model system for understanding the molecular basis of symbiosis between different types of bacteria. Previous work has described four putative symbiosis genes (Cag1919, Cag1920, Cag0614, Cag0616) of the epibiont which were recovered by suppression substractive hybridization and bioinformatic approches. These four genes are constitutively transcribed and do not occur in genomes of non-symbiotic relatives of the epibiont. Cag1919 contains a haemolysin-type Ca^{2+}-binding region with several RTX repeats. RTX-type toxins so far have been found in Gram-negative bacterial pathogens and Cag1919 may have been recruited by the epibiont via lateral gene transfer. To facilitate localization of the proteins, Cag1919 was cloned in its entirety into the vector pQE60. Interestingly, expression of Cag1919 was deleterious to *E. coli* strains causing the formation of extremely long, filamented cells. Expression of the recombinant protein was achieved in *E.coli* strain *XL1-Blue*. Recombinant Cag1919 is used to produce antibodies for immunogold labelling and tyramide signal amplification to establish the location of the protein in the *C. chlorochromatii* in the free-living and symbiotic states. Efforts to identify and express suitable fragments of the giant Cag0614 and Cag0616 gene products will also be described.

SIP006
The symbiotic gut microbiota of termites and cockroaches: Are there evolutionary patterns in the dictyopteran lineage?

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Previous studies on the intestinal microbiota of termites have identified numerous clusters of bacteria that seem to occur exclusively in termite guts. Some of these termite-specific clusters are also affiliated with sequences originating from their closest relatives, the cockroaches. However, the microbial diversity in cockroach guts has not been studied in any detail, and it is therefore not clear whether this exclusiveness reflects properties of the particular niches in the dictyopteran gut (e.g., habitat preferences or diet-related factors) or whether these are even evolutionary patterns (i.e., elements of the gut microbiota are cospeciating with their dictyopteran hosts). To address these points, we investigated the diversity of the bacterial gut microbiota in numerous representatives of the dictyopteran lineage using 454 pyrosequencing. Total DNA was extracted from the microbe-packed hindguts of 35 insect species, comprising 16 termites, 15 cockroaches, 1 mantid, and 3 insect species outside the *Dictyoptera*. The 16S rRNA genes were sequenced after PCR amplification with a modified primer set targeting the V3-V4 region (ca. 450 bp) to exploit the full capacity of the Titanium technology. The resulting sequences (3,000-15,000 per species) were processed using a pipeline combining Naïve Bayesian classification with a manually curated reference database. OTU assignment, statistical and phylogenetic analyses are being performed using MOTHUR, R, and ARB software. Preliminary results indicate that the gut microbiota of termites and cockroaches contains phylotypes typical of the gut environment in general, and particular lineages are apparently cospeciating with their dictyopteran host. The presence of bacterial groups occurring exclusively in soil-feeding or fungus-feeding species suggests a participation of these groups in the degradation of the respective diet.

SIP007
Will not be presented!

SIP008
Development of lineage-specific gut microbial communities during termite evolution

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Termites feed on diets consisting of lignocellulosic or humic substrates. They are divided into two groups - the more primitive lower termites, which possess cellulolytic gut flagellates, and the evolutionarily advanced higher termites, which lack such flagellates and have developed novel strategies to digest their respective diets. While the lower termites feed almost exclusively on wood, higher termites (family Termitidae), which make up about 80% of all termite species, comprise several feeding guilds of fungus-cultivating, soil-feeding, and secondarily wood-feeding forms. We conducted a comprehensive comparative analysis of the bacterial gut microbiota in representatives of all subfamilies and feeding guilds, combining 454 pyrosequencing of the V3-V4 region with additional Sanger

sequencing of the 16S rRNA genes for hitherto underrepresented termite taxa. Clear differences in community structure between the bacterial gut microbiota of lower and higher termites reflect the importance of the fiber-digesting flagellates as a bacterial habitat (e.g., the loss of the abundant endosymbiotic Endomicrobia). The strong increase of potentially cellulolytic Fibrobacteres in wood-feeding taxa and the decrease of Spirochaetes and the concomitant appearance of apparently alkali-adapted Firmicutes in soil-feeding taxa correlate with the nutritional specialization of their termite host. Our results document the evolution of specific gut microbial communities in each lineage of higher termites and will help to better understand the function of the gut microbiota in the digestive process.

SIP009

Analysis of Anthraquinones biosynthesis in *Photorhabdus*

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The entomopathogenic bacterium *Photorhabdus luminescens* lives in symbiosis with nematodes of the genus *Heterohabditis*. Among other natural products, *P. luminescens* produces anthraquinones (AQ). Earlier studies in our group have shown that a type II polyketide synthase (PKS) is responsible for the biosynthesis of AQ, which is only the second example of type II PKS production in Gram-negative bacteria[1]. Unlike expected from the heptaketide backbone of AQ, Brachmann et al. have shown that AQ are derived from an octaketide precursor, as deletion of gene encoding a cyclase/aromatase AntH resulted in the formation of octaketides, which were already known as shunt-products in in the octaketide natural product actinorhodin from *Streptomyces coelicolor* A3(2)[2].
We continued to investigate the AQ biosynthesis by deletion of cyclase encoding gene *antA,* which resulted in production of additional typical polyketide shunt-products. Furthermore, AQ biosynthesis was successfully reconstituted in *Escherichia coli*. Thus, partial heterologous expression of the *ant* cluster allowed the detailed investigation of the AQ biosynthesis. Recently, the type II PKS components were expressed in *E. coli* and purified for the activity assays. Therefore, the mechanisms of the biosynthesis and specificities of the proteins can now be investigated *in vitro*[3]. The results of the analysis will be presented on our poster.

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SIP010

Losing the partner - now what? Effects of host loss on transcription in arbuscular mycorrhizal fungi

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Arbuscular mycorrhizal fungi are an ancient fungal phylum (Glomeromycota) that coevolved with plants for the last 400 million years, assisting the colonization of land masses by higher plants. Today, these fungi associate with 70-90% of all plant roots and form intimate and, in most cases, mutualistic symbioses. Collectively referred to as arbuscular mycorrhiza (AM), this association is regarded as the most widespread terrestrial symbiosis. The interaction is mainly characterized by fungal arbuscules, i.e. tree-shaped subcellular structures within plant root cells that are the main site of nutrient exchange between the fungal and plant symbiotic partners. The fungal partner provides water, phosphate and other nutrients which are taken up via its extensive hyphal network from the soil. In return AM fungi obtain carbohydrates from their plant partner. Up to 20% of the photosynthesis products of terrestrial plants (roughly 5 billion tonnes of carbon per year) are estimated to be consumed by AM fungi. Therefore, the AM symbiosis contributes considerably to global phosphate and carbon cycling and influences primary productivity in terrestrial ecosystems.
While the symbiosis is well characterized in regard to nutrient and signal exchange, little is known regarding the senescence (ageing and death) of AM fungi. The life time of extraradical hyphae (i.e. hyphae outside the root) has been shown to be relatively short, on average 5-6 days, and intraradical hyphae (hyphae inside the root) as well as arbuscules have been described to be turned over equally rapidly. The aim of our experiments is to characterize this turnover on a molecular level. Using carrot root cultures colonized by *Glomus irregulare* (DAOM197198) we induced fungal senescence by separating AM fungi from their plant partner. Host loss eventually resulted in the termination of fungal cytoplasmic streaming after several days. Currently, we compare mRNA extracts of vital and senescent fungal cultures by suppressive subtractive hybridization to detect differentially expressed genes. Key mRNA transcripts defined in this project will be used as molecular markers in field studies directed at assessing the presence, abundance, and activity of AM fungi in various ecosystems and under simulated conditions of climate and land use change.

SIP011

Detection of Differences between Specific Bacterial Groups of the Intestinal Flora of Adipose Persons compared to Normal Weighted Persons

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Obesity epidemic is global phenomena, affecting both the developed and developing world. Development of obesity is due to many factors such as less exercise, excessive nutrition, genetics or illnesses. Recent work has shown that the intestinal flora may also have an influence on the energy balance of the host due to interactions of the intestinal flora and the host. For understanding these observation in more detail stool samples were collected of 10 normal weighted (mean body mass index (kg/m²) of 22.84) and 11 obese (mean body mass index (kg/m²) of 40.54) healthy persons in a homogeneously group regarding to age and gender. After extraction of the DNA from the stool samples real-time quantitative PCRs were performed with different primers sets detecting mayor groups of intestinal flora e. g. *Bacteroidetes* and *Firmicutes*. Further detailed characterizations of the community were done using the same primer sets for cloning and PCR-SCCP with subsequent sequencing of the 16S rRNA gene of the DNA-bands or the vector inserts. First results showed significantly higher *Firmicutes* 16S rRNA gene targets in the obese group compared to the control group whereas no differences in the target numbers were found between the groups by primer sets for *Bacteroidetes* and total *Bacteria*. DNA-Band pattern of PCR-SCCP with *Firmicutes* specific primer showed a prominent band in the samples of the obese.

SIP012

Biosynthesis of xenocyloins, secondary metabolites from X*enorhabdus bovienii*

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Xenorhabdus are Gram-negative bacteria belonging to the family of Enterobacteriaceae. They live in a symbiotic association with soil dwelling nematodes of the genus *Steinernema*. Once the nematodes infect an insect larvae, the bacteria start to proliferate in the hemocoel of the insect and produce bioactive secondary metabolites to inhibit the insect immune system and to protect their food source from other microorganisms [1]. Former work demonstrated that indole derived compounds show strong antifungal and antibacterial activity [2].
As we are interested in the biosynthesis of bioactive compounds from these bacteria [3] we searched for the biosynthesis gene cluster of these indole derivatives that we named xenocyloins in the producer strain *X. bovienii* SS-2004. We assumed, an acetolactate synthase like enzyme must be the key-step to the xenocyloin biosynthesis and subsequently could identify one biosynthesis gene cluster, which was only present in *X. bovienii* but not in non-producers like *X. nematophila* or related *Photorhabdus* species. Heterologous expression of the predicted xenocyloin biosynthesis gene cluster in *E. coli* DH10B confirmed that this cluster is indeed responsible for xenocyloin biosynthesis. Structure elucidation of known and new derivatives was obtained by detailed NMR and HPLC-MS experiments. Their absolute stereochemistry was determined by CD spectroscopy. Expression of this gene cluster into a transaminase-deficient *E. coli* strain DL39 allowed the detailed elucidation of the biosynthesis via specific feeding experiments [4] and allowed the differentiation between the incorporation of amino acids and their respective keto acids. Recently we could demonstrate that not the putative esterase but a gene encoding a putative beta-oxoacyl(ACP)synthase III is involved in the esterification of hydroxylated xenoxyloins. Moreover, results from deletion of acetolactatesynthases in *X. bovienii* genome will be presented on our poster.

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SIP013
The protein phosphatase MtPP2C2 as regulator of the symbiotic receptor-like kinase MtDMI2 in *Medicago truncatula*

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The mutualistic arbuscular mycorrhizal (AM) symbiosis is one of the evolutionary oldest symbioses of plants, formed between fungi of the Glomerymycota and roots of the majority of vascular flowering plants. The establishment of the AM symbiosis requires a specific signal exchange between both partners. In the last years a large progress has been made in identifying signal molecules and pathways involved. The plant receptor-like kinase DMI2 (Does not make infections 2) which is located to the plasma membrane plays an important role in the recognition of AM fungi. DMI2 is part of the common symbiotic signaling pathway (SYM) shared by AM fungi and Rhizobium bacteria. Using the kinase domain of *Medicago truncatula* DMI2 as bait in a yeast-two-hybrid assay, we could identify a protein phosphatase typ 2C (MtPP2C2) as a DMI2 interacting partner. The specificity of this interaction was confirmed in yeast by testing other kinases as well as another phosphatase. Bimolecular fluorescence complementation assays in *Nicotiana benthamiana* and *in vitro* pull-down assays revealed also a specific interaction between DMI2 and PP2C2. Expression analyses in mycorrhized roots by quantitative PCR showed that *MtPP2C2* expression is independent of mycorrhization. Inactivation experiments using RNA interference and overexpression studies are in progress to investigate the role of PP2C2 during mycorrhiza formation. Plant PP2Cs are known as regulators of signal transduction pathways involved in growth, development, responses to hormones and abiotic stress as well as in defense responses. In particular, PP2C2 has been recently being shown as regulator of receptor kinases involved in pathogenic interactions. Therefore a role for MtPP2C2 as regulator of the DMI2-dependent symbiotic signaling pathway in mycorrhiza is proposed.

SIP014
Diversity and distribution of methanogens in higher termites

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Termites produce significant amounts of methane. Preliminary analysis indicated that in phylogenetically higher termites, which emit more methane than lower termites, archaeal diversity and community structure is higher and more complex. While the methanogenic community in lower termites seems to consist exclusively of Methanobacteriales colonizing the gut wall and the cytoplasm of certain gut flagellates, little is still known about the diversity, location, and distribution of methanogens in the different taxa of higher termites. Our clonal analysis of archaeal 16S rRNA genes revealed highest diversity in the guts of soil-feeding *Cubitermes* and *Apicotermes* species, with nearly all major lineages of methanogens represented; diversity of methanogens in fungus-cultivating *Odontotermes* and grass-feeding *Trinervitermes* species was lower. In addition, we recovered also a deeply branching lineage of Euryarchaeota distantly related to uncultivated Thermoplasmatales from all the termites investigated. Presently it is not clear whether members of this group are also methanogenic. *mcrA* gene analysis yielded three clusters of this functional marker of methanogenic archaea: one affiliated with Methanobacteriales, the second with Methanomicrobiales and a third representing a deeply branching and hitherto uncultivated lineage, which is subject of further investigations. Analysis of community structure showed heterogeneous distribution of archaeal populations in the highly compartmentalized gut of a *Cubitermes* species, apparently reflecting the varying physic-chemical conditions encountered in the different compartments. The highly alkaline anterior gut regions were predominantly colonized by Methanosarcinales and the posterior gut regions by Methanobacteriales, Methanomicrobiales and the Thermoplasmatales related lineage. An apparently termite-specific cluster of Crenarchaeota was found to colonize the crop and the rectum.

SIP015
Isolation of secreted *Glomus intraradices* signals activating *Medicago truncatula* mycorrhiza-specific early induced genes

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Arbuscular mycorrhizal (AM) fungi form long-term symbiosis with roots of more than 80% of all land plants and are obligate biotrophs. The establishment and maintenance of a mutualistic symbiosis requires constant signal exchange between both partners to avoid the host defense reactions that would jeopardize the association. In the AM symbiosis, is the delivery of fungal effectors molecules, termed Myc-factors, from earliest fungal life cycle stages the way to initiate the symbiotic program even before both organisms contact. Although our understanding of the molecular dialogue between AM fungi-host has been improved in the recent years, specially with the identification of the plant signal and some clues about the nature of the Myc-factors, still little is know about the effect *in planta* or the signal transduction pathway used to decode the fungal signal.
In our group, it has been recently shown that some *Medicago truncatula* genes are specifically induced at early stages by diffusible signals produced by the fungus *Glomus intraradices*. While the gene activation is partially travelling through the symbiotic transduction pathway (SYM pathway) we have shown that a second cascade is required for the activation of some of those early genes. This suggests that probably several Myc-factors are secreted at the same time by the fungus. Changes in the expression pattern of those early-induced genes will be monitored by real time RT-PCR upon contact with different fungal exudates in order to isolate and characterize the different fungal substances. Furthermore, the use of SYM-mutant plant lines will allow distinguishing the signalling cascade that leads to the activation of each gene for each compound.

SIP016
Generation of molecular tools for functional genomics of bifidobacteria

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Bifidobacteria represent an important group of the human gut microbiota and many strains were shown to have probiotic properties such as inhibition of pathogens, reinforcement of intestinal barrier function or anti-inflammatory effects. However, the molecular mechanisms are largely unknown and tools for the genetic characterization of these effects are scarce. Thus, there is a need to develop tools for functional genomics of bifidobacteria.
Here, we report the generation of a range of *E. coli-Bifidobacterium* shuttle vectors based on the previously published pMDY23 plasmid [1]. We constructed plasmids with different antibiotic resistance including chloramphenicol (pMGC), erythromycin (pMGE), ampicillin (pMGA) and spectinomycin (pMGS). Furthermore, different promoters were cloned into these plasmids which should allow inducible or constitutive expression of proteins in various *Bifidobacterium* strains. Moreover, plasmids were shown to replicate stably over at least 100 generations in the absence of selective pressure and hence can be used *in vitro* as well as in *in vivo* studies.
To test their functionality, different proteins were cloned under the control of various promoters. The fluorescent protein Pp1 of *Pseudomonas putida* was cloned under control of bile (P_{bs}) and starch (P_{st}) inducible promoters in pMGS and the resulting plasmids were transformed in *B. bifidum* S17, *B. breve* S27 and *B. longum*/infantis E18. Our results indicate that expression from P_{st} can be induced in both *E. coli* and *Bifidobacterium* species by adding 1% starch to the growth medium as detected by fluorescent microscopy. pMGS containing Pp1 under the control of P_{st} will be further tested *in vivo* to assess colonization dynamics of bifidobacteria in the gastrointestinal tract of mice.
Moreover, these plasmids can be used for overexpression of proteins potentially involved in the probiotic effects of bifidobacteria. BopA, a cell surface protein involved in adhesion of *B. bifidum* to intestinal epithelial cells [2] was cloned as a His-tagged fusion under control of P_{BAD} for arabinose-inducible expression. The fusion protein was successfully expressed in *E. coli* DH5a and purified by Ni-NTA affinity chromatography. Purified protein was analyzed in adhesion experiments in competition to whole *B. bifidum* S17.

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SIP017

Impact of diet on the gut microbiota of the cockroach *Shelfordella lateralis*

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The dietary requirements of termites and cockroaches are distinctly different. While termites consume a highly specialized diet of lignocellulose that is digested with the help of a specialized gut microbiota, cockroaches are omnivorous and opportunistic feeders. Our analysis of the bacterial community in the hindgut of *Shelfordella lateralis*, revealed a diverse gut microbial community that comprised many lineages clustering with sequences from termite gut, reflecting the close phylogenetic relationship between cockroaches and termites. It is not clear, however, whether the hindgut community is also influenced by diet. Here we examine the effects of different diets on the colonic gut microbiota of *Shelfordella lateralis.* The cockroaches were fed one of four diets: chicken food (balanced), soy (protein-rich), bran, and bran-cellulose (fibre-rich). Although colon weight was significantly greater in cockroaches that were fed a high fibre diet, there were no significant effects of diet on volatile fatty acid concentrations or methane production. Analysis of bacterial community structure by terminal-restriction-fragment length polymorphism and 454 pyrosequencing of 16S rRNA genes revealed a high individual variability but little impact of diet. Each cockroach seems to maintain a core gut microbiota that is insensitive to dietary shifts.

SIP018

A gene of the multidrug and toxic compound extrusion (mate) family in the ectomycorrhizal fungus T*richoloma vaccinum*

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All over the world fungi can be found in different habitats and in interaction with a multiplicity of organisms. This widespread distribution and the contact to other organisms have a lot of advantages but also bear the risk of having contact to antagonistic defense mechanisms including toxic compounds. To prevent their cells from these substances a lot of facilities are given. One possibility is the extrusion via multidrug transporters. These proteins can transport toxic substances out of the cell and save the cells from damages. Because of the high number of transporters in the membrane only a part of these proteins is well investigated yet. A new familiy of multidrug transporters are the proteins from the multidrug and toxic compound extrusion (MATE) family. For some orthologes in human, bacteria and plants their role in detoxification is understood. They can transport e.g. chemotherapeutics, antibiotics and secondary plant metabolites. In fungi only ERC1 (ethionine conferring resistance) from *S. cerevisiae* is described as being responsible for accumulation of ethionine when it is overexpressed in the cell. Like most fungi yeast has two MATE paraloges. Both strains were used for heterologues expression experiments with a MATE gene from the ectomycorrhizal fungus *Tricholoma vaccinum, mte1*. It could be shown, that Mte1 is responsible for the detoxification of different compounds as metals, xenobiotics, dyes and secondary plant metabolites.

SIP019

Effect of associated *Pseudomonas* bacteria and their secondary metabolites on the resistance of black alder against pathogenic *Phytophthora alni*

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Since two decades, *Phytophthora alni* (*Oomycetes*) causes the disease of alder (*Alnus* spp.) decline in Europe and has been posing a serious threat to young, adult riparian and forest alder stands. The disease is distributed by mobile zoospores in water systems and thus could establish itself in whole Central Europe within short time. In Germany, the pathogen is present in the most riparian and forest alder stands. An effective control against *P. alni* is currently not available. However, a stagnating disease progress can be observed in some areas in the meantime. Beside climatic and genetic factors, it is assumed that the native soil microflora contributes to the regulation of the pathogen and disease decline.
Our *Phytophthora alni* isolates, grown on different culture media, were associated regularly with bacteria, which have been isolated and identified as *Pseudomonas veronii*-like strain PAZ1 and *Pseudomonas* sp. PAZ43. *In-vitro* and *in-vivo* plant tests as well as antagonist tests clearly revealed that these *Pseudomonas* strains and their secondary metabolites support the growth of alder roots and inhibit the growth of *P. alni*, respectively. The treatment of plantlets resulted in a distinct promotion of root and shoot growth under sterile conditions and a slower infection course by the pathogen although differences between the alder clones existed. Under greenhouse conditions, the infection of plants was reduced by the half after the cultivation time of 12 months. This study demonstrated the positive effects of associated *Pseudomonas* and their metabolites on the promotion of constitutive resistance of black alder against *P. alni*. Because of that, we are especially interested on these *Pseudomonas* strains and their metabolites.
The structure of secondary metabolites of associated *Pseudomonas* strains has been elucidated by means of LC-ESI-Q-TOF-MS and -MS/MS as well as H/D-Exchange-MS/MS and -Pseudo-MS^3. About 50 cyclic lipodepsipeptides (with 9 or 8 amino acids) were found from *Pseudomonas veronii*-like strain PAZ1. More than 50 % of them have been detected for the first time and belong to the group of the antibiotic active main CLP viscosin. 37 cyclic lipodepsipeptides were found from *Pseudomonas* sp. PAZ43. 30 of them belong to the group of novel cyclic lipodepsidecapeptides with 3-hydroxydecanoic acid as lipid moiety.

SIP020

A natural prodrug-mechanism in secondary metabolism

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Bacteria of the genus *Xenorhabdus* live in symbiosis with entomopathogenic nematodes of the genus *Steinernema* and are pathogenic against numerous insect larvae. By producing insect-toxic proteins and other unknown factors the insect larvae is killed within 24h post-infection [1-3]. As there have been hints that secondary metabolites produced by the bacterium are either involved in the pathogenesis against the insect or play an important role in the symbiosis towards the nematode [4], we investigated the biosynthesis of secondary metabolites produced by these bacteria with a special focus on non-ribosomal peptide synthetases (NRPS) and polyketide synthesis (PKS).
Xenocoumacin-1 (XCN-1), a potent antibiotic and antifungal compound and the only weakly active XCN-2 are the main antibiotics produced by *Xenorhabdus nematophila* [5].
During our effort to understand the xenocoumacin biosynthesis, we could identify and characterize four new derivatives and the corresponding biosynthesis gene cluster. Additionally, we confirmed that XCN-2 is derived from XCN-1, representing a novel mechanism for pyrrolidine ring formation [6]. Additionally, deletion of *xcnG* encoding a bifunctional protein with a peptidase and transmembrane domains led to a complete loss of XCN production. Instead, five new compounds, extended XCN derivatives with a D-Asn and a fatty acid, named prexenocoumacins (PreXCN) were produced.
Encouraged by these results, we postulated the following model: PreXCN, which are not active and act as a prodrug for XCN are formed inside the cytoplasm. While exported into the periplasm by XcnG, all PreXCN are cleaved into the active XCN-1, which kills competing bacteria. As *X. nematophila* itself is sensitive to XCN-1 [7], XCN-1 is converted into XCN-2.

Such a prodrug-activation mechanism seems to be more widespread in nature as we could already identify several other biosynthesis gene clusters in different bacterial genera indicating a highly similar mechanism.

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SIP021
Evolution and biogeography of deep-sea bathymodioline mussel symbioses

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Mytilid mussels of the subfamily Bathymodiolinae host endosymbiotic bacteria and are key species in habitats such as hydrothermal vents, hydrocarbon seeps, sunken wood and whale bone falls. Their symbionts oxidize reduced compounds such as sulfide and methane. The gained energy is used for assimilation of carbon that they transfer to their hosts, thus contributing significantly to their hosts′ nutrition. Many host species are associated with sulfur oxidizers, others harbor methane oxidizers, and a number of species host both functional types co-occurring in a dual symbiosis.
Bathymodiolinae are known from all ocean basins, although most species are bound to a specific habitat type depending on their type of symbionts and adequate substrate availability. It was hypothesized that the subfamily originated in reducing shallow water habitats and immigrated into the deep-sea hydrothermal vent habitats by using sunken wood, whale falls and hydrocarbon seeps as stepping stones. In order to test this hypothesis, we analyzed the phylogenies of the hosts and their symbionts and integrated them with ecological data such as biogeography, habitat type, substrate availability, depth and types of associated symbionts of a specific host species to test for the presence of correlations. We are examining mussel species from vents and seeps worldwide. For reconstruction of the host phylogeny we have analyzed three marker genes, the mitochondrial cytochrome oxidase subunit I (COI) and the NADH-dehydrogenase subunit 4 (ND4) genes, and the nuclear 28S rRNA gene. The phylogeny of the bacterial endosymbionts is based on analysis of the 16S rRNA. The results of these analyses will give us insight into the evolution of the symbiotic interactions and colonization history of chemosynthetic habitats.

SRV001
Molecular mechanisms governing the three-component system HbpS-SenS-SenR from *Streptomyces reticuli*

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The novel three-component signalling system HbpS-SenS-SenR from the cellulose degrader *Streptomyces reticuli* has been shown to sense redox signals and to provide this bacterium with an efficient defence system against oxidative stress [1]. The heme-binding and oligomer-forming protein HbpS is extracellularly located. It interacts with the membrane-embedded sensor kinase SenS from the two-component system SenS-SenR. Further analyses revealed that the octameric assembly of HbpS is essential for the interaction with SenS [2]. Furthermore, HbpS has been shown to modulate the phosphorylation state of the sensor kinase SenS as, in the absence of oxidative stress conditions, HbpS inhibits SenS autophosphorylation whereas the presence of heme or iron ions and redox-stressing agents enhances it [2].
Using a number of genetic, biochemical, structure and biophysical approaches including site-directed mutagenesis, FRET, CD spectroscopy, fluorescence spectroscopy and immunoblotting, we have demonstrated that iron-mediated oxidative stress induces both secondary structure and overall intrinsic conformational changes within HbpS. We showed in addition that HbpS is oxidatively modified, leading to the generation of highly reactive carbonyl groups and tyrosine-tyrosine bonds [3]. Therefore, it can be proposed that iron-mediated oxidative modifications causing structural and conformational changes in HbpS are responsible for the control of the HbpS-SenS-SenR signalling cascade.

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SRV002
Anoxygenic photosynthesis and photooxidative stress: A particular challenge for *Roseobacter*

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Roseobacter clade aerobic anoxygenic photosynthetic bacteria are abundant in photic zone environments of marine ecosystems. These bacteria perform anoxygenic photosynthesis under oxic conditions, a situation known to generate singlet oxygen ($^1O_2$) in the closely related anoxygenic phototroph *Rhodobacter sphaeroides* [1]. Compared to *Rhb. sphaeroides*, photosynthetic membranes of *Roseobacter denitrificans* generated three fold more $^1O_2$ during light exposure and consequently the key regulator genes *rpoE* and $rpoH_{II}$ [2, 3] of *Rsb. denitrificans* were much stronger induced in response to $^1O_2$ stress compared to *Rhb. sphaeroides*. The regulon controlled by RpoE was different in *R. denitrificans* and *Rhb. sphaeroides* and patterns of synthesized soluble proteins strongly changed upon high light exposure in *Rsb. denitrificans*, but not in *Rhb. sphaeroides*. Changes in the proteome were not further promoted by artificial $^1O_2$ generation, which indicates that light alone generates high levels of $^1O_2$ in *Rsb. denitrificans*. The strong increase of the small RNA RDs2461 by photooxidative stress [4] implies a role of sRNAs in post-transcriptional regulation of the response to $^1O_2$ in *Rsb. denitrificans*. Overall, our data reveal similarities but also significant differences in the response of *Rsb. denitrificans* and *Rhb. sphaeroides* to $^1O_2$, most likely a consequence of their different life styles [5].

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SRV003
The apoptosis inducing factor (AIF)-like mitochondrial oxidoreductase (*aifA*) mediates reistance towards the *Penicillium chryosgenum* antifungal protein PAF in *Aspergillus fumigatus*

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The antifungal protein PAF from *Penicillium chrysogenum* is a secreted low-molecular weight, cysteine-rich and cationic protein that inhibits the growth of the zoopathogen *Aspergillus fumigatus*. Thus PAF represent a promising candidate for the development of novel antimycotic strategies and a detailed characterization of its mode of action is essential.
By the use of a genome wide gene expression analysis in *A. fumigatus* a deregulation of genes involved in oxidative phosphorylation and oxidative stress response after exposure to PAF was determined. Indeed, we observed an increased level of reactive oxygen species (ROS) in PAF-treated hyphae. The determination of the mitochondrial respiration efficiency, the ATP production and the copy number of mitochondrial DNA indicated that the deregulation of mitochondrial genes in response to PAF primarily resulted from a mitochondrial malfunction but not from a reduction of the mitochondrial number. The deletion of the mitochondrial *aifA* gene resulted in the hypersensitivity of *A. fumigatus* towards PAF which underlines the function of AIFA in the detoxification of PAF-induced ROS. From these results we conclude that induction of oxidative stress and mitochondrial malfunction are central features of PAF toxicity which can finally lead to programmed cell death as previously shown[1].

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SRV004

A novel type of DNA photolyase containing an iron sulfur cluster

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Photolyases and cryptochromes are evolutionarily related flavoproteins with distinct functions. While photolyases can repair UV-induced pyrimidine dimers on the DNA in a light dependent manner, cryptochromes regulate growth, development and the circadian clock in plants and animals. Here we report about a photolyase related protein, named PhrB, found in the phytopathogen *Agrobacterium tumefaciens*. Phylogenetic studies showed that PhrB belongs to a new class which we designate bacterial cryptochrome and photolyase proteins (BCP). It contain FAD as a catalytic cofactor and a second chromophore that absorbs in the short wavelength region, but with spectral properties distinct from other known photolyase antenna cofactors. Alignment of protein sequences suggests that the classical photoreduction pathway consisting of three tryptophans, is absent in PhrB. Moreover, structure modelling revealed four cystein residues that seem to be clustered possibly for the coordination of an iron sulfur cluster and the presence of iron in a 4:1 stochiometry was confirmed experimentally. Although PhrB is clearly distinguished from other photolyases it is required for photorepair of UV-lesions in *A. tumefaciens*. We thus propose that PhrB is a functional photolyase which represents the first member of this protein family that contains an iron-sulfur cluster.

SRV005

Specific control of hypochlorite resistance by the redox-sensing MarR/DUF24-type regulator HypR in *Bacillus subtilis*

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Bacillus subtilis encodes several redox-sensing MarR-type regulators of the OhrR and DUF24-families that are conserved among Firmicutes and control oxidative stress resistance and virulence functions in pathogenic bacteria via thiol-based redox-switches. While most characterized members of the OhrR family respond to organic hydroperoxides, the DUF24-family regulators YodB, CatR and HxlR were shown to sense specifically electrophiles such as diamide, quinones or aldehydes. However, the genome of *Bacillus subtilis* encodes additional DUF24 family regulators of unknown functions and we were interested whether any of these is involved in oxidative stress resistance mechanisms. We used DNA microarray analysis to analyse expression changes in *B. subtilis* in response to the strong oxidant hypochloric acid (HOCl) which is present in house-hold bleach. The overall transcriptional response of *B. subtilis* to HOCl is indicative of disulfide stress and overlapping to the response provoked by the thiol-oxidizing electrophile diamide. The glyceraldehyde 3-phosphate dehydrogenase GapA was most strongly oxidized to an intramolecular disulfide by HOCl stress among cytoplasmic proteins as shown by redox proteomics and mass spectrometry. We further identified an unknown DUF24-type transcriptional regulator as novel hypochlorite-specific redox sensor which we accordingly renamed as HypR. HypR controls positively an oxidoreductase (HypO) that confers specific protection against HOCl stress in *B. subtilis*. The conserved N-terminal Cys residue of HypR is essentiell for activation of *hypO* transcription by HOCl stress *in vitro* and *in vivo*. HypR resembles a 2-Cys type redox sensing regulator of the DUF24 family that is activated by intersubunit disulfide formation in response to HOCl stress *in vitro* and *in vivo* as confirmed by mass spectrometry. Crystallization trials and structural refinements of oxidized and reduced HypR proteins are in progress to support the thiol-disulfide switch model for this novel transcriptional activator. Collectively our studies have revealed that the conserved MarR/DUF24 family is able to sense selectively electrophiles (diamide, quinones and aldehydes) and strong oxidants such as HOCl. Bleach is not only present in the soil environment of *B. subtilis* but also released by activated macrophages upon the infection process. Thus, the function of the DUF24 family among pathogenic Gram-positives could be to protect cells against the host immune defense.

SRV006

Structural studies on the Iron core formation in *Marinobacter hydrocarbonoclasticus* Dps

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Iron is an essential element for the vast majority of organisms. Among other characteristics, its capacity to cycle between two (or more) redox states (Fe^{2+} or Fe^{3+}) made it an attractive element to use in the catalytic active site of several enzymes [1]. In the reduced Fe^{2+} ferrous state iron is relatively soluble. In the oxidized Fe^{3+} form, however, it becomes insoluble and consequently its bioavailability in our modern oxidative atmosphere is severely decreased [2]. Additionally, in the presence of oxygen, iron sites can become a source of unwanted oxygen reactive species such as superoxide or hydrogen peroxide. To overcome this problem, iron must be kept in a non-toxic reduced form, in the cell. Dps proteins (DNA-binding protein from starved cells), widely spread in bacteria, are highly important in the bacterial response against oxidative stress. They are members of the ferritin superfamily but, contrary to ferritins that are only involve in the biomineralization and iron storage, Dps proteins have the capacity to detoxify the cell by removing hydrogen peroxide and ferrous iron and therefore the ability to protect DNA against oxidative damage [3]. Dps proteins are dodecamers with a two-fold symmetry in the dimer. The ferroxidase center lies at the interface between two monomers and has a highly specific and conserved motif among Dps proteins; a HW pair in helix I and H-14-DXXXE in helix II where the histidines, aspartate, and glutamine residues are the iron ligands [4,5]. Despite the high conservation of these iron ligands, the occupancy of the two metal binding site differs significantly in known crystal structures [6]. Using X-Ray crystallography in combination with spectroscopic data we are investigating the intermediates stages of iron core formation in a Dps protein from *Marinobacter hydrocarbonoclasticus*.

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SRV007

Characterisation of the oxidative stress response in *C. glutamicum*

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Aerobic bacteria are exposed to oxidative stress as daily problem because of the permanent endogenous formation of reactive oxygen species like hydrogen peroxide (H_2O_2). The cellular sources of ROS are manifold and because of the damage of cellular components the enzymatic removal of ROS, e.g. by catalase, plays a pivotal role in the bacterial oxidative stress response. Interestingly, knowledge on oxidative stress response of *C. glutamicum* is scarce in spite it is applied in large scale industrial fermentations and exposed to rigorous variations of the oxygen supply. Interestingly, the catalase of *C. glutamicum* has an extraordinary high activity promoting its industrial production. Why does *C. glutamicum* possess a highly active catalase and does the non-constitutive expression of the catalase gene cause metabolic limitations?
We addressed the oxidative stress response of *C. glutamicum* and the contribution of the catalase by comparing a catalase mutant and wild type cells. Whereas wild type cells tolerate exposure to 1 M H_2O_2 cells of the mutant are highly sensitive and can not grow in presence of 1 mM H_2O_2. Additionally, an increased sensitivity towards alkaline pH and increased iron availability was found. By using *in vitro* experiments the significant impact of low amounts of ferrous iron on protein oxidation was shown by the Oxyblot™ technique and compared with the impact of other divalent

cations. Besides the catalase dependent damage of proteins the H_2O_2 dependent decomposition of DNA was analysed. To address the variety and the extent of H_2O_2 induced oxidative modifications at the proteome level the catalase mutant was applied as a tool in LC-MS/MS studies. Interestingly, numerous oxidative modifications were found even for wild type cells under *in vivo* conditions during fermentation of *C. glutamicum* in controlled bioreactors. In particular enzymes of central metabolic pathways were identified as targets. Our results underline the continuous formation of ROS and unravel their deleterious effects on the physiological performance of *C. glutamicum* in spite of the presence of a highly active catalase enzyme.

SRV008
On the multitude of mechanisms that establish high-level heavy metal resistance in an aggregate forming bacterium

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In the last decades chromium has become a wide spread pollutant in the environment. This is mainly due to anthropogenic factors, namely an often inadequate toxic waste management in leather tannery, dye-, car- and steel-industry. Consequently chromium has become the most important heavy metal pollutant in the European Union. The toxicity of chromium is dependent on its oxidation state. Cr(VI) is the most toxic and bioavailable form, whereas Cr(III) is only sparsely soluble and therefore less toxic.
In this study the chromate resistance strategy of a new *Leucobacter* species (*L. chromiiresistens*) was investigated [1]. This species is capable of tolerating more than 300 mM chromate and shows a distinct correlation between the chromate concentration in the medium and the production of aggregates. Formation of these aggregates accompanies with the enhanced production of extracellular polymeric substances (EPS), mainly extracellular DNA (eDNA) and sugars. Extracellular DNA was shown to be essential for the structural integrity of the aggregates. Inhibition of aggregate formation via DNaseI treatment resulted in an almost complete loss of resistance against potassium chromate. Our hypothesis regarding the role of EPS production and cell aggregation is that these factors result in decreased Cr(VI) uptake and therefore reduce intracellular Cr(VI) concentrations.
Besides aggregate formation, *Leucobacter chromiiresistens* produces a carotene-related pigment in the membrane as a response to chromium stress. Carotenes are known to function as radical quenchers in photosynthetic organisms. In *L. chromiiresistens* they might protect the cell from lipid peroxide formation triggered by chromium radicals. Last but not least we could measure a soluble cytoplasmic chromate reductase activity. NAD(P)H serves as electron donor for this enzyme.
We suggest that aggregate formation, carotene production and chromate reductase expression serve in an orchestrated way to protect the cell from oxidative stress caused by chromium(VI) or chromium radicals.

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SRV009
Osmotic stress response in *Bacillus subtilis* - integration of the fluxome with the regulatory networks

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Bacillus subtilis is one of the major industrial working horses in biotechnology. In industrial production environments it typically experiences high osmolarity, making this an important parameter to be investigated. The specific osmotic stress response of Bacillus has been elucidated in detail [1] but information about the integration into the regulatory network of *B. subtilis* is still incomplete. Protection against an osmotic challenge is primarily conferred by a specific adaptational response that controls the uptake, synthesis and accumulation of osmoprotective substances. In addition to the uptake of compatible solutes, e.g. glycine betaine, *Bacillus subtilis* is able to synthesize amino acids *de novo* especially glutamate and proline to counteract the external osmotic pressure. Furthermore, this specific osmoadaptation response is integrated with the SigB-dependent general stress response, because genes such as *opuD* and *opuE* are subject to overlapping control by SigB.
In the present work, the response of *Bacillus subtilis* 168 trp^+ to osmotic stress was assessed by a polyomics approach, integrating the fluxome as functional network output of *Bacillus subtilis* with its cellular components involving metabolome, proteome and transcriptome analysis. For this purpose, cells were grown in glucose-limited chemostats at NaCl concentrations up to 1.2M. At metabolic steady-state, samples were analyzed for systems-wide metabolome, transcriptome, proteome and fluxome analysis. This should unravel regulatory interactions between the different functional layers of the cell [2].

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SRV010
Post-transcriptional activation of the SacP phosphatase counteracts phosphosugar stress in enterobacteria

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Question: The small regulatory RNA SgrS is well known to counteract phosphosugar stress, a process that involves the post-transcriptional targeting of the ptsG mRNA, coding for the major glucose transporter [1]. Bacterial non-coding RNAs have now been established to control the expression of multiple target genes rather than single transcripts. In this study we aimed to elucidate the target profile of SgrS in the model pathogen Salmonella Typhimurium.
Methods: To investigate the role of SgrS in *S.* Typhimurium we made use of a pulse-expression approach that combines tightly controlled expression of an sRNA from an inducible promoter with whole genome microarrays analysis [2].
Results: Our analysis revealed an extended SgrS regulon, displaying a larger set of repressed mRNA targets, but also up-regulation of a single transcript, termed *sacP*. Interestingly, *sacP* is the 2nd gene of a polycistronic messenger, however SgrS mediated gene activation is limited to sacP and does not render the expression of other members of this operon. Mechanistically, this up-regulation involves RNA duplex formation of SgrS with distal parts of the preceding *pldB* mRNA and requires the action of the RNA chaperone Hfq and the RNase E ribonuclease. Biocomputational and biochemical analysis have shown that SacP belongs to the group of HAD-phosphatases that display high affinity towards phosphorylated sugar substrates, including Glucose-6-phosphate [3]. Indeed, under phosphosugar stress conditions, post-transcriptional up-regulation of SacP by SgrS is critical for cellular replication, suggesting that SacP activation is required to decrease the intracellular amount of phosphorylated sugars.
Conclusions: We present a sophisticated mechanism of discoordinate operon expression that leads to induction of the conserved sugar phosphatase SacP. SacP is required to dephosphorylate accumulated sugar compounds and required for counteraction of phosphosugar stress in bacteria.

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SRV011
The Phage-Shock Protein LiaH of *Bacillus subtilis*

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The LiaRS two-component system (TCS) is part of the cell envelope stress response in *Bacillus subtilis*, which is triggered by compounds that affect the integrity of the cell wall [1, 2]. The main target of the response regulator LiaR is the *liaI* promoter, resulting in a strong induction of the *liaIH* operon, which encodes a small putative membrane protein and a member of the

Psp/IM30 protein family [2, 3]. Phage-shock proteins are widely conserved in bacteria, archaea, cyanobacteria and plants. LiaH forms large oligomeric ring structures reminiscent of those observed for PspA (*E.coli*) or Vipp1 (*A.thaliana*). Comprehensive phenotypic profiling of *lia* mutants only revealed weak sensitivities against cell envelope and oxidative stress conditions [3]. To gain a mechanistic insight into the physiological role of *B.subtilis* LiaH, we searched for potential interaction partners. Bacterial two hybrid assays revealed a complex protein-protein interaction network in which LiaH is embedded. Moreover, we were able to demonstrate that LiaH plays an important role in protein secretion. Our collective data indicates that the *lia* system of *B.subtilis* has adopted a function similar to the proteobacterial phage-shock response, despite significant regulatory differences.

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SRV012
Characterization of the farnesol-induced stress response in *Aspergillus nidulans*

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Farnesol is a sesquiterpene alcohol representing the first identified quorum sensing molecule in eukaryotic organisms. It is produced by the human pathogenic fungus *Candida albicans* responsibly inhibiting the yeast-to-hyphae switch and biofilm formation. Furthermore, it represses growth of filamentous fungi by triggering apoptosis as demonstrated for the model organism *Aspergillus nidulans*. We aimed to identify the molecular targets of farnesol an thus carried out comparative proteome analysis. *Aspergillus nidulans* was grown in minimal medium over night and 50 µM farnesol was added 3 hours before harvesting. After preparing the protein extracts we compared farnesol-induced and non-induced conditions by 2D-DIGE. We identified 53 proteins showing at least 1,5 fold significant alteration in relative spot volume. Due to farnesol treatment many proteins involved in cell cycle (Cdc48), morphogenesis (HexA) and general stress response were up-regulated (HSP and ROS-detoxifying proteins). In addition we identified a highly up-regulated protein of unknown function with a dehydrin-like motif. Further proteome and northern blot analysis showed its involvement in an early response to farnesol. The corresponding deletion mutant exhibited no increased sensitivity to farnesol. However, the dehydrin-like protein is involved in osmoadaptation and sexual development which exemplifies an additional target of farnesol in filamentous fungi.

SRV013
Structural und functional insight into pilus sensing by the Cpx envelope stress system

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Two-component signal transduction systems (TCS) are the predominant adaption machineries of bacteria to cope with environmental changes. In many TCSs auxiliary proteins enable responses to additional stimuli. The Cpx-TCS is the global modulator of cell envelope-stress that integrates very different signals. It consists of the kinase CpxA, the regulator CpxR and the auxiliary protein CpxP. CpxP both inhibits activation of CpxA and is indispensable for the quality control system of P pili that are crucial for uropathogenic Escherichia coli during kidney colonization. However, it is not clear how these two essential biological functions of CpxP are linked.
We have solved the crystal structure of CpxP to 1.45 Å resolution with two monomers being interdigitated like „left hands" forming a cap-shaped dimer (1). Our combined structural and functional studies suggest that CpxP inhibits the kinase CpxA through direct protein-protein interaction.
It has been proposed that Cpx pathway activation is caused by titrating CpxP away from CpxA (2). A prerequisite of this scenario is the detection of unfolded proteins by CpxP which might result from a chaperone-like activity (3). We will not only provide evidence for a chaperone-like activity of CpxP but also corroborate the functionality of an extended hydrophobic cleft on the convex surface of CpxP as a recognition site for misfolded pilus subunits. Therefore, we analyzed the capacities of the CpxP single-site mutants to promote pili degradation in vivo.
From our combined results we propose a model that elucidates both functions of CpxP. Accordingly, the structural details of CpxP provide a first insight how a periplasmic TCS inhibitor blocks its cognate kinase and is released from it.

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SRV014
In vivo phosphorylation patterns of key stressosome proteins define a second feedback loop that limits activation of *Bacillus subtilis* σ^B

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The *Bacillus subtilis* stressosome is a 1.8 MDa complex that orchestrates activation of the σ^B transcription factor in response to environmental signals. It comprises members of the RsbR co-antagonist family and the RsbS antagonist, whose similar STAS domains form a core that sequesters the RsbT serine-threonine kinase. Stress-induced phosphorylation of the STAS domains by RsbT is associated with its release from core, allowing RsbT to activate a downstream regulator. Here we investigate the *in vivo* phosphorylation of RsbRA and its RsbRB, RsbRC and RsbRD paralogs, whose STAS domains share two conserved threonine residues. In unstressed cells these RsbR proteins are known to be phosphorylated on their more N-terminal threonine, exemplified by RsbRA T171. T171 phosphorylation is thought to be prerequisite but not the trigger for activation, which correlates instead with stress-induced serine phosphorylation of RsbS. We show here that all the initial threonine modifications require RsbT kinase. Also, phosphorylation on the more C-terminal threonine, exemplified by RsbRA T205, had not been detected *in vivo*. We find (i) RsbRA is additionally phosphorylated on T205 following strong stresses; (ii) this modification depends on RsbT; and (iii) T205A substitution greatly increases post-stress activation of σ^B. We infer that T205 phosphorylation constitutes a second feedback mechanism that limits σ^B activation, operating in addition to the RsbX feedback phosphatase. Loss of RsbX function greatly increases the fraction of phosphorylated RsbS and doubly phosphoylated RsbRA in unstressed cells. Thus RsbX both maintains the ready state of the stressosome prior to stress, and restores it post-stress. Because similar RsbR-S-T modules are found in diverse bacteria, our results may have broad application.

SRV015
Signal perception and transduction by the transcriptional activator CadC of *Escherichia coli*

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Adaptation of *E. coli* to acidic stress is mediated by the concerted action of several proteins, among them the inducible amino acid decarboxylase systems. One of these systems is the Cad system that is induced at low external pH and concomitantly available lysine. The transcriptional activator CadC of the Cad system belongs to the ToxR-like proteins that are characterized by a common topology. These proteins possess a periplasmic sensor domain, a single transmembrane helix and a cytoplasmic DNA-binding domain. Recent data revealed that the periplasmic domain of CadC is responsible for pH sensing, while lysine signaling is mediated by an interaction of CadC with the lysine permease LysP. We are interested in elucidating how the inner-membrane protein CadC is able to perceive and transduce these signals across the membrane and subsequently activates transcription of the *cadBA* operon.

Based on the recently solved 3D-structure of the periplasmic domain of CadC and a large scale site-directed mutagenesis approach, a negatively charged patch was identified that is essential for pH detection. This patch is located at the dimer interface manifesting the role in proton sensing and signal transduction.
A bioinformatics approach revealed that almost all ToxR-like regulators are very similar with respect to the cytoplasmic domain that is composed of a winged helix-turn-helix DNA-binding domain and a large unstructured loop. To investigate the role of the loop between the transmembrane domain and the DNA-binding domain, this part of the protein was gradually truncated or elongated. Our results reveal that the large unstructured loop is important for transducing the pH signal, but unimportant for lysine signaling.

SRV016
Signal transduction and gene regulation in response to surfactant stress in *Pseudomonas aeruginosa*

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Biofilms and cell aggregates are considered to be the predominant form of microbial life in nature. The formation of these multicellular structures often proceeds in a sequential manner and is usually a response to the prevailing environmental conditions by means of signal transduction pathways. Our understanding of the essential molecular mechanisms underlying these complex regulatory events is currently limited.
We previously reported that cell-cell aggregation in response to surfactant stress provides a strategy to increase fitness for *Pseudomonas aeruginosa* under unfavourable environmental conditions [1, 2]. Mutagenesis approaches, overexpression studies and comparative microarray analysis further demonstrated, that the second messenger cyclic di-guanosine monophosphate (c-di-GMP) and a small set of genes, including *cupA*, *psl*, *cdrAB*, PA4623 and the novel signal transduction system *siaABCD,* are essential for surfactant-induced aggregation [2, 3].
In order to decipher the corresponding mechanisms for signal transduction and target gene expression, we performed a systematic mutational analysis of the *siaABCD* operon. Transcriptional-, biochemical- and physiological characterisation of these mutants uncovered that the protein encoded by *siaB* represents a repressor of the SiaABCD signalling pathway. Loss of SiaB function was found to increase cell aggregation in response to surfactant stress. In contrast, the overexpression of *siaB* on a multicopy plasmid completely abolished cell-cell aggregation during growth in the presence of surfactant. Even more interestingly, the non-aggregative phenotype of a Δ*siaD* mutant strain could be complemented by a secondary mutation in the *siaB* gene. This suggests that the SiaABCD signal transduction pathway can regulate surfactant-induced aggregation by a bifunctional mechanism. One, which is dependent on SiaD, a putative di-guanylate cyclase involved in the synthesis of c-di-GMP, and one which is independent of SiaD but most likely requires a functional *siaA* gene, encoding a putative PP2C-like phosphatase. A model for the regulatory mechanism of signal transduction, target gene expression, and the interconnection of the SiaABCD pathway with other global regulatory systems will be discussed.

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SRP001
Glycogen deficiency affects the response to nitrogen starvation in the cyanobacterium *Synechocystis* sp. PCC 6803

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Glycogen is a branched polymer of glucose that is present as a carbon and energy reserve compound in many organisms. Cyanobacteria usually synthesize this storage carbohydrate during the day and catabolize it during the night. The polymer accumulates massively under conditions of unbalanced growth, e.g. when cells are starved for nitrogen. Furthermore, the most abundant cyanobacterial protein complexes, the light-harvesting phycobilisomes, are degraded in order to supply amino acids for synthesis of proteins that may be essential under these conditions. This process is commonly designated chlorosis.
The particular role of glycogen in the interconnected carbon and nitrogen metabolism in cyanobacteria is not fully understood yet. A detailed analysis of glycogen-deficiency via the analysis of knockout mutants provided new insights into the cyanobacterial carbon metabolism. Mutants of the model organism *Synechocystis* sp. PCC 6803, defective in genes of ADP glucose pyrophosphorylase and glycogen synthases, respectively, were impaired in phycobilisome degradation under nitrogen starvation (non-bleaching phenotype). Moreover, glycogen-deficient mutants massively excreted pyruvate and 2-oxoglutarate. The latter organic acid is the key metabolite sensor of the cyanobacterial nitrogen response. Glycogen deficiency like heterotrophic growth on glucose might originate a metabolic switch in *Synechocystis* sp. PCC 6803. The properties of the glycogen-deficient mutants suggest that an as yet unknown metabolic signal is involved in the cyanobacterial nitrogen response. The impact of this putative metabolic signal on transcription and expression of key proteins involved phycobilisome degradation was further examined with respect to the action of sRNA´s and transcription factors.

SRP002
The two sides of the medal: impact of carbon dioxide on pH homeostasis and anaplerotic reactions in *Corynebacterium glutamicum*

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During industrial fermentations e.g. glutamate and lysine production using *C. glutamicum*, increased CO_2 concentrations occur [1]. This phenomenon is caused by high hydrostatic pressure resulting in a higher solubility of CO_2 and by insufficient mixing at the bottom region of large bioreactors. It is well known that this causes acidification of the medium, however, the impact of CO_2 on the internal pH of bacterial cells is scarcely understood. At neutral and alkaline pH, *C. glutamicum* tolerates up to 20% CO_2 [2]. Under acidic conditions the spontaneous reaction of CO_2 with H_2O leading to HCO_3^- and H^+ should cause an additional decrease of the internal pH. We established a method to monitor changes in pHi by measuring the fluorescence of GFP variants and applied the technique at different external CO_2 concentrations. We show that under acidic conditions, pH homeostasis fails in a CO_2 dependent manner. Subsequently, we address the role of the carbonic anhydrase, responsible for the conversion of CO_2. A deletion mutant of *C. glutamicum* lacking the ß-type carbonic anhydrase *cg2954* did not show improved pH homeostasis at low pH and high CO_2 concentrations but, is unable to grow unless the CO_2 concentration is raised to 10%. This is in agreement with earlier findings at neutral pH [3]. In conclusion, two aspects have to be considered. On the one hand CO_2 is required in particular for anaplerotic reactions but, on the other hand high CO_2 concentrations trigger the collapse of pH homeostasis. We will discuss whether the enzymatic formation of HCO_3^- from CO_2 is essential for growth, especially at low pH and whether the lack of carbonate is a bottleneck for *C. glutamicum* under acidic stress conditions.

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SRP003
Stress responses in the soil bacteria *Bradyrhizobium japonicum* relating to temperature, pH and salt

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Bradyrhizobium japonicum is able to establish a symbiotic interaction with soybean and is used for inoculation of this crop. During symbiosis, bacteria reduce atmospheric nitrogen to ammonia, which is used by the plant as nitrogen source. The natural habitat of *B. japonicum* is the soil, a complex and dynamic ecological system with changing parameters like pH, salt concentration, nutrition availability and a temperature gradient between day and night. Because these parameters may influence symbiosis, a whole genome microarray (AffymetrixGeneChip®) was used for studying the transcriptome of *B. japonicum* in response to heat shock, heat and salt stress, pH 4.0 and pH 8.0. This revealed global as well as specific stress responses. The pH of the growth medium strongly influenced the expression pattern. After incubation for four hours at pH 8.0, more than 1600 genes were

differentially expressed if compared to data at pH 6.9 (fold change ≥2). At pH 4.0 the response was less pronounced with about 120 genes being differentially expressed. 48 genes reacted to both extreme pH values, with 16 genes being up-regulated at pH 8.0 and down-regulated at pH 4.0. The two-component system RegSR seems to be involved in the regulation of several of these genes. In the presence of 80 mM NaCl and similar to other bacteria, *B. japonicum* exhibits an up-regulation of genes involved in synthesis of osmotic protectants, e.g., trehalose and of genes encoding transport systems. After heat shock for 15 min at 43°C, 654 genes were down-regulated and 279 genes were up-regulated. This included the well-known heat shock genes. Several hundred genes were differentially expressed after cultivation for 48 hours at 35.2°C. Four genes were up-regulated under all tested stress conditions. Therefore, these genes might be involved in the general stress response of *B. japonicum*. One of the genes (*gscR*) is likely to encode a transcriptional regulator involved in general stress response. To test this hypothesis, we created a *gscR* mutant and analysed its transcriptome under various stress conditions.

SRP004
γ-eccompensates for the loss of glutathione in *Escherichia coli*

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Partially reduced oxygen forms very toxic Reactive Oxygen Species (ROS). The presence of too much ROS in cells is called oxidative stress. The glutathione (GSH) system is an important system that protects cells from oxidative stress. GSH is the main thiol-redox buffer in many organisms including the model organism *Escherichia coli*. It is thought to protect cells against the negative effects of ROS, such as damage of DNA, lipids, or proteins, by maintaining the thiol-redox state of cells. A change in the ratio of reduced and oxidized (GSSG) glutathione has also been observed in several diseases. A Δ*gshB E. coli* mutant strain, with a disrupted biosynthesis of glutathione, however, shows no apparent growth phenotype under standard conditions, when compared to wildtype. This suggested to us that other Low Molecular Weight Thiols (LMWT) in *E. coli* could be compensating for the loss of GSH in this mutant. Our HPLC analyses confirmed the absence of GSH and showed an increased level of γ-glutamyl-cysteine(γ-EC), a GSH-precursor, in the Δ*gshB*-mutant. Enzymatic tests with glutathione reductase revealed that γ-EC, unlike other LMWT commonly found in *E. coli*, including cysteine and homocysteine, is a substrate of this enzyme with a $K_m$of 604 µM. Although degradation and redox stability experiments showed that glutathione is more stable when compared to γ-EC *in vitro*, stress-experiments showed an equivalent resistance of the Δ*gshB*-mutant against 3 mM H_2O_2 stress and an even better resistance against 125 µM paraquat stress when compared to the wildtype. We also detected protein modifications by γ-EC in the mutant comparable to protein-glutathionylation in the wildtype, which is known to serve as a protection system against protein damage under oxidative stress. These experiments suggest that γ-EC can partially assume the function of glutathione in *E. coli*.

SRP005
Identification of redox regulated proteins upon peroxynitrite stress in *Escherichia coli*

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Peroxynitrite is a reactive nitrogen species (RNS) that is generated in cells of the mammalian immune system to fight off pathogens. Reactive nitrogen species are known to damage a wide range of biomolecules. We are specifically interested in protein modifications that occur in bacteria that are subjected to peroxynitrite stress. It has been shown that tyrosins are modified by peroxynitrite and form nitro-tyrosin. With Nitro-tyrosin specific antibodies we could detect a peroxynitrite-concentration-dependent increase in modified tyrosins in *Escherichia coli*. But Peroxynitrite also targets cysteines. This can lead to a modification of the thiol redox state by the formation of disulfides, S-nitrosylation and S-hydroxylation. Because these thiol modifications are reversible *in vivo* and could therefore play a potential role in redox-signalling, we additionally investigated the consequence of peroxynitrite on the thiol-redox proteome in *E. coli*. Thus, we used an ICAT (isotope coded affinity tag) based method, which allows us to investigate the thiol redox state of proteins *in vivo*. With this method, we were able to identify several proteins that are significantly more oxidized in *E. coli* upon treatment with 1 mM peroxynitrite: the glutathione-dependent formaldeyhde dehydrogenase (FrmA), the asparagine synthetase (AsnB), the malic enzyme (MaeB) and YjgF, a protein of unknown function. Deletion strains in genes encoding these proteins showed a significant defect in cell growth and cell survival under peroxynitrite stress, indicating a direct or indirect role of the identified genes in cell defense mechansims against reactive nitrogen species.

SRP006
Sensing of osmotic stress by salt dependent protein-nucleic acid interaction in the cyanobacterium *Synechocystis* sp. PCC 6803

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Under osmotic stress conditions most bacteria accumulate compatible solutes by uptake or *de novo* synthesis. Whereas the osmotic stress response by regulation of gene expression was investigated extensively understanding of the immediate response by biochemical activation of enzymes is scarce. In the cyanobacterium *Synechocystis* sp. PCC6803 synthesis of the main compatible solute glucosylglycerole (GG) is triggered by salt stress in a transcription independent manner. The key enzyme is the glucosylglycerole-phosphate synthase (GgpS) for which a novel mechanism of the activity regulation was found. The protein is inhibited by binding to the backbone of nucleic acids by an electrostatic interaction. Liberation of GgpS is salt dependent and activates the enzyme. Inhibition of GgpS occurs by non competitive inhibition indicating inhibitor binding apart from the substrate binding pocket. In order to identify the interaction site or nucleic acid binding biotinylation of the protein in absence and presence of nucleic acids was performed and a subsequent analysis by mass spectrometry. Residues covered by nucleic acids are protected against biotinylation and the according peptides show no specific increase in mass. Residues putatively involved in inhibitor binding were exchanged by site directed mutagenesis of the *ggpS* gene and the impact of these modifications on nucleic acid binding and enzyme activity will be discussed.

SRP007
Systemic analysis of bacterial aconitase deletion mutants reveals a strong selection pressure for secondary mutations inactivating citrate synthase

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Aconitase, a 4Fe-4S cluster containing protein, catalyses the second step of the tricarboxylic acid cycle, the reversible isomerisation of citrate to isocitrate [1]. In the past years it was shown that the aconitase gene *acn* of the *Corynebacterium glutamicum*, a member of the actionbacteria, is subject to a complex expression control by four different transcriptional regulators [2-5]. In order to better understand the causes for this elaborate regulation, a *C. glutamicum* Δ*acn* mutant was analysed regarding growth, proteome, transcriptome, and secretion of organic acids. The mutant was glutamate-auxotrophic, showed a strong growth defect and secreted large amounts of acetate. Importantly, none of these phenotypes could be complemented by plasmid-encoded aconitase, suggesting the presence of a secondary mutation. In fact, a point mutation within the *gltA* gene encoding citrate synthase was identified, which caused degradation of this protein and an almost complete lack of its enzymatic activity. Subsequently, 27 further, independent Δ*acn* clones were isolated and 15 of them were found to contain mutations in the *gltA* gene causing loss of citrate synthase activity. Elevated intracellular citrate concentrations were considered to be the main cause of this selection pressure. Citrate toxicity was subsequently investigated by citrate pulse experiments with a *C. glutamicum* strain overexpressing the citrate carrier CitH. In fact, rapid citrate uptake by cells not adapted to this substrate elicited a complete, though temporary growth inhibition. According to these results, the tight control of aconitase synthesis might have evolved due to the necessity to avoid toxic citrate levels on the one hand and the excessive synthesis of a labile protein requiring both iron and sulphur on the other hand.

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SRP008
Anisin1, a defensin-like protein in *Aspergillus nidulans*, senses oxidative stress and balances asexual development

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In the genome of *A. nidulans* a defensin-like protein, Anisin1, was annotated that exhibits 51% amino acid identity to the mosquito *Aedes* (*Ae.*) *aegypti* defensin AaDefA1. Although defensins are widely distributed in nature and their function in higher eukaryotes is well characterized, no studies exist so far on defensins that originate from filamentous ascomycetes. We, therefore, started to characterize the Anisin1 encoding gene in *A. nidulans*. Expression studies in submers cultures indicated that elevated levels of intracellular reactive oxygen species (ROS) triggered the *anisin1* expression. We used specific mutants of the histidine-to-aspartate signal transduction pathway to show that *anisin1* expression was strongly induced in D*srrA*, which suffers from a defect to detoxify ROS. In contrast, *anisin1* was repressed in *A. nidulans* strains that efficiently respond to oxidative stress. In *A. nidulans* wild-type surface cultures, however, the *anisin1* transcription correlated with that of the central regulator for asexual development, *brlA*, and with *catB*. This co-regulation was deregulated in D*srrA* which might explain the sporulation defect in this mutant. The phenotype analysis of an *anisin1* deletion mutant revealed an increased oxidative stress sensitivity, a defect in mitospore development and lower conidial counts at 42°C compared to the wild-type. Taken together, our results suggest that in *A. nidulans anisin1* plays an important role in sensing oxidative stress, in balancing conidiogenesis and in supporting thermotolerance during asexual development. In analogy to a multiple function of defensins in higher eukaryotes, Anisin1 could therefore contribute to the fitness of *A. nidulans* under unfavourable growth conditions.

SRP009
Repair potential in natural drinking water biofilms after water treatment

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Biofilms are present in drinking water distribution systems despite water treatment and disinfection at waterworks. They are a possible niche for hygienic relevant bacteria, and therefore a main concern for water industries. Up to now knowledge about survival strategies of bacteria during their regeneration process in biofilms after disinfection treatments has been limited. To get a deeper understanding of this problem, biofilms of ground water and surface water were investigated in different waterworks.
In each waterworks the same pilot scale, built up with different standard pipe materials, was used to simulate a household water distribution system. The water that flowed through the pilot scale was exposed to disinfection methods such as UV and chlorine dioxide. Three month old biofilms were compared using RNA and DNA based methods.
When stress markers on RNA level were investigated, UV disinfection was found to be responsible for the up-regulation of *recA*-mediated dark repair in natural biofilms. The highest *recA* induction in biofilms was associated with copper, confirming previous investigations from other waterworks. No or only low *recA* expression was found in biofilms gained from the waterworks in which drinking water was not disinfected or treated with ClO_2.
The total amount of bacteria present in the biofilms did not depend on the different materials or disinfection processes. But DGGE analysis showed a significant shift in the bacterial population when different materials and disinfection treatments were used, showing e.g. an interesting species selection when grown on copper.

SRP010
Analysis of antimicrobial peptides, their use for biofilm protection and the general stress response in fungi

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Microbial biofilms are ubiquitously found and the chosen life form of most of the microorganisms. Bacteria, fungi but also algae and protozoa benefit symbiotically from each other, while they are surrounded by an extracellular matrix. Some of the biofilms are unwanted, as they cause disease or destroy technical systems. To prevent biofilm formation on surfaces, we constructed modified fungal hydrophobins fused to antimicrobial peptides. Hydrophobins are small proteins, which self assemble at any hydrophilic-hydrophobic interface into extremely stable amphipathic monolayers. Cationic antimicrobial peptides (AMPs) are only 9 to 50 amino acids in size and are proven to be active against bacteria and even against yeasts and filamentous fungi [1]. They are an alternative to antibiotics and do not affect human cells. Different cationic antimicrobial peptides (AMPs) were fused to A. Nidulans hydrophobin DewA and expressed in E. coli.
A second aspect is the analysis of the general stress response in A. nidulans when these fungi are exposed to those AMPs. To this end, we are currently analyzing different His-Asp phosphorelay signaling systems (also known as two component systems), which consist of response regulators (rr) and histidine kinases (hk). A. nidulans has 4 rrs and 15 hks [2], for all of which we have corresponding deletion strains, which will be analyzed for their sensitivity against the cationic antimicrobial peptides.

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SRP011
Iron-binding properties of the redox sensor protein HbpS of the three-component system HbpS-SenS-SenR from *Streptomyces reticuli*

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The novel three-component signalling system HbpS-SenS-SenR from the cellulose degrader *Streptomyces reticuli* has been reported as an example of a redox sensing pathway in bacteria. This system senses redox stress signals in form of toxic concentrations of hemin, iron ions or other redox-active compounds and regulates genes involved in oxidative stress response [1]. During these processes, the extracellular oligomer-forming protein HbpS acts in concert with the two-component system SenS-SenR. Moreover, heme-binding and heme-degradation as well as iron-binding properties of HbpS have been shown to play an important role in the signalling cascade [2,3].
Recently, iron-binding motifs (D/E-X-X-E) have been identified in HbpS [3]. In order to get more insights as to their functional role, a set of mutant proteins will be generated and analysed *in vitro* and *in vivo*. Furthermore, by using of the 3D crystal structure of HbpS the localization of these motifs will be addressed. Homologues to HbpS exist in a number of Gram-positive and Gram-negative bacteria. Based on the structure of HbpS, modelling of their structure will be pursued. The obtained results will be discussed in frame of this presentation.

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SRP012
A new facet in the adaptation of *Bacillus subtilis* to high salinity: The cell wall hydrolase YqiI

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The habitat of the Gram-positive bacterium *Bacillus subtilis* is the upper layer of the soil. In this environment, *B. subtilis* has to cope with a multitude of environmental challenges. Increases in the salinity of the soil severely impair growth of *B. subtilis* and trigger adaptive countermeasures of the cell that are aimed at maintaining proper hydration of the cytoplasm and turgor [1]. Genome-wide transcriptional profiling studies of salt-stressed cells revealed novel aspects of the acclimatization of *B. subtilis* to high salinity environments [2, 3]. This included the up-regulation of genes that encode cell-wall modifying enzymes, suggesting that alterations in the cell wall are taking place when *B. subtilis* cells are exposed to high salinity surroundings.
We observed a drastic change in the morphology of *B. subtilis* cells that were grown in a high salinity minimal medium, indicating a re-arrangement of the cell wall peptidoglycan. To modulate the architecture of the cell wall, *B. subtilis* possesses various hydrolases [4]. Among them is a cell wall hydrolase homologue YqiI protein, whose structural gene was osmotically inducible. YqiI is a new cell wall hydrolytic enzyme that possesses an amidase-3 domain (Pfam data base), a domain that is typically found in N-acetylmuramoyl-L-alanine amidases. However, YqiI lacks the characteristic cell wall binding domain of these hydrolases. RT-PCR allowed us to demonstrate the *yqiI* gene is part of an operon: *yqiH-yqiI-yqiK*. Via Primer extension analysis we identified an osmotically regulated and SigA-dependent promoter located upstream of this operon. Reporter gene fusion (*yqiI-treA*) revealed that the *yqiI* gene is induced by high salinity but only if the salt concentration of the growth medium exceeds 0.7 M NaCl. Furthermore, we observed a strong induction of the transcription of the *yqiI-treA* reporter gene fusion in cultures that had entered the decay phase. Taken together, our data suggest that the YqiI hydrophilic cell wall amidase serves an important function in *B. subtilis* cells that are exposed to high salinity and in cells that are dying. Hence, the modification of the cell wall in response to the osmotic changes of the environment appears to be a new facet in the osmo acclimatization process of *B. subtilis*.

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SRP013
Proline biosynthesis in *Bacillus subtilis*: by-passing of the ProA-bottleneck

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In its soil habitat, *Bacillus subtilis* is exposed to high salinity conditions due to dessication. Its major osmotic stress response relies on the synthesis of the compatible solute proline. *B. subtilis* possess two pathways for proline production: (1) anabolic proline biosynthesis is mediated by the ProB-ProA-ProI enzymes and (2) osmoregulatory proline biosynthesis is mediated by ProJ-ProA-ProH enzymes. Hence, the *proA*-encoded γ-glutamyl-phosphate-reductase interlinks both proline biosyntetic routes.
We found that a *proBA* deletion strain was still able to form micro-colonies on minimal agar plates lacking proline. An additional mutation in *rocD* was required to create a tight Pro-auxtotroph. RocD is an enzyme involved in arginine degradation and produces the same reaction product as the ProA enzyme: γ-glutamyl-semialdehyde. We detected faster-growing Pro^+-suppressor mutants in the *proBA* deletion background. The genetic alterations in these suppressors were genetically mapped to the *rocR-rocDEF* region. RocR is an activator of the expression of the *rocDEF* operon. Upon inducer binding (e.g. arginine or proline), RocR acts in concert with the alterantive transcription factor SigL to induce *rocDEF* transcription from a -12 -24 type promotor. Four of the Pro^+-suppressors carried single amino acid substitutions in RocR and in all likelyhood lead to inducer independent RocR variants.
The *rocDEF* regulatory region carries a cryptic SigA-type promotor that lacks the so-called „invariant T" residue in a putative -10 region. Six of the studied Pro^+-suppressors carried single base-pair insertions that create a novel -10 region positioned with an appropriate spacing to a typical -35 sequence. Hence, these types of mutations activate cryptic SigA-type promotors and thereby allow *rocDEF* transcription even in the absence of an inducer for the RocR activator.
Taken together, the Pro^+-suppressor mutants genetically by-passe the *proA* deletion by allowing the RocD catalyzed synthesis of γ-glutamyl-semialdehyde that then can be further converted via ProI or ProH to proline. Our studies thus revealed an interesting physiological connection between the degradation of newly synthesized arginine and the biosynthesis of proline in *B. subtilis*.
Funding for this study was provided by the BMBF via the BaCell-SysMO2 consortium.

SRP014
Activation of RpoE by ChrR-proteolysis in the photooxidative stress response of *Rhodobacter sphaeroides*

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Singlet oxygen can be generated in the presence of photosynthetic pigments and is toxic as it leads to the damage of cellular macromolecules. Therefore photosynthetic organisms have to mount an adaptive response to this photooxidative stress. One major player of the photooxidative stress response in *Rhodobacter sphaeroides* is the alternative sigma factor RpoE, which is inactivated under non-stress conditions by its cognate anti-sigma factor ChrR. In this study we present data on RpoE and ChrR levels *in vivo*. We demonstrate that RpoE is activated by rapid proteolysis of ChrR upon exposure to singlet oxygen as well as organic peroxide. Specifically under singlet oxygen stress ChrR proteolysis is triggered by RSP_1090, a protein to which only a putative function was assigned so far. The specific involvement of RSP_1090 in ChrR proteolysis under singlet oxygen stress indicates that the response to singlet oxygen and organic peroxide is transmitted in part by different mediators. Based on our results we provide a model for RpoE activation in *R. sphaeroides*.

SRP015
Physiological role of rhomboid proteases in *Corynebacterium glutamicum*

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Rhomboid proteases are intramembrane proteases and belong to the class of serine proteases. In contrast to other intramembrane proteases, they differ functionally by releasing their cleavage products to the periplasm, rather than to the cytosol [1]. Rhomboids are found in eukaryotes and prokaryotes. While eukaryotic rhomboid proteases are known to participate in signal transduction in *D. melanogaster* or the host invasion by parasites, the general function of these proteases in prokaryotes remains still elusive. So far only for *P. stuartii* an involvement of rhomboids in generating a cell communication signal is known [2].
For our model organism *C. glutamicum* two rhomboid proteases named Cg0049 and Cg2767 are predicted. Apparently, the two rhomboids are differently regulated, because previous work revealed an increase of Cg2767 after heat stress, whereas Cg0049 was not detectable. To ascertain the function of Cg2767 during heat stress response and to find natural substrates, a proteomic approach using LC-ESI-MS/MS technology using the wild type and a *cg2767* deletion mutant was performed. Preliminary data indicate that before heat stress as well as after stress conditions some proteins are differently regulated in the *cg2767* deletion mutant in contrast to the wild type. Interestingly, about 20 proteins were exclusively identified in the wild type or the deletion mutant independent of temperature conditions. In contrast some proteins seem to be particularly affected by an increased expression of Cg2767, as about 10 proteins in the mutant or the wild type were only detected after heat stress. To put these data on a solid statistical basis further mass spectrometric investigations are in process. To elucidate, if the identified proteins are directly or indirectly regulated by Cg2767 biochemical assays are carried out.

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SRP016
Effect of the sRNA repeat RSs0680a-d on global gene regulation in *Rhodobacter sphaeroides*

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In bacteria small RNAs (sRNAs) play an important role in response to stress situations by means of posttranscriptional gene regulation. Although there are few sRNAs that bind proteins, common sRNAs bind to target messenger RNAs (mRNAs) and modulate the stability and/or translation of the mRNA [1]. There are two classes of sRNAs interacting with mRNAs, the so called cis-encoded antisense RNAs that show perfect base pairing with their target mRNAs and the trans-encoded sRNAs which show limited complementarity to their target mRNAs. To facilitate the interaction, the hexameric protein Hfq is needed in case of trans-encoded sRNAs to overcome the limited base pairing [1]. In *Rhodobacter sphaeroides* the expression of several such sRNAs is related to oxidative stress [2]. One sRNA that shows increased expression levels under oxidative stress is RSs0680a, which is cotranscribed with 3 homologous sRNAs (RSs0680b-d) and one hypothetical protein (RSP_6037). To realize stress dependent induction, the RSP_6037/RSs0680a-d operon is controlled by an $RpoH_I/RpoH_{II}$-dependent promoter [3]. We could show that constitutive overexpression of RSs0680a-d in *R. sphaeroides* leads to enhanced resistance to oxidative stress. Transcriptome and proteome analyses revealed serveral mRNAs and proteins with a changed abundance in the *R. sphaeroides* RSs0680a-d overexpression strain. Combination of those experiments with bioinformatic approaches revealed putative target mRNAs. Most of them show a putative relation to sugar transport or to aerobic respiration, which is a major source for oxidative stress. Especially a putative operon of four genes (RSP_2876 - RSP_2879) shows lower levels of both the expressed mRNAs as well as the respective proteins. The genes in this operon are subunits of a putative aerobic carbon monoxide dehydrogenase and one hypothetical protein. A possible function of the genes in this operon is related to the oxidation of cytochrome *b*561, which is part of the membrane bound electron transport chain. Interestingly possible binding sites for RSs0680a can be detected 2-6 bp upstream of the AUGs of the four genes. Presently in vitro sRNA:mRNA interaction studies including Hfq are performed, in order to prove the putative binding of RSs0680a to the mRNA of the putative operon.

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SRP017
Transcriptional analyses of steady-state cells of *Clostridium acetobutylicum* in a chemostat culture

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Clostridium acetobutylicum is well known for its acetone-butanol (AB) fermentation. Using phosphate limited chemostat cultures at pH 5.7, *C. acetobutylicum* was kept at a steady state in the acidogenesis, whereas at pH 4.5, the cells showed stable solventogenesis without sporulation. We investigated steady-state transcriptomes of pH 5.7 and pH 4.5 using DNA micro array analyses to provide new insights into the metabolic changes (1). Stable growth rates and constant exogenous parameters during the chemostat fermentation process enabled homogeneity of bacterial cells and the pH as single parameter was changed to switch from acidogenesis to solventogenesis.
All in all, 53 genes were significantly repressed, while 95 genes showed a significant upregulation in the steady-state of the solventogenesis at pH 4.5. The respective genes are suitable candidates for a basic mathematic model of the solventogenic shift, which will be developed within the COSMIC2 project (www.sysmo.net).
Furthermore, a transcriptional analysis of butanol stressed steady-state cells of the acidogenesis will be presented using DNA micro array analyses enabling the differentiation of genes, which transcription pattern was influenced by lowering the pH value or by butanol stress.

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SRP018
Peptide antibiotic sensing and detoxification modules in Firmicutes bacteria: Co-evolution of ABC-transporters and two component systems

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The genome of *Bacillus subtilis* contains three loci (*bceRSAB, psdRSAB*, *yxdJKLM*), which are very similar in gene organization and in sequence, and involved in resistance to various peptide antibiotics. The encoded systems are comprised of a two-component regulatory system (TCS) and an ATP-binding-cassette (ABC) transporter. Both the permease and sensor kinase components of these modules show unusual domain architecture: the permeases contain ten transmembrane helices with a large extracellular loop between helices 7 and 8, while the sensor kinases lack any obvious input domain. Strikingly, in the Bce and Psd modules the ABC-transporter and TCS have an absolute and mutual requirement for each other in both sensing of and resistance to their respective antimicrobial compounds. A search of several non-redundant protein databases revealed the existence of 265 ABC-transporters with homology to BceB and PsdB, 80% of which were associated with a TCS homologous to BceRS and PsdRS. All but four of these were found in Firmicutes bacteria. Parallel phylogenetic analysis of the permease and sensor kinase components revealed a tight evolutionary correlation, displayed as a congruence of the two phylogenetic trees. Our findings suggest direct protein-protein interactions between the ABC-transporters and TCSs in mediating resistance. Based on this correlation, we could identify putative corresponding two-component systems for transporters lacking a regulatory system in their immediate neighborhood. Taken together, our results show that these types of ABC-transporters and TCSs have co-evolved to form self-sufficient detoxification modules against antimicrobial peptides, restricted to and conserved among Firmicutes bacteria.

SRP019
Will not be presented!

SRP020
Guanidino-ectoine: a new member of the incompatible solute family

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The guanidinium function has been identified as a protein-backbone interacting group which displays the opposite effect to that of compatible solutes [1, 2]. This also seems to apply for guanidinium groups in amino acids like arginine [3]. In previous studies, inhibitory effects of the guanidinium compound creatine on bacterial growth have been demonstrated. Under elevated salinities, creatine is „mistaken" for the structurally related compatible solute betaine and taken up with the help of betaine transport systems. Despite its negative effect on cellular metabolism, creatine is accumulated to high cytoplasmic concentrations. Such a solute has since been named an incompatible solute.
To investigate whether other compounds which serve as compatible solutes also lose their protecting feature when a guanidinium group is introduced, a new derivate of the compatible solute ectoine, 2-amino-3,4,5,6-tetrahydro-4-pyrimidinecarboxylic acid (abbreviated guanidino-ectoine), was synthesized and growth experiments were performed in media supplemented with the new substance. The effect of guanidino-ectoine on bacterial growth, its impact on the intracellular solute-pool and the uptake systems of *E. coli* were characterized.
Contrary to ectoine, guanidino-ectoine did not support cell growth at elevated salinity. Instead, increasing concentrations led to enhanced inhibitory effects. In the presence of both solutes, the compatible and

incompatible form seemed to compensate each other. We therefore consider guanidino-ectoine a new member of incompatible solutes characterized by a guanidinium group [4].

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SRP021
Etherlipid biosynthesis in myxobacteria

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Myxobacteria are Gram-negative, motile, soil-dwelling δ-proteobacteria which are known for their complex life cycle including the formation of complex spore-containing fruiting bodies as well as their richness in diverse secondary metabolites formed by partly unique biosynthetic pathways. Many of these compounds exhibit antibacterial, antifungal or cytotoxic biological activities, which makes them interesting as drug candidates). Non-ribosomal [1] peptide synthethases (NRPS) and polyketide synthases (PKS) as well as NRPS/PKS hybrids are prevalently involved in the synthesis of many of these secondary metabolites.
A detailed analysis of *Myxococcus xanthus* cells exposed to various stresses showed that the formation of lipid vesicles is a common response of those cells towards environmental adversities [2] whereas starvation induced lipid vesicles proved to contain substantial amounts of unusual branched chain fatty acid-derived ether lipids. Those ether lipids make up at least one third of all lipids found in mature myxospores [3].
When investigating the biosynthesis of these ether lipids we discovered a gene encoding a multifunctional PKS/NRPS-like enzyme, designated as *elbD*, which is part of a five gene operon. This operon can be found in all myxobacteria sequenced so far. After its inactivation, a strong reduction of ether lipid formation in *M. xanthus* and a complete loss of ether lipid formation in *S. aurantiaca* under vegetative growth and starvation conditions was observed. Additionally, the speed of fruiting body formation is being affected in the respective mutants.
Therefore we cloned, heterologously expressed and purified ElbD in order to investigate the function of this protein by the means of *in vitro* activity assays and MALDI-TOF MS.

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SRP022
Significance of aspartokinases for the regulation of ectoine biosynthesis

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The biosynthetic pathway of ectoine-type compatible solutes is an off-branch of the metabolic network leading to the aspartate family amino acids. Lysine, methionine, threonine and isoleucine share the precursors aspartyl phosphate and aspartic acid semialdehyde as starting point in their biosynthesis. A strict feedback regulation of the aspartokinases [2] was reported to cause a metabolic bottle-neck in heterologous production of ectoines, when using ectoine biosynthetic genes from *Marinococcus halophilus* [1].
As we learn from latest reports [5] these findings are not valid for all heterologous production systems. Interestingly, some of the ectoine biosynthesis gene clusters have an aspartokinase in close proximity (e.g. in *Pseudomonas stutzeri*, [5]) which indicates a possible evolutionary and functional correlation [3] while others have not [4]. In addition, some ectoine producers have multiple enzymes of the aspartokinase type, whereas in others only one gene encodes for this function (e.g. *Halomonas elongata* and *Chromohalobacter salexigens*).

Here we present kinetic and regulation studies of *H. elongata* and *C. salexigens* aspartokinases and compare them with our previous findings for the *P. stutzeri* enzymes [6]. These results provide a first glance into the importance of different aspartokinase constellations in ectoine biosynthesis.

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SRP023
Solvent accessible surface characteristics of compatible solutes for the prediction of their protein-stabilizing potential

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Compatible solutes are small osmolytes that influence the equilibrium of the protein folding reaction (U ↔N). They raise the free energy of the unfolded state (U) and, in doing so, stabilize the native conformation (N) [1]. From the work of the Bolen group it is known that such solutes operate predominantly on the protein backbone, and that the backbone transfer free energy (ΔG_{tr}) from water to osmolyte solution is a good predictor for a compatible solute´s relative strenght as a protein stabilizer [2]. Structures of compatible solutes can differ considerably, it is therefore a challenge to predict their stabilizing potential from structural properties. Recently, Street et al. [3] tried to correlate structural properties of solutes and proposed a minimal model in which the fractional polar surface area served as an indication for ΔG_{tr} and hence the solute´s stabilizing power (the smaller the relative polar surface the better).
In this work, the concept by Street et al. [3] is put to the test by applying it to the natural ectoine-type compatible solutes, ectoine and 5-hydroxy-ectoine, the latter distinctly more polar than the former, as well as to the hitherto unavailable peptide-type compatible solute N-acetylglutaminyl glutamine-1-amide (NAGGN). NAGGN is characterized by an unusually large fractional polar surface area, which according to the polar surface concept should result in week stabilizing properties. The characterization of solute surface properties serves as a tool to enlarge the knowledge of interaction mechanisms between compatible solutes and biomolecules.

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SRP024
A carbon monoxide dehydrogenase-similar protein in *Clostridium acetobutylicum*

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A machinery for the scavenging of molecular O_2 and its reactive derivatives (ROS) is essential for the survival of strict anaerobic bacteria under aerobic condition. Previous studies with *Clostridium acetobutylicum* proved a robust defense mechanism under oxidative stress which leads to a protection of multiple cellular processes. The analysis of the transcriptome revealed a highly induced expression of an open reading frame *cac0116* after exposure to air (1). The deduced amino acid sequence of *cac0116* is annotated as a carbon monoxide dehydrogenase (CODH) and shows similarities to the respective homologue from *Carboxydothermus hydrogenoformans,* a type IV CODH. It has been speculated that this enzyme plays an important role in the oxidative stress response of this organism, e. g., by providing electrons from CO to rubrerythrin to reduce H_2O_2 to water (2).
To analyze the role of *cac0116* in *C. acetobutylicum*, we constructed a strain with altered expression of this gene. The CODH knock-out mutant is hypersensitive to oxygen, leading to a decreased ability to cope with O_2. Furthermore, the putative role of CODH in the electron transfer chain from NADH + H^+ or H_2 to O_2 and ROS will be elucidated using the purified

CODH after overexpression in *E. coli* together with the other components possibly involved (rubredoxin, ferredoxin, reverse rubrerythrin).

SRP025
Cold stress in Antarctic fungi targets enzymes of the glycolytic pathway and tricarboxylic acid cycle

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The ability of microorganisms to survive and thrive within hostile environments depends on rapid and robust stress responses. Antarctic fungi had to develop molecular mechanisms of adaptation to extreme low temperatures, but little is known about the effect of cold stress on the expression of key enzymes of the basic metabolic pathways. To investigate the role of those enzymes in cold tolerance two Antarctic fungal strains (psychrotrophic *Penicillium* sp. 161 and mesophilic *Aspergillus glaucus* 363) grown at the optimal temperature (20 and 25°C, respectively) were subjected to temperature downshift (10 and 4°C), and several enzymes involved in carbon metabolism, including hexokinase (HK; EC 2.7.1.1), glucose-6-phosphate dehydrogenase (G6PDH; EC 1.1.1.49), glyceraldehyde-3-phosphate dehydrogenase (GAPDH; EC 1.2.1.12), lactate dehydrogenase (LDH; EC 1.1.1.27), succinate dehydrogenase (SDH; EC 1.3.99.1), isocitrate dehydrogenase (IDH; EC 1.1.1.42), and malate dehydrogenase (MDH; EC 1.1.1.37) were assessed. While the activity of the HK was decreased, the activity of G6PDH was increased at low temperature showing a switch from Embden-Myerhoff pathway (EMP) to pentose phosphate pathway (PPP). Enhanced GAPDH activity support the hypothesis for its crucial role in antioxidant cell response. Modulation of LDH, a biomarker of oxidative stress, depends on temperature characteristic of the model strains. The same tendency was found about enzymes of Tricarboxylic Acid Cycle.

Acknowledgements: This work was supported by the European Social Fund, Operational Programme Human Resources Development (grant **BG051PO001-3.3.04/32) and** National Scientific Fund of the Ministry of Education and Science, Bulgaria (grant VU-B-205/06).

SRP026
Regulation of RpoS proteolysis by multiple input signals during the growth phase in *Escherichia coli*

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The RpoS (σ^S) subunit of RNA polymerase is the master regulator of the general stress response in *Escherichia coli*. Regulation of RpoS, which responds to many different environmental and cellular stresses, occurs at the levels of transcription, translation, proteolysis and protein activity [3]. RpoS degradation, which has become a paradigm of proteolytic regulation in bacteria, is initiated by binding of phosphorylated RssB, a response regulator acting as a proteolytic targeting factor. This interaction results in a structural rearrangement that exposes the ClpX6-binding site close to the RpoS N-terminus. Using ATP hydrolysis, RpoS is then unfolded, threaded into the proteolytic chamber formed by the ClpP14 part of the ClpXP complex and completely degraded, whereas RssB is released [2].
In recent work, we have observed that successive RpoS stabilization and accumulation during the post exponential and beginning stationary phase of *Escherichia coli* is a complex multistep process, with terminal stabilization in early stationary phase. Mechanistically, RpoS stabilization is based on changes in the ratio between free RpoS and phosphorylated RssB (which is limiting for the RpoS degradation rate [4]) by titration, competition and/or sequestration of either binding partner [2]. Thus, RssB can be titrated by a sudden massive increase in RpoS synthesis (e.g. in response to certain stresses). Alternatively, RpoS can be protected from proteolysis by increased binding to RNA polymerase, which is mutually exclusive with RssB binding [5]. Moreover, RssB can be sequestered by Ira proteins [1]. Furthermore, ClpX6 not only has to unfold RpoS, but also to strip it from tightly bound phosphorylated RssB, indicating that RpoS proteolysis has a particularly high ATP requirement. Consistently, we observed that successive RpoS stabilization correlates to decreasing cellular ATP levels, suggesting that energy starvation seems to trigger RpoS stabilization by reducing the intracellular ATP pool below a threshold required for terminal unfolding, RssB release and degradation of RpoS.

[1] Bougdour, A. et al (2008): Multiple pathways for regulation of sigmaS (RpoS) stability in *Escherichia coli* via the action of multiple anti-adaptors. Mol. Microbiol. 68: 298-313.
[2] Hengge, R. (2009): Proteolysis of σ^S (RpoS) and the general stress response in *Escherichia coli*. Res. Microbiol. 160: 667-676.
[3] Hengge, R. (2010): The general stress response in Gram-negative bacteria. In: G. Storz and R. Hengge (ed.), Bacterial Stress Responses (2nd edition). ASM Press, Washington, D.C. pp. 251-289.
[4] Pruteanu, M. and R. Hengge-Aronis (2002): The cellular level of the recognition factor RssB is rate-limiting for σ^S proteolysis: Implications for RssB regulation and signal transduction in σ^S turnover in *Escherichia coli*. Mol. Microbiol. 45:1701-1714.
[5] Typas, A. et al (2007): Stationary phase reorganisation of the *Escherichia coli* transcription machinery by Crl protein, a fine-tuner of sigmas activity and levels. EMBO. 26:1569-1578.

SRP027
Structural insides of the envelope stress sensor kinase CpxA - What causes the specificity of two component systems?

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The predominant family of signaling proteins in bacteria is the two-component signal transduction system (TCS). In general it consists of a sensor histidine kinase (HK) that after autophosphorylation transfers the phosphoryl group to its cognate response regulator (RR), which than effects changes in bacterial physiology. TCSs are essential for bacteria for sensing their environment during infection enabling optimal virulence factor production and protection against the host immune response. Although TCSs have remarkable similarities in sequence and structure, only small crucial differences seems to have a major impact, which not only results in a specific regulatory readout but also prevents unwanted cross-talk between non-cognate signalling systems (1). Structural information on signal transduction proteins are a prerequisite to identify the crucial attributes that guarantee specificity.
Here, we present a structural model of the catalytic cytosolic part of the envelope stress HK CpxA in contact with its cognate RR CpxR based on the HK/RR co-crystal structure solved by the group of Marina (2). The structural model of CpxA and CpxR enabled us to identify critical amino acids located in the interface of CpxA to CpxR that contribute specificity between HK and RR (1). To corroborate the functionality of these residues, we analyzed the capacities of single, double, triple or quadruple substitutions in the interface of CpxA on the efficiency to bind CpxR. Therefore, we preformed *in vivo* crosslinking with these different variants of the membrane anchored HK CpxA (Membrane-SPINE) to monitor the impact of the identified residues on the protein-protein interaction between the HK and the RR. We could confirm the predicted effects on RR binding by the substitution of essential amino acids for the first time *in vivo*.
Altogether, the structural insides of CpxA in complex with CpxR will strikingly contribute to a better understanding of these central signal transduction systems.

[1] Capra et al (2010): PLoS Genet. e1001220.
[2] Casino et al (2009): Cell 139: 325.

SRP028
New aspects in the regulation of the acid stress response system Cad in *Escherichia coli*

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On their way from the stomach to the gut enterobacteria are exposed to substantial change in the pH range. As the stomach can reach a pH value as low as 1.0, neutrophilic bacteria had to evolve several strategies to survive this extreme stress condition while maintaining their internal homeostasis. One acid response system is the lysine dependent Cad system. It consists of the enzyme CadA which catalyzes the decarboxylation of lysine to cadaverine while consuming a cytoplasmic proton and releasing CO_2. This reaction results in the increase of the internal pH. Furthermore, the lysine/cadaverine antiporter CadB, the membrane-integrated protein CadC and the lysine permease LysP are involved in the Cad system [2; 3]. Under non-inducing conditions, the secondary lysine transporter LysP represses the transcriptional activator CadC, whereas under low pH, anaerobiosis and in presence of lysine CadC is released and can act as an activator of transcription of the *cadBA* operon. A second repressor of the *cadBA* operon is the small histon-like molecule H-NS [1]. To make the picture more complex, several other proteins such as the lysine-2,3-aminomutase YjeK,

the lysyl-tRNA synthetase YjeA, the small RNA binding protein Hfq and YjeJ, a protein with an unknown function, were identified to play a role in the regulation of the Cad system. Mutants of these genes were either unable to express the genes of the *cadBA* operon or - in the case of YjeJ - the expression was higher as in the wild-type. Posttranslational modification is one interesting aspect which could be involved in the regulation process. Another aspect is the control of the acid response system at the transcriptional level by small RNAs with the help of Hfq. It is discussed how these components extend the regulation network of the Cad system.

[1] Shi, X.et al (1993): Modulation of acid induced amino-acid decarboxylase gene expression by H-NS in *Escherichia coli*. *J Bacteriol* 175: 1182-1186.
[2] Tetsch, L. et al (2008): The membrane-integrated transcriptional activator CadC of *Escherichia coli* senses lysine indirectly via the interaction with the lysine permease LysP. *Mol Microbiol* 67: 570-583.
[3] Watson, N. et al (1992): Identification of elements involved in transcriptional regulation of the *Escherichia coli cad* operon by external pH. *J Bacteriol* 174: 530-540.

SRP029
Osmotic stress induces different stress responses in *Enterococcus faecalis* and *Enterococcus faecium*

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Bacterial encounter changing environments, where they have to cope with limited nutrients, temperature shifts and other stresses. Thus, bacterial survival and fitness is dependent on an adequate stress response. The stress responses of the opportunistic pathogens *Enterococcus faecium* and *Enterococcus faecalis* in respect to osmotic stress were investigated via (i) genomic fingerprinting and (ii) gene expression analyses of a specific stress marker. To investigate general osmotic stress induced genome alterations via genomic fingerprinting, RAPD (randomly amplified polymorphic DNA) - PCR was applied. Short, unspecific binding primers were used in a PCR reaction and the generated fingerprints were compared. Polyphosphate kinase (PPK) encoded by *ppk* gene, catalyses the synthesis of polyP in bacteria and plays an important role in stress tolerance, virulence and survival. Expression of the *ppk* gene was assayed as specific stress marker. Enterococci in the early stationary growth phase were transferred into 0.5M NaCl solution and incubated for three, four and five days respectively. Osmotic stress did not change the genomic fingerprint of *Enterococcus faecium*, indicating its robustness, whereas RAPD-PCR of *Enterococcus faecalis* showed variations on the genome level, indicating the strains osmosensitivity. In parallel RNA from both enterococci was extracted and transcribed into cDNA using random hexamers. Expression analyses of the *ppk* gene in comparison to16S ribosomal housekeeping gene were performed. In both enterococci the stress responsive target *ppk* was constantly expressed during salt stress application.Whereas in *Enterococcus faecalis* the 16S rRNA was also constantly expressed, the amount of ribosomal 16S rRNA in *Enterococcus faecium* decreased significantly upon salt stress. It is likely that the reduction of 16S is caused by ribosomal disassembly, associated with a degradation of the ribosomal RNA. Despite their close relationship to each other the two enterococci show different stress responses upon osmostress.

SRP030
The regulatory interplay between the membrane-integrated transcriptional activator CadC and the lysine transporter LysP in *Escherichia coli*

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The Cad system is involved in the acid tolerance response of *E. coli* and helps to maintain the cytosolic pH within the physiological range. The system is composed of the lysine decarboxylase CadA, the lysine/cadaverine antiporter CadB and the membrane-integrated transcriptional activator CadC. Both, the consumption of a cytoplasmic proton during decarboxylation of lysine and the excretion of the more alkaline polyamine cadaverine, result in an increase of the intra- and extracellular pH. CadC regulates expression of the *cadBA* operon and induces the transcription under conditions of low external pH (5.8) and concomitantly available lysine. CadC co-senses the exogenous lysine signal in an interplay with the lysine-specific transporter LysP. LysP inhibits CadC activation at a low external lysine concentration presumably via a direct interaction with the transmembrane domain of CadC. To gain more insights into the molecular mechanism of the interconnectivity between CadC and LysP we applied site-directed and random mutagenesis. Both methods generated several LysP derivatives with single amino acid replacements that altered CadC mediated *cadBA* expression. To elucidate whether transport of LysP is the prerequesite for co-sensing, we investigated transport activity of these variants *in vivo* by measuring L-^{14}C-lysine uptake in an *E. coli* strain lacking all lysine transporters. These analyses revealed a functional coupling of the regulatory and transport activities of LysP. It is still unclear, whether amino acid replacements in LysP affect lysine binding and/or the mediation of protein-protein-interactions. In another approach transmembrane interactions between LysP and CadC were analyzed with the BACTH system. First results indicate an interaction between transmembrane helix three of LysP and the transmembrane helix of CadC.

SRP031
Strand specific transcriptomes of *Escherichia coli* O157:H7 EDL933 revealed by RNA-sequencing

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Escherichia coli O157:H7 EDL933 is an important human pathogen. Infection leads to hemorrhagic diarrhea and can cause a hemolytic uremic syndrome (HUS). This bacterium is transmitted by food, including produce. Its genome was sequenced in 2001. Due to the progresses in Next Generation Sequencing we were able to sequence the total transcriptome of this pathogen under six different conditions. Cells were harvested from LB medium, LB medium at pH9, LB medium with nitrite, minimal medium, homogenized spinach and the surface of raddish shoots. The sequenced transcripts (SOLiD4.0) were mapped to the reference genome and compared among the six different conditions.
The data give insights into gene usage under different conditions. Beside many known genes, we have evidence for transcription of several hypothetical genes. Owing to different expression patterns these putative genes can now be attributed with functional involvements. In addition, these data sets uncovered yet unknown transcripts. Some of those show very similar structures to known sRNAs and asRNAs, others may code for proteins. Several genetic elements of the *E. coli* O157:H7 EDL933 genome can now be re-annotated or mapped with higher precision, respectively. This includes major and minor transcriptional start sites or operon configurations under different conditions. Taken together, the data allow to better understand the mechanisms of environmental persistence and infection of different vectors.

SRP032
Identification of the molecular mode of action of Carolacton, a novel biofilm inhibitor

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Biofilm forming bacteria are often significantly more resistant to drug treatments than their planktonic counterparts and are associated to various pathological conditions in humans as e.g. cystic fibrosis, colonisation of indwelling medical devices and dental plaque formation. To this end new substances and therapies aiming to erase biofilms are urgently needed. Carolacton, a secondary metabolite isolated from the myxobacterium *Sorangium cellulosum* was proven to effectively kill *S. mutans* biofilm cells in a wide range of concentrations while showing only minor toxic effects on planktonically living cells [1]. A severe membrane damage, caused by carolacton, was verified by the analysis of the protein and DNA content in the supernatant of carolacton treated cells and the comparison to untreated reference biofilms. Utilisation of a ß-galactosidase reporter strain revealed cytoplasmically localised ß-galactosidase to be present in large extent extracelluarly. Furthermore it was shown that carolacton interferes with the acid resistance of *S. mutans*. In order to evaluate the carolacton affected transcriptome and to get insights into the molecular mode of action a comparative time series microarray analysis using treated and untreated biofilm cells was performed. Up to 28% of all 1961 ORFs of *S. mutans* were identified to be differentially expressed (log FC > +/- 0.8; $p < 0.001$) upon carolacton pertubation. Regulated genes include numerous coding for

bacteriocines, proteins involved in cell wall metabolism, cell division, pyrimidine metabolism and metabolism of amino acids. The two component signal transduction systems CiaRH, VicKRX, RelRP, comDE and SMU.1037/1038 were significantly affected by carolacton. While comDE is the only positively regulated TCS, VicKRX shows the earliest response to carolacton. Among the other differentially expressed genes many known to be regulated by VicKRX were identified, implicating a central role of the VicKRX-system in the mode of action of carolacton.
An analysis of the sensitivity of all 13 viable HK-mutants of *S. mutans* to carolacton using Live/Dead Bacterial Viability staining showed that all mutants were susceptible. Recently analysed regulon of eukaryotic type serine/threonine kinase pknB showed a strong overlap with the carolacton affected genes. Furthermore pknB is predicted to modulate the activity of the VicKRX system and interferes with the acid resistance. A pknB mutant was proved to be insensitive to carolacton using Live/Dead staining and Cfu-counts, indicating pknB as the potential target.

[1] Kunze, B. et al (2010): Damage of Streptococcus mutans biofilms by carolacton, a secondary metabolite from the myxobacterium *Sorangium cellulosum*. BMC.Microbiol. 10:199.

SRP033
Extracellular proteolysis of *A. fumigatus* and eIF2α kinase signaling - is there a connection?

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Aspergillus fumigatus is a ubiquitous mould colonizing soil and decomposing organic matter. It produces small conidia that are distributed by the air and reach the alveoli of the lung when inhaled. In immunocompromised patients, this opportunistic pathogen can cause several forms of disease, the most severe form is called Invasive Aspergillosis (IA). Several determinants contribute to pathogenicity of *A. fumigatus*, e.g. its nutritional versatility and the ability to react on fast changing environmental conditions. The *prtT* gene product is a global regulator of extracellular proteolytic activity in *A. fumigatus* and is therefore involved in degradation of polymeric proteinaceous substances of the surrounding environment. This transcription factor regulates expression of several secreted proteases (*alp1, mep, pep1*); however, it is not a virulence determinant of pulmonary invasive aspergillosis in leukopenic mice. Expression of PrtT appears to be regulated posttranscriptionally, also supported by a long 5' leader region of the *prtT* transcript. Furthermore, *A. fumigatus* exhibits eIF2α-kinase signaling to counteract environmental stress conditions as it expresses two functional kinases for this initiation factor of translation: CpcC, an integral component of the so-called Cross-Pathway Control system of amino acid biosynthesis, and IfkB (*i*nitiation *f*actor *k*inase *B*) with as yet unknown cellular function. To further investigate any regulatory role of the *prtT* leader region, we generated 5'*prtT::gfp*-reporter strains in genetic deletion backgrounds for either or both eIF2α kinases. CpcC and IfkB seem to influence translation of the reporter upon a shift from minimal medium to BSA as sole nitrogen source with the presence of either sensor kinase being apparently sufficient for expression. These data imply a mechanism of translational regulation of PrtT expression via two redundant eIF2α kinases, which links extracellular proteolysis of *A. fumigatus* to this conserved regulatory cascade.

SRP034
Yeast protein kinase C does not relocate to mitochondria upon membrane stress: evidence for experimental artefacts

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Protein kinase C (Pkc1) of the Baker's yeast *Saccharomyces cerevisiae* is a key component of the cell wall integrity (CWI) signalling pathway, which governs cell wall biosynthesis upon cell surface stress. This pathway is essential for survival under normal growth conditions (i.e. in the absence of osmotic stabilization) and constitutes an ideal target for the development of antifungal agents. In addition to its role in activating the central MAP kinase module of the CWI pathway, Pkc1 influences the dynamics of the actin cytoskeleton and is involved in the secretory pathway.
A Pkc1-GFP fusion protein has been reported to relocate from the cytoplasm to mitochondria upon treatment of yeast cells with the oxidative stress agent farnesol (Fairn et al. 2007: J. Biol. Chem., 282, 4868-4874). In order to verify these data, we also constructed a similar Pkc1-GFP fusion in our laboratory strain and used it in combination with a mCherry-fusion of the mitochondrial marker succinate dehydrogenase. We found that Pkc1-GFP primarily localizes to the yeast bud neck during cytokinesis and does not relocalize to mitochondria upon treatment with farnesol, tea trea oil or detergents like Tween20 or Tween40. However, the nonionic detergent Nonidet P-40, which was used by the authors cited above as a solvent for farnesol, led to an accumulation of Pkc1-GFP at mitochondrial structures, even in the absence of farnesol.We conclude that the reported effect of oxidative stress on Pkc1 localization is an experimental artefact.
Currently, we are investigating the involvement of Pkc1 in the regulation of yeast cytokinesis.

SRP035
A proteomic signature library: Gene expression of *Staphylococcus aureus* under various growth-restricting conditions

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Since decades, gel-based proteomics has been used to get deeper insights into physiological processes of living cells. Now we present an inter-experimental comparison of different protein synthesis pattern induced in *Staphylococcus aureus* COL by nine various stressors or growth limiting factors as hydrogen peroxide, diamide, paraquat, nitric oxide, heat, puromycin, mupirocin, and oxygen limitation in the presence or absence of nitrate. Based on a cytoplasmic reference map with 698 identified protein spots (521 different proteins) common and specific features of the individual stress responses were analyzed. Whereas only the synthesis of UspA (SACOL1759) was generally induced in six out of the nine experiments, several other proteins showed stress-specific expression profiles. For instance, synthesis of Rex-controlled proteins (*e.g.* Ldh1, SrrAB) was clearly induced only after oxygen limitation or nitrosative stress. Exposure to H_2O_2 led to a stepwise adaptation that affects mainly expression of proteins involved in DNA repair and nucleotide metabolism. Furthermore, expression data of more than 70 so far uncharacterized proteins are available. This might provide initial indications of their possible physiological role. All data generated in this study are stored in a custom-made online resource which might be helpful for the interpretation of new (e.g. *in vivo* derived) expression data in future.

Personalia aus der Mikrobiologie 2010

Habilitationen

Ute Warnecke-Eberz habilitierte sich am 4. Mai 2009 an der Universität zu Köln (Responseprädiktion der neoadjuvanten Therapie beim Ösophaguskarzinom: Evaluierung molekularer Marker und Nachweisverfahren für den klinischen Einsatz).

Lars Blank habilitierte sich am 13. Januar 2010 an der Technischen Universität Dortmund (Systems Biotechnology & Biocatalysis).

Ralf Heermann habilitierte sich am 20. Januar 2010 an der Ludwig-Maximilians-Universität München (Molekulare Mechanismen der bakteriellen Signaltransduktion an Beispielen der Enterobakterien *Escherichia coli* und *Photorhabdus luminescens*).

Kürsad Turgay habilitierte sich am 19. Mai 2010 an der Freien Universität Berlin in Mikrobiologie (Regulatorische und Generelle Proteolyse in *Bacillus subtilis*).

Ingo Schmidt habilitierte sich am 31. Mai 2010 an der Universität Bayreuth (Metabolism of inorganic and organic substrates by the facultative chemolithoautotrophic ammonia oxidizer *Nitrosomonas europaea* as basis of its energy conservation).

Bernhard M. Fuchs vom Max-Planck-Institut für Marine Mikrobiologie in Bremen habilitierte sich am 22. Juni 2010 an der Universität Bremen (Cytometric Analyses of Marine Picoplankton Populations).

Matthias Brock habilitierte sich am 16. August 2010 an der Universität Jena (Metabolismus als Grundlage der Virulenz: Stoffwechselphysiologie pathogener Pilze).

Simone Bergmann habilitierte sich am 15. November 2010 an der Technischen Universität Braunschweig (Vom Kommensalen zum Pathogen: Aufklärung von Interaktionen zwischen *Streptococcus pneumoniae* und Wirtsfaktoren der Blutgerinnung und der extrazellulären Matrix).

Wiebke Hansen habilitierte sich am 2. Dezember 2010 an der Universität Duisburg-Essen (Charakterisierung und Modulation regulatorischer T-Zellfunktionen).

Rufe

Jörg Overmann von der Ludwig-Maximilians-Universität München übernahm am 1. Februar 2010 die Position des geschäftsführenden Direktors des Leibnitz-Institutes Deutsche Sammlung von Mikroorganismen und Zellkulturen sowie eine W3-Professur für Mikrobiologie an der Technischen Universität Braunschweig.

Heike Brötz-Oesterhelt von der AiCuris GmbH & Co. KG, Wuppertal übernahm am 1. März 2010 die W2-Professur für den Lehrstuhl Pharmazeutische Biologie an der Universität Düsseldorf.

Heike Krebber von der Universität Marburg übernahm am 1. April 2010 die W2-Professur für Molekulare Genetik an der Universität Göttingen.

Bernd Kreikemeyer von der Universität Rostock übernahm am 1. April 2010 die W2-Professur für Molekulare Bakteriologie an der Universität Rostock, Institut für medizinische Mikrobiologie, Virologie und Hygiene.

Katharina Riedel von der Universität Zürich übernahm am 1. Juli 2010 die W2-Professur Mikrobielle Proteomforschung für den Lehrstuhl Mikrobiologie an der Technischen Universität Braunschweig.

Jörg Simon von der Universität Frankfurt übernahm am 1. August 2010 die W2-Professur für Mikrobiologie an der Technischen Universität Darmstadt.

Peter Schupp von der Universität Guam, USA übernahm am 1. Oktober 2010 die W2-Professur Umweltbiochemie an der Universität Oldenburg.

Emeritierungen/ Pensionierungen

Arnold Geis vom Institut für Mikrobiologie und Biotechnologie am Max-Rubner-Institut in Karlsruhe wurde am 28. Februar 2010 pensioniert.

Kurt Mendgen vom Institut für Phytopathologie an der Universität Konstanz wurde im Mai 2010 emeritiert.

Georg Auling vom Institut für Mikrobiologie an der Universität Hannover wurde am 30. September 2010 pensioniert.

Ulrich Fischer vom Institut für Marine Mikrobiologie an der Universität Bremen wurde am 1. Oktober 2010 pensioniert.

Wolfgang Schumann vom Institut für Genetik an der Universität Bayreuth wurde am 1. Oktober 2010 pensioniert.

Rainer Borriss vom Institut für Biologie an der Humboldt-Universität zu Berlin wurde am 1. Oktober 2010 emeritiert.

Jürgen Kreft vom Institut für Mikrobiologie an der Universität Würzburg wurde am 1. Oktober 2010 pensioniert.

Wissenschaftliche Preise 2010

Roland Lill vom Max-Planck-Institut für Terrestrische Mikrobiologie in Marburg erhielt 2010 von der Feldberg Foundation for Anglo-German Scientific Exchange den Feldberg Foundation Preis für seine Arbeiten über die Biosynthese von Eisen-Schwefel-Proteinen in Eukaryoten.

Katrin Breitinger von der Universität Ulm erhielt am 5. Februar 2010 den Frauenförderpreis für ihre Arbeiten über Vergleichende Funktionsanalyse von RamB aus *Corynebacterium glutamicum* und Rv0465c aus *Mycobacterium tuberculosis*.

Markus Bröcker von der Technischen Universität Braunschweig erhielt am 8. Februar 2010 den Preis der Vereinten Stiftung der Technischen Universität Braunschweig für Studierende und Doktoranden für seine Promotionsarbeit zum Thema Function and Structure of the Light-Independent Protochlorophyllide Oxidoreductase.

Bärbel Friedrich von der Humboldt-Universität zu Berlin erhielt am 12. Februar 2010 den Frontiers in Biological Chemistry Award der Max-Planck-Gesellschaft für ihre Arbeiten über Sauerstoff-tolerante Hydrogenasen und deren Anwendungspotenzial auf dem Weg zur biosolaren Wasserstoffproduktion.

Garabed Antranikian neuer Präsident der TU Hamburg-Harburg

Der Mikrobiologe Prof. Dr. rer. nat. Dr. h.c. Garabed Antranikian wird neuer Präsident der TUHH. Der 59-jährige Wissenschaftler wurde einstimmig vom Hochschulrat gewählt und vom akademischen Senat bestätigt. Antranikian übernimmt das Amt am 1. April 2011 für die Dauer von sechs Jahren. Antranikian studierte Biologie in Beirut und promovierte 1980 an der Universität Göttingen, wo er sich 1988 auch habilitierte. 1989 folgte er dem Ruf an die TU Hamburg-Harburg und lehrte dort zunächst als Professor für Technische Mikobiologie und seit 2003 als Leiter des gleichnamigen Instituts auf dem Gebiet der Mikrobiologie und Biotechnologie.

Iris Chaberny von der Medizinischen Hochschule Hannover, Institut für Medizinische Mikrobiologie und Krankenhaushygiene, erhielt am 17. März 2010 den Hufeland-Preis für Präventivmedizin für ihre Arbeiten über Methicillin-resistente *Staphylococcus aureus* (MRSA) im Krankenhaus: Surveillance, Management und Intervention im Sinne der Patientensicherheit.

Andreas Peschel von der Universität Tübingen erhielt am 28. März 2010 den Hauptpreis der Deutschen Gesellschaft für Hygiene und Mikrobiologie (DGHM) für seine Arbeiten über *Staphylococcus aureus*.

Karolin Graf von der Medizinischen Hochschule Hannover, Institut für Medizinische Mikrobiologie und Krankenhaushygiene, erhielt am 18. April 2010 den Projektpreis 2010 der Deutschen Gesellschaft für Krankenhaushygiene e.V. (DGKH) für ihre Arbeiten über Analyse und Reduktion tiefer sternaler Wundinfektionen in der Herzchirurgie mit Hilfe umfangreicher Infektionspräventionsmaßnahmen.

Ralf Conrad vom Max-Planck-Institut für Terrestrische Mikrobiologie in Marburg erhielt am 18. Mai 2010 den Einstein-Professorship-Preis der Chinese Academy of Sciences für seine Arbeiten über den biogeochemischen Kreislauf von atmosphärischen Spurengasen.

Regine Hengge von der Freie Universität Berlin erhielt am 1. Juni 2010 den Advanced Researcher Grant vom European Research Council für ihre Arbeiten über Cyclic-di-GMP: New Concepts in Second Messenger Signaling and Bacterial Biofilm Formation.

Marco Kai von der Universität Rostock erhielt am 2. Juli 2010 den Joachim-Jungius-Preis der Universität Rostock für seine Arbeiten über Analyse und Wirkungen flüchtiger Metabolite von *Serratia odorifera* Rx13.

Ingrid Waege von der Universität Regensburg erhielt am 8. Juli 2010 den GBM-Diplom-/Masterpreis 2010 für ihre Arbeiten über Genetische Transformationsexperimente in *Pyrococcus furiosus* und *Thermococcus kodakaraensis*.

Christian Riedel von der Universität Ulm erhielt am 19. Juli 2010 den Wissenschaftspreis der Stadt Ulm für seine Arbeiten über Molekulare Mechanismen der Interaktion von Bifidobakterien und Epithelzellen im menschlichen Darm.

Bo Barker Jørgensen vom Max-Planck-Institut für Marine Mikrobiologie in Bremen erhielt am 27. August 2010 den Jim Tiedje Award für sein herausragendes Lebenswerk auf dem Gebiet der mikrobiellen Ökologie.

Marc Strous vom Max-Planck-Institut für Marine Mikrobiologie in Bremen erhielt am 27. August 2010 den ISME Young Investigators Award für seine bedeutenden Arbeiten zur mikrobiellen Ökologie.

Kurt Mendgen von der Universität Konstanz erhielt im September 2010 die Anton de Bary-Medaille der Deutschen Phytomedizinischen Gesellschaft für seine herausragenden wissenschaftlichen Leistungen bei der molekularbiologischen und immunhistologischen Erforschung der Wirt-Parasit-Interaktionen von Rostpilzen.

Thomas Opfermann vom Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie – Hans-Knöll-Institut in Jena – erhielt von der Firma Siemens am 8. September 2010 den Siemens Award Excellence in Preclinical Imaging at WMIC für seine Arbeiten über molekulare Bildgebung durch PT/CT.

Lubos Polerecky vom Max-Planck-Institut für Marine Mikrobiologie in Bremen erhielt am 11. September 2010 den Biomaris-Forschungspreis für die Entwicklung neuer Techniken für die Meeresforschung.

Hauke Harms und **Mona Wells** vom Helmholtz-Zentrum für Umweltforschung UFZ und **Jan Roelof van der Meer** von der Universität Lausanne, Schweiz, erhielten am 16. September 2010 den Erwin-Schrödinger-Preis 2010 für ihre Arbeiten über Bioreporterbakterien – Einfachanalyse von Arsen und anderen Umweltschadstoffen.

Hendrik Kortmann von der TU Dortmund erhielt am 14. Oktober 2010 den Klaus-Goerttler-Preis, Deutsche Gesellschaft für Zytometrie (DGFZ) für seine Arbeiten über Einzelzellanalyse.

Tobias J. Erb, VAAM-Promotionspreisträger 2010, von der University of Illinois, Urbana, USA erhielt am 20. Oktober 2010 den Hans-Grisebach-Preis für seine Arbeiten über The Ethylmalonyl-CoA Pathway: A Novel Acetyl-CoA Assimilation Strategy.

Ein Team Bielefelder Studenten (**Simon Unthan, Frieder Hänisch, Eva Brombacher, Jonas Aretz, Timo Wolf, Nikolas Kessler, Armin Neshat, Frederik Walter, Nils-Christian Lübke und Jonas Marschall**) erhielt im November 2010 bei der International Genetically Engineered Machine competition (iGEM), einem Wettbewerb zu Synthetischer Biologie in Boston, Massachuetts, USA, einen Gold Award für ihr Modulated Acetosyringone Receptor Sensor System (MARSS).

Munisch-Kumar Wadwa erhielt am 09. November 2010 von der Universität Duisburg-Essen den Preis für Absolventen mit Migrationshintergrund für seinen Masterabschluss (M. Sc. Medizinische Biologie) in der Fakultät Biologie/Geographie.

Tanja Schneider von der Universität Bonn erhielt am 12. November 2010 den Robert-Koch-Postdoktoranden-Preis für Mikrobiologie für ihre Arbeiten über die Entwicklung neuer Antibiotika.

Sandra Bruns, Olaf Kniemeyer und Andreas Thywißen vom Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie – Hans-Knöll-Institut in Jena – erhielten von der Firma medac am 13. Dezember 2010 den medac-Forschungspreis für ihre Arbeiten über die Immunantwort gegen *Aspergillus fumigatus*-Infektionen.

Thorger Lincke, Swantje Behnken, Keishi Ishida und Martin Roth vom Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie – Hans-Knöll-Institut in Jena – erhielten von der Firma medac am 13. Dezember 2010 den medac-Forschungspreis für ihre Arbeiten über das erste Antibiotikum aus *Clostridium*.

Gerald Lackner vom Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie – Hans-Knöll-Institut in Jena – erhielt von der Firma medac am 13. Dezember 2010 den medac-Forschungspreis für seine Arbeiten über die Struktur und Funktion von Lipopolysacchariden endofungaler Bakterien.

Promotionen 2010

Universität Bayreuth

Stefan Gilch: Ammonium-Monooxygenase aus *Nitrosomonas europaea*: Charakterisierung und Isolierung des nativen Enzyms
Betreuer: Ortwin Meyer

Freie Universität Berlin

Britta Kraushaar: Ein konjugatives Typ IV-Sekretionssystem in der Gattung *Yersinia*: Verbreitung und Variabilität
Betreuer: Bernd Appel (Bundesinstitut für Risikobewertung), Rupert Mutzel

Sascha Brunke: Molekularbiologische Untersuchungen zur Pigmentsynthese von humanpathogenen Hefen
Betreuer: Bernhard Hube (Hans Knöll-Institut Jena), Rupert Mutzel

Susan Busse: Unorthodoxe Mechanismen der Regulation innerhalb von Zweikomponentensystem in *Escherichia coli*
Betreuer: Regine Hengge, Kürsad Turgay

Humboldt-Universität zu Berlin

Alexander Schwarze: Light-driven H_2 production by connecting O_2-tolerant [NiFe]-hydrogenases from *Ralstonia eutropha* H16 with the cyanobacterial photosystem I
Betreuerin: Bärbel Friedrich

Anke Licht: Charakterisierung von zwei ABC-Importsystemen im Acarbose-Metabolismus von Actinomyceten
Betreuer: Erwin Schneider

Universität Bielefeld

Christoph Hellweg: Untersuchung der Antwort des symbiontischen Bodenbakteriums *Sinorhizobium meliloti* 1021 auf azidischen pH-Stress
Betreuer: Alfred Pühler

Monika Flügel: Transkriptomanalysen zur Interaktion von *Clavibacter michiganensis subsp. michiganensis* mit seiner Wirtspflanze
Betreuer: Rudolf Eichenlaub

Ghazaleh Nematollahi: Zelltypspezifisch exprimierte Gene in der Grünalge *Volvox carteri*
Betreuer: Armin Hallmann

Anja Doebbe: Biosolar hydrogen as a CO_2-free renewable energy source: optimization of proton and electron supply to increase the H_2-production rates in green algae
Betreuer: Olaf Kruse

Universität Bochum

Lena Gaubig: Regulation des *Escherichia coli ibpAB*-Operons unter Hitzestress-Bedingungen
Betreuer: Franz Narberhaus

Sonja Klüsener: Charakterisierung und physiologische Relevanz des Membranlipids Phosphatidylcholin für das pflanzenpathogene Bakterium *Agrobacterium tumefaciens*
Betreuer: Franz Narberhaus

Juan Carlos Lorenzo Fajardo: Funktionelle Charakterisierung eines potenziellen Regulators der Häm-Homöostase in *Pseudomonas aeruginosa*
Betreuerin: Nicole Frankenberg-Dinkel

Björn Gisk: Biochemische und biophysikalische Untersuchungen an pflanzlichen und bakteriellen Hämoxygenasen
Betreuerin: Nicole Frankenberg-Dinkel

Jens Kortmann: Structure and function of novel RNA thermometers
Betreuer: Franz Narberhaus

Sandra Bloemendal: Molecular genetics of fruiting body formation in the filamentous fungus *Sordaria macrospora*: Identification of interaction partners of developmental proteins *in vitro* and *in vivo*
Betreuer: Ulrich Kück

David Löper: DNA-bindende Proteine des Antibiotika-Produzenten *Acremonium chrysogenum*: Biochemische Charakterisierung und molekulargenetische Funktionsanalysen
Betreuer: Ulrich Kück

Universität Bonn

Bettina Franz: Untersuchungen zum Sox-Multienzymkomplex in *Allochromatium vinosum* und zur Verwertung von Elementarschwefel in phototrophen Schwefeloxidierern
Betreuerin: Christiane Dahl

Ute Selan: Biochemische Untersuchungen zum DsrC Protein und zum DsrEFH Heterohexamer von *Allochromatium vinosum*
Betreuerin: Christiane Dahl

Fabian Grein: Biochemical, biophysical and functional analysis of the DsrMKJOP transmembrane complex from *Allochromtatium vinosum*
Betreuerin: Christiane Dahl

Technische Universität Braunschweig

Katja Böhme: Identification and characterization of regulatory factors and regulatory RNA elements controlling the expression of the primary invasion factors invasin and YadA in *Yersinia pseudotuberculosis*
Betreuerin: Petra Dersch

Markus Bröcker: Function and Structure of the Light-Independent Protochlorophyllide Oxidoreductase
Betreuer: Dieter Jahn

Boyke Bunk: Comparative and Functional Genomics of *Bacillus megaterium* DSM319
Betreuer: Dieter Jahn

Julia Garbe: Isolation of *Pseudomonas aeruginosa* phages and their application for the analysis of lipopolysaccharides
Betreuer: Dieter Jahn

Ines Gruner: Die funktionelle Charakterisierung des anaeroben Regulators Fnr und die Regulation der anaeroben Genexpression in *Bacillus subtilis*
Betreuer: Dieter Jahn

Johannes Klein: Bioinformatics of gene regulatory networks in pathogenic bacteria
Betreuer: Dieter Jahn

Anika March: Die Regulation der Acetoinbiosynthese in *Bacillus subtilis* durch den transkriptionellen Regulator AlsR
Betreuer: Dieter Jahn

Andreas Roth: Vektorsystem für die Produktion und Reinigung von rekombinanten Proteinen in *Aspergillus niger*
Betreuerin: Petra Dersch

Claudia Schulz: Characterisation of Enzymes involved in Tetrapyrrole Biosynthesis
Betreuer: Dieter Jahn

Simon Stammen: Genetic tools for high yield protein production with *Bacillus megaterium*
Betreuer: Dieter Jahn

Universität Bremen/MPI für Marine Mikrobiologie

Christina Liliana Moraru: Fluorescence *in situ* hybridization of genes in environmental microbiology
Betreuer: Rudolf Amann

Caroline Verna: Phylogeny and diversity of symbionts from whale fall invertebrates
Betreuerin: Nicole Dubilier

Paola Gomez: Marine Bacteroidetes: distribution patterns and role in the degradation of organic matter
Betreuer: Rudolf Amann

Regina Schauer: Diversity and function of microbial communities in sediments from different deep-sea habitats
Betreuer: Rudolf Amann

Stefanie Grünke: Diversity of Mat-forming Sulfide-oxidizing Bacteria at Continental Margins
Betreuer: Rudolf Amann

Karina Stucken: Physiogenomics of *Cylindrospermopsis raciborskii* and *Raphidiopsis brookii* (Cyanobacteria) with Emphasis on Evolution, Nitrogen Control and Toxin Biosynthesis
Betreuer: Rudolf Amann

Caroline Rühland: Characterization of bacterial endo- and ectosymbionts of oligochaete worms from marine sediments: Phylogeny and metabolic potential
Betreuer: Rudolf Amann

Lars Schreiber: Assessing the Genetic Potential of Uncultivated Sulfate Reducing Bacteria
Betreuer: Rudolf Amann

Luciana Raggi: Bacterial-bivalve associations, from an asphalt cold seep to shallow waters
Betreuerin: Nicole Dubilier

Mohammad Al-Najjar: Flow of light energy in benthic photosynthetic microbial mats
Betreuer: Bo Barker Jørgensen

Laura Wehrmann: Biogeochemical processes in sediments associated to cold-water coral ecosystems – From living reefs to ancient mounds
Betreuer: Bo Barker Jørgensen

Katharina Kohls: Diversity, salinity adaptation, and role in carbon cycling of microbial communities inhabiting the oxic layer of intertidal hypersaline microbial mats
Betreuer: Friedrich Widdel

Thomas Holler: Mikrobiologische Studien zur anaeroben Oxidation von Methan (AOM)
Betreuer: Friedrich Widdel

Ilaria Pizzetti: Abundance, distribution and diversity of planktonic *Planctomycetes* in coastal zones
Betreuer: Rudolf Amann

Rita Dunker: Motility of the giant sulfur bacterium *Beggiatoa*
Betreuer: Bo Barker Jørgensen

International Jacobs University Bremen/MPI für Marine Mikrobiologie

Angélique Gobet: Microbial Community Ecology of Temperate Coastal Sands
Betreuerin: Antje Boetius

Sandra Schöttner: Bacterial Habitat Differentiation in Cold- and Warm-water Coral Reef Ecosystems
Betreuerin: Antje Boetius

Melissa Beth Duhaime: Exploring the Marine Virosphere
Betreuer: Frank-Oliver Glöckner

Technische Universität Dortmund

Rainer Gross: Catalytic Biofilms in Membrane Reactors: Continuous Asymmetric Epoxidation of Styrene and Regioselective Hydroxylation of Alkanes
Betreuer: Katja Bühler, Andreas Schmid

Technische Universität Dresden

Anne Kretzschmar: Die Beeinflussung der Succinatproduktion durch die veränderte Aktivität der Succinyl-CoA-Synthetase und der Pyruvat-Carboxylase in *Yarrowia lipolytica*
Betreuer: Gerold Barth

Johannes Wollbold: Attribute Exploration of Discrete Temporal Transitions
Betreuer: Reinhard Guthke, Bernhard Ganter

Universität Duisburg-Essen

Nina Schmidt: The serine protease HtrA1 is a novel regulator of cell division and plays an important role in the malignant transformation
Betreuer: Michael Ehrmann

Linda Trübestein: Structural and biochemical characterization of the human serine protease HtrA1
Betreuer: Michael Ehrmann

Juliane Weski: The network of the periplasmic protein quality control in *Escherichia coli*
Betreuer: Michael Ehrmann

Christiane Lütticke: Characterization of the putative periplasmic metalloproteases YfgC and YggG of *E. coli*
Betreuer: Michael Ehrmann

Andre Plagens: Characterisation of the CRISPR/Cas system of the hyperthermophilic Archaeum *Thermoproteus tenax*
Betreuer: Reinhard Hensel

Britta Huber: Mikrobielle Derivatisierung von Bismut im Maus-Modell und *in vitro*-Analysen der Biogenese von Trimethylbismut
Betreuer: Reinhard Hensel

Universität Düsseldorf

Tanja Hanke: Studies on central carbon metabolism and respiration of *Gluconobacter oxydans* 621H
Betreuer: Hermann Sahm

Jens Nickel: Identifizierung und Charakterisierung von Regulatoren der Acyl-CoA-Carboxylasen in *Corynebacterium glutamicum*
Betreuer: Hermann Sahm

Jan van Ooyen: Systemische Analyse des Zitratzyklus in *Corynebacterium glutamicum*
Betreuer: Michael Bott

Han Min Woo: Characterization of the phosphate starvation response of *Corynebacterium glutamicum* using matabolomics
Betreuer: Michael Bott

Meike Baumgart: Novel insights into characteristics, relevance and regulation of corynebacterial aconitase
Betreuer: Michael Bott

Norma Stäbler: Untersuchungen zur Bildung von D-Aminosäuren mit *Corynebacterium glutamicum*
Betreuer: Michael Bott

Universität Erlangen-Nürnberg

Marcus Krüger: Informationsübertragung im Tetrazyklin-Repressor als Grundlage für den allosterischen Induktionsmechanismus
Betreuer: Wolfgang Hillen

Dagmar Goeke: Novel oligopeptides controlling TetR repressor-based gene regulation
Betreuer: Wolfgang Hillen

Britta Beyerlein: Konstruktion von Reporter-Regulator-Komponenten mit dem Transkriptionsinduzierenden Peptid TIP in *Salmonella enterica*
Betreuer: Wolfgang Hillen

Christina Danke: A novel regulatory system for tightly controlled and long-term inducible transgene expression in human cell lines
Betreuer: Wolfgang Hillen

Johannes Amon: Mining the genomes of actinomycetes: Identification of metabolic pathways and regulatory networks
Betreuer: Andreas Burkovski

Kristin Hasselt: Biochemische und molekularbiologische Untersuchungen zu AmtR, dem Stickstoffregulator in *Corynebacterium glutamicum*
Betreuer: Andreas Burkovski

Nadine Rehm: Biochemische und molekularbiologische Untersuchungen zur Verstoffwechselung von Ammonium und Glutamin in *Corynebacterium glutamicum*
Betreuer: Andreas Burkovski

Xandra Grünz: Generierung von Mauslinien zur Doxyzyklin-regulierbaren Expression von Transgenen in B-lymphoiden Zellen
Betreuer: Wolfgang Hillen

Universität Frankfurt am Main

Michael Fritz: Identifizierung und Charakterisierung eines V_O-F_O-Hybridmotors in der Na^+-F_1F_O-ATP-Synthase aus *Acetobacterium woodii*
Betreuer: Volker Müller

Michael Dambeck: Regulation der Genexpression in halophilen Archaea
Betreuer: Jörg Soppa

Britta Meyer: Struktur und Funktion des Ribosomenbiogenese-Faktors Nep1
Betreuer: Karl-Dieter Entian

Universität Freiburg

Christine Kaimer: Two Bacterial DNA translocases coordinate chromosome segregation and cell division
Betreuer: Peter Graumann

Walter Hugo Ramos Vera: Aufklärung und Regulation des autotrophen CO_2-Fixierungsweges in *Thermoproteales*
Betreuer: Georg Fuchs

Hana Smejkalova: Methanol Assimilation in *Methylobacterium extorquens* AM1
Betreuer: Georg Fuchs

Liv Rather: Structure and mechanism of benzoyl-CoA epoxidase BoxAB from *Azoarcus evansii*
Betreuer: Georg Fuchs

Jan Zarzycki: The 3-Hydroxypropionate Bi-Cycle: Missing Enzymes, Distribution, and Various Functions
Betreuer: Georg Fuchs

Universität Gießen

Jasmin Weisel: RNA prozessierende Enzyme und ein BoxS/D-sRNA enthaltender Ribonukleoprotein-Komplex in *Halobacterium salinarum* NRC-1
Betreuerin: Gabriele Klug

Sebastian Metz: Blaulichtabhängige Genregulation in *Rhodobacter sphaeroides:* Untersuchungen zur physiologischen Funktion der Blaulichtrezeptoren AppA, CryB und LOV
Betreuerin: Gabriele Klug

Sobha Rani Basineni: Processing and turn-over of the small non-coding RNA OxyS and post-transcriptional regulation of RpoS levels by the sRNAs OxyS and DsrR and the Hfq protein in *E. coli*
Betreuerin: Gabriele Klug

Aaron Nuss: Alternative sigma factors in the photooxidative stress response of *Rhodobacter sphaeroides*
Betreuerin: Gabriele Klug

Universität Göttingen

Florian Schulze: The role of Pcl5p and Pcl7p in the Gcn4 stability regulation of *Saccharomyces cerevisiae*
Betreuer: Gerhard Braus

Nicole Rachfall: Translational control of the ribosomal protein Asc1p7Cpc2p in *Saccharomyces cerevisiae*
Betreuer: Gerhard Braus

Yasmine Bernhards: Untersuchung der Fruchtkörperentwicklung bei dem Hyphenpilz *Sordaria macrospora*
Betreuerin: Stefanie Pöggeler

Lope Florez Weidinger: Systems biology in *Bacillus subtilis*: databases for gene function and software tools for pathway discovery
Betreuer: Jörg Stülke

Britta Herzog: Metabolic and developmental function of the transcription factor Gcn4p of *Saccharomyces cerevisiae*
Betreuer: Gerhard Braus

Daniela Justa-Schuch: Regulation of septum formation by RHO4 GTPase signalling in *Neurospora crassa*
Betreuer: Gerhard Braus

Volker Klix: Analyse des Kreuzungstyp-Locus des filamentösen Ascomyceten *Sordaria macrospora*
Betreuerin: Stefanie Pöggeler

Nico Pietack: Investigation of glycolysis in *Bacillus subtilis*
Betreuer: Jörg Stülke

Michael Rachinger: Stammdesign in *Bacillus licheniformis*
Betreuer: Rolf Daniel

Haitham Saad Eddin: Investigation of molybdenum iron protein expression and activity in *Wollinella succinogenes*
Betreuer: Oliver Einsle

Sebastian Schmidl: Pathogenicity of a minimal organism: Role of protein phosphorylation in *Mycoplasma pneumoniae*
Betreuer: Jörg Stülke

Marco Schwarzer: Physiologische Untersuchungen zur Regulation des Aminosäure-Stoffwechsels von *Bacillus licheniformis* DSM 13
Betreuer: Wolfgang Liebl

Antje Wollherr: Komparative Genomanalyse zur Stammoptimierung produktionsnaher *Bacillus*-Stämme
Betreuer: Wolfgang Liebl

Universität Greifswald

Jessica Rehdorf: Discovery of novel Baeyer-Villiger monooxygenases and their application in organic synthesis
Betreuer: Uwe Bornscheuer

Helge Jochens: Entwicklung neuer Konzepte für das Protein-Engineering am Beispiel von Enzymen mit a/ß-Hydrolasefaltung
Betreuer: Uwe Bornscheuer

Matthias Höhne: Synthese optisch aktiver Amine mit Transaminasen
Betreuer: Uwe Bornscheuer

Kristian Geitner: Untersuchungen zur Vergrößerung des Reaktionsmaßstabes am Beispiel einer enantioselektiven enzymatischen Baeyer-Villiger-Oxidation
Betreuer: Uwe Bornscheuer

Sebastian Bartsch: Proteindesign einer Phenylalanin-Ammoniak-Lyase: Veränderung des Substratspektrums und Untersuchungen zum Mechanismus
Betreuer: Uwe Bornscheuer

Anna Schließmann: Protein engineering of a *Pseudomonas fluorescens* esterase: Alteration of substrate specificity and stereoselectivity
Betreuer: Uwe Bornscheuer

Kristina Hempel: Entwicklung und Anwendung von Massenspektrometrie-basierten Methoden zur Identifizierung und Quantifizierung von Zelloberflächen-assoziierten Proteinen des humanpathogenen Bakteriums *Staphylococcus aureus*
Betreuer: Michel Hecker

Stephan Fuchs: Physiologische und molekularbiologische Untersuchungen zur Adaptation von *Staphylococcus aureus* an anaerobe Bedingungen
Betreuer: Michel Hecker

Universität Halle-Wittenberg

Simone Hahn: Analyse des molekularen Mechanismus der Aktivität des Typ-III-Effektors AvrBs3 aus *Xanthomonas campestris* pv. *Vesicatoria*
Betreuer: Dietrich Nies

Felix Berthelmann: Untersuchungen zur intrazellulären Verteilung der Komponenten des Tat-Systems in *Escherichia coli*
Betreuer: Thomas Brüser

Daniel Thieme: Alte Faktoren mit neuen Funktionen – Kupferentgiftung in *Escherichia coli*
Betreuer: Dietrich Nies

Annegret Boch: Funktionelle Charakterisierung von ZIP-Transportproteinen aus *Schizosaccharomyces pombe* und *Arabidopsis*
Betreuer: Dietrich Nies

Nadine Taudte: Eisen und Mangan: Wechselwirkungen und Homöostase in *Escherichia coli* mit dem Schwerpunkt ZupT
Betreuer: Gregor Grass

Universität Hamburg

Catur Sriherwanto: Studies on the Solid State Fermentation of Cassava Bagasse for Animal Feed
Betreuer: Bernward Bisping

Tri Erny Dyahningtyas: Potency of Chitosan as a Bioactive Edible Coating for Preservation of Meat of Common Shrimps (*Crangon crangon*)
Betreuer: Bernward Bisping

Universität Hannover

Patrick Stolle: Nachweis eines neuartigen Tyrosyl-$Mn^{III}Mn^{III}$ gekoppelten Spinsystems in der Mn-Ribonucleotid-Reduktase von *Corynebacterium ammoniagenes* und *Corynebacterium glutamicum*
Betreuer: Georg Auling

Medizinische Hochschule Hannover

Claudia Dürr: Negative regulatory mechanisms of intestinal epithelial innate immune activation
Betreuer: Mathias Hornef

Katrin Janik: Functional characterization of the chlamydial effector protein CT166 and studies revealing the influence of *in vitro* culture conditions onto *in vivo* virulence of *Chlamydophila pneumoniae* in a mouse infection model
Betreuer: Andreas Klos

Anna Leybo: Interaction of Helicobacter flagellins with human and murine cells: TLR5-independent signalling and role of alternative innate immune receptors
Betreuerin: Christine Josenhans

Claudia Moccia: Mechanisms of *Helicobacter pylori* to generate genetic variability during chronic infection
Betreuer: Sebastian Suerbaum

Universität Jena

Sandra Kreher: Anaerobe O-Demethylierung in *Acetobacterium dehalogenans*: Untersuchungen zu den etherspaltenden Methyltransferasen I – Zinkbindung und Substratspezifität
Betreuerin: Gabriele Diekert

Lidan Ye: Studies on the microbial halogen cycle: Reactions of fungal peroxidases and bacterial reductive dehalogenases
Betreuerin: Gabriele Diekert

Theodore Asiimwe: Molecular characterization of a fungal aldehyde dehydrogenase in the *Tricholoma vaccinum*-spruce ectomycorrhiza
Betreuerin: Erika Kothe

Daniela Siegel: Black slate – surface alteration due to fungal activity
Betreuerin: Erika Kothe

Judith Behnsen: Interaktion von *Aspergillus fumigatus* mit dem Immunsystem unter besonderer Berücksichtigung des Komplementsystems
Betreuer: Axel Brakhage

Christian Fleck: Untersuchungen zur Redundanz im Pilzmetabolismus am Beispiel von *Aspergillus nidulans*, *Aspergillus fumigatus* und *Saccharomyces cerevisiae*
Betreuer: Axel Brakhage, Matthias Brock

Marcel Thön: Redoxregulierung des CCAAT-bindenden Komplexes (AnCF) von *Aspergillus nidulans*
Betreuer: Axel Brakhage

Sebastian Bergmann: Regulation von Sekundärmetabolit-Genclustern in *Aspergillus nidulans*
Betreuer: Axel Brakhage

Martin Richter: Biochemische Charakterisierung multifunktioneller Oxygenasen aus mikrobiellen Sekundärmetabolit-Biosynthesewegen
Betreuer: Christian Hertweck

Daniela Albrecht: Integration von Transkriptom- und Proteomdaten human-pathogener Pilze
Betreuer: Reinhard Guthke, Axel Brakhage

Benjamin Busch: Functional analysis of the non-canonical biosynthetic pathways of aureothin and rhizoxin
Betreuer: Christian Hertweck

Michelle Unger: Molekularbiologische Grundlage der Cervimycin-Biosynthese
Betreuer: Christian Hertweck

André Schmidt: Die Stressantwort von *Aspergillus fumigatus* auf Eisenmangel: Vergleichende Proteomanalysen und die Charakterisierung Eisen-regulierter Proteine
Betreuer: Axel Brakhage

Katharina Gropp: Auseinandersetzung zwischen der humanpathogenen Hefe *Candida albicans* und dem angeborenen Immunsystem des Wirts
Betreuer: Peter Zipfel

Mirko Ludwig: Funktionelle Charakterisierung des Faktor H verwandten Proteins CFHR3
Betreuer: Peter Zipfel

Technische Universität Kaiserslautern

Ilka Zerfaß: PBP2x-Mutationen in *Streptococcus pneumoniae*: Auswirkung auf β-Laktam-Resistenz und Zellphysiologie
Betreuerinnen: Regine Hakenbeck, Dalia Denapaite

Yvonne Schähle: Genomische Diversität und Evolution von Virulenzdeterminanten in *Streptococcus* spp.
Betreuerinnen: Regine Hakenbeck, Dalia Denapaite

Katja Todorova: β-Lactam-Resistenz in *Streptococcus* spp.: Eine neue Resistenzderminante murE
Betreuer: Regine Hakenbeck, Patrick Maurer

Universität Karlsruhe, KIT

Christian Kastner: Regulation der Sporenkeimung und des Sekundärmetabolismus durch Licht in *Aspergillus nidulans*
Betreuer: Reinhard Fischer

Uta Deiting: Klonierung und heterologe Expression der Nitrilhydratase aus *Rhodococcus* spec.
Betreuer: Reinhard Fischer

Sylvia Müller: Untersuchung des Lichtregulatorkomplexes in *Aspergillus nidulans*
Betreuer: Reinhard Fischer

Nicole Helber: Analysis of the sugar transport and metabolism in the arbuscular mycorrhizal symbiosis
Betreuerin: Natalia Requena

Gyu-Sung Cho: Determination and expression analysis of functional genes in *Lactobacillus plantarum*
Betreuer: Charles Franz

Universität Kiel

Nancy Weiland: Screening for quorum sensing interfering compounds using a metagenomic approach: A novel strategy to prevent biofilm formation
Betreuerin: Ruth Schmitz-Streit

Anja Büttner: Transcriptional responses upon oxygen exposure in the methanogenic archaeon *Methanosarcina mazei* strain Gö1
Betreuerin: Ruth Schmitz-Streit

Leibniz-Institut für Meereswissenschaften IFM-GEOMAR, Kiel

Marcus Tank: Ecological and phylogenetic studies on purple sulfur bacteria based on their *pufLM* genes of the photosynthetic reaction center
Betreuer: Johannes F. Imhoff

Universität Köln

Tina Radespiel: Transport von organischen Säuren als Vorstufen der Aminosäurebiosynthese in *Corynebacterium glutamicum*
Betreuer: Reinhard Krämer

Tobias Mohrbach: Untersuchungen zum Export von Tryptophan in *Escherichia coli*
Betreuer: Reinhard Krämer

Jens Novak: Regulation der Glucosylglycerol-Phosphat-Synthase aus dem Cyanobacterium *Synechocystis* sp. PCC 6803
Betreuer: Reinhard Krämer

Inga Wadenpohl: The cell division protein FtsL of *Bacillus subtilis* and its proteolysis
Betreuer: Reinhard Krämer

Universität Konstanz

Nicolai Müller: Reversed electron transport in syntrophic degradation of glucose, butyrate, and ethanol
Betreuer: Bernhard Schink

Julia Roeder: Syntrophe Oxidation der Fettsäure Acetat und den biogenen Amins Cadaverin (1,5-Diaminopentan) durch definierte methanogene Kokulturen
Betreuer: Bernhard Schink

Felix ten Brink: Acetylene hydratase from *Pelobacter acetylenicus* – functional studies on a gas-processing tungsten, iron-sulfur enzyme by site directed mutagenesis and crystallography
Betreuer: Peter M. H. Kroneck

Sonja Weinitschke: New intermediates, pathways, enzymes and genes in the microbial metabolism of organosulfonates
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Redaktion:
Dr. Christine Schreiber
Redaktion BIOspektrum
Spektrum Akademischer Verlag
Springer-Verlag GmbH
Tiergartenstraße 17
D-69121 Heidelberg
Tel.: +49 (0)6221 - 487 8043
Fax: +49 (0)6221 - 487 68043
christine.schreiber@springer.com
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Verlag:
Spektrum Akademischer Verlag
Springer-Verlag GmbH
Tiergartenstraße 17
D-69121 Heidelberg
Tel.: +49 (0)6221 - 487 8043
Fax: +49 (0)6221 - 487 68043
Spektrum Akademischer Verlag GmbH ist ein Imprint von Springer Science+Business Media

Geschäftsführer:
Derk Haank, Martin Mos, Peter Hendriks

Anzeigen:
top-ad Bernd Beutel
Hammelbächer Str. 30
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Tel.: +49 (0)6201-290 92 0
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Dieses unterhaltsame Buch bietet einen ungewöhnlichen und faszinierenden Blick auf die Beziehung zwischen Menschen und Bakterien. *„Möge Dr. Koneman für das, was er für Euch, die Bakterien getan hat, glücklich und zufrieden leben, ohne sich je eine Infektion einzufangen. Für uns, seine Mitmenschen, hat er das wahrscheinlich witzigste und unterhaltsamste Buch verfasst, das je über die faszinierende Welt der Mikroorganismen geschrieben wurde."*
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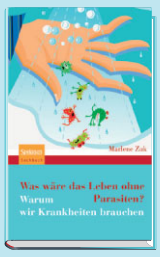

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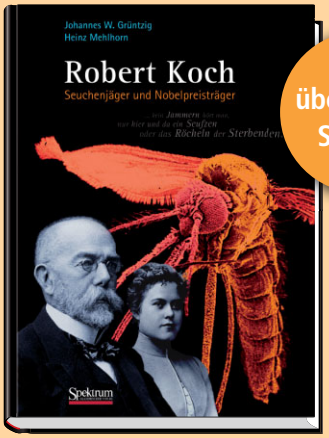

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Johannes W. Grüntzig und Heinz Mehlhorn, zwei erfolgreiche Sachbuchautoren, schlagen in dieser profunden Biografie den Bogen von den Pest-Epidemien des 14. Jh. bis hin zum Zeitalter der deutschen Kolonien, in dem Robert Koch die wissenschaftliche Weltbühne betritt. Seine bahnbrechenden Forschungen und seine gefahrvollen Expeditionen nach Ägypten, Indien, Neuguinea und Afrika werden mit erstmals veröffentlichten Quellen, Auszügen aus Privatbriefen und zahlreichen farbigen Bildern dokumentiert. So entsteht ein lebendiges Zeitzeugnis zum Leben von Robert Koch, das auch die Reaktionen des bigotten gesellschaftlichen Milieus Berlins auf Kochs zweite Ehe mit einer deutlich jüngeren Frau nicht ausblendet. Erstmals wird hier auch die Biografie von Hedwig Koch-Freiberg geschrieben, einer starken, emanzipierten Frau an der Seite Robert Kochs, die ihn forderte und förderte. **Eine überaus spannende Biografie eines der bedeutendsten deutschen Wissenschaftler!**

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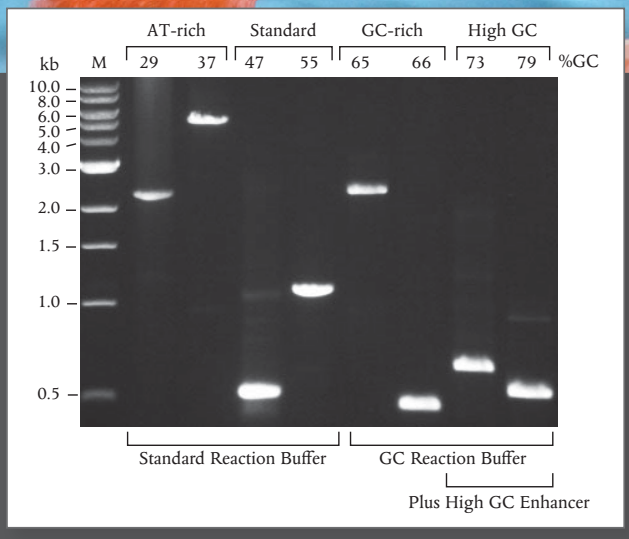

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