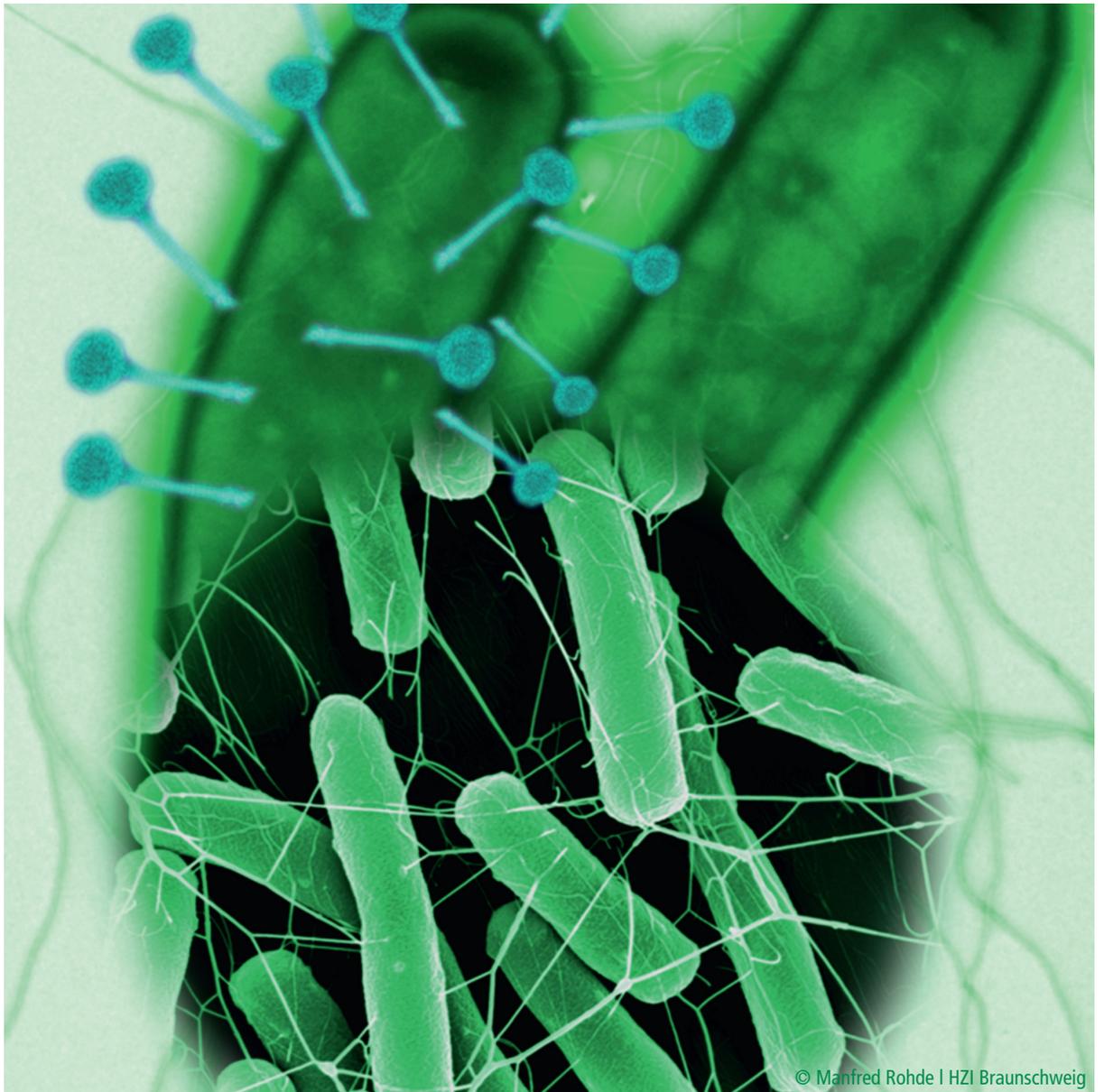


# ANNUAL CONFERENCE

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# 2018



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**15-18 APRIL 2018** WOLFSBURG | GERMANY

**ABSTRACTS**



H. König, G. Uden, J. Fröhlich  
**Biology of Microorganisms on Grapes,  
in Must and in Wine**

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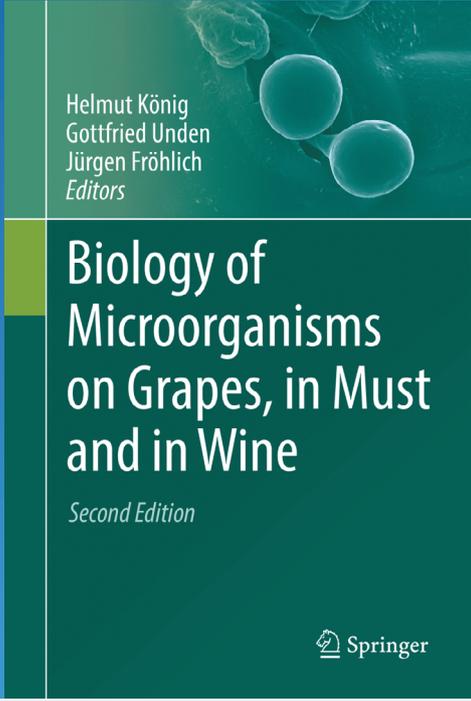
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Helmut König  
Gottfried Uden  
Jürgen Fröhlich  
*Editors*

## Biology of Microorganisms on Grapes, in Must and in Wine

*Second Edition*

 Springer

# Highlight

- This is the first book with a clear and exclusive focus on the different biological aspects of wine-related microbes
- The editors and authors are well-known experts, researchers and teachers in wine science, oenology and viticulture from different wine-growing countries
- Helmut König, Institute of Microbiology and Wine Research, Johannes Gutenberg University of Mainz, is involved in the International Wine Education Program of the Global Network "Great Wine Capitals"

The second edition of the book begins with the description of the diversity of wine-related microorganisms, followed by an outline of their primary and energy metabolism. Subsequently, important aspects of the secondary metabolism are dealt with, since these activities have an impact on wine quality and off-flavour formation. Then chapters about stimulating and inhibitory growth factors follow. This knowledge is helpful for the growth management of different microbial species. The next chapters focus on the application of the consolidated findings of molecular biology and regulation the functioning of regulatory cellular networks, leading to a better understanding of the phenotypic behaviour of the microbes in general and especially of the starter cultures as well as of stimulatory and inhibitory cell-cell interactions during wine making. In the last part of the book, a compilation of modern methods complete the understanding of microbial processes during the conversion of must to wine. This broad range of topics about the biology of the microbes involved in the vinification process could be provided in one book only because of the input of many experts from different wine-growing countries.

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## Special Groups

## Oral/Poster ID

Regulation and Signal Transduction.....	RSV-FG
Environmental Microbiology .....	EMV-FG
Water and Sewage.....	WAV-FG
Symbiotic Interactions .....	SIV-FG
Fungal Biology and Biotechnology .....	FBV-FG
Identification and Systematics .....	ISV-FG
Food Microbiology .....	FMV-FG
Microbial Pathogenicity.....	MPV-FG

## Abstract Topics

Archaea .....	ARV; ARP
Biochemistry .....	BCV; BCP
Biotechnology.....	BTV; BTP
Environmental Microbiology and Ecology.....	EMV; EMP
Eukaryotic Microbes .....	EuP
Food Microbiology .....	FMV; FMP
Fungal Biology.....	FBV; FBP
Gene Regulation .....	GRV; GRP
Infection Biology .....	IBV; IBP
Membranes and Transport .....	MTV; MTP
Metabolomics .....	STMV; MetP
Microbial Cell Biology .....	MCBV; MCBP
Microbial Diversity and Evolution.....	MD(M)V; MDEP
Microbiota.....	MDMV; MBP
Secondary Metabolomics .....	BSMV; SMP
Systems Biology .....	STMV; SBP

## Opening Lecture 15 April 2018 • 15:15–16:00

### IL01

#### Systems Ecology of Microbiomes

P. Wilmes\*<sup>1</sup>

<sup>1</sup>University of Luxembourg, Luxembourg Centre for Systems Biomedicine, Esch-sur-Alzette, Luxembourg

Microbiomes represent highly dynamic and heterogeneous systems, which exhibit tremendous complexity at all levels. High-throughput molecular measurements are allowing unprecedented insights into microbiome composition, physiology, ecology and evolution *in situ*. Our group has pioneered a comprehensive toolbox comprising wet- and dry-lab methodologies to enable systematic measurements of microbiomes over space and time, the integration and analysis of the resulting multi-omic data, and experimental models for rapidly testing hypotheses *in vitro*. The application of these methods has culminated in essential new insights regarding niche ecology and resulting lifestyle strategies of specific microbial populations *in situ* as well as the discovery of keystone functional genes. In the context of the human gut microbiome, it has highlighted the disruption of ecosystem services in the context of human disease (type 1 diabetes). To study the ramifications of such perturbations, a microfluidics-based model of the gastrointestinal human-microbe interface has been developed called HuMiX. HuMiX allows the probing of the molecular interactions between human and microbial cell contingents and has highlighted the importance of microbial metabolism in governing human cell physiology in the gut. The developed toolbox is also particularly pertinent for the exploration of the vast expanse of unknown features in microbiomes, ranging from uncharacterised organisms to proteins of unknown function and small molecules. Thereby, Microbial Systems Ecology offers great potential for the discovery of new biological functions in the future.

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## VAAM Honorary Award 15 April 2018 • 16:05–16:40

### IL15

#### A tale of a tail: how bacteria assemble their motility organelle

M. Erhardt\*<sup>1</sup>

<sup>1</sup>Humboldt-Universität zu Berlin, Bakterienphysiologie, Berlin, Germany

Life has evolved diverse protein machines and bacteria provide many fascinating examples.

Flagella are the primary organelles of motility in bacteria and enable movement towards nutrients and away from harmful substances, a process known as chemotaxis. Flagella-mediated motility is also important for many pathogens – including *Salmonella enterica* – and allows the bacteria to reach the site of infection, facilitate host-pathogen interactions, and promote biofilm formation.

The bacterial flagellum is a remarkable complex nanomachine. It is several times longer than a bacterial cell body and made through self-assembly of a few tens of thousands individual building blocks. The assembly and function of the flagellum and the evolutionary related virulence-associated injectisome relies on protein export via a type III secretion system (T3SS). The T3SS utilizes the proton motive force as the primary energy source to

translocate substrate proteins across the inner membrane. Exported substrates travel through a narrow channel within the structure and self-assemble at the tip of the growing flagellum.

During the talk, recent advances in our understanding of the self-assembly and protein export mechanisms of this fascinating nanomachine will be discussed.

---

## Plenary Lecture Microbial Reprogramming and Interaction 15 April 2018 • 17:30–19:00

### IL02

#### Bacterial microcompartments and their redesign for biotechnological purposes

M. J. Warren\*<sup>1</sup>

<sup>1</sup>University of Kent, School of Biosciences, Canterbury, United Kingdom

One of the defining differences between prokaryotes and eukaryotes is the presence of specific organelles and subcellular structures in the cytoplasm of the latter. The advantages of compartmentalisation are multiple and include an ability to provide a physical barrier for part of the cell allowing separation from the cytoplasmic milieu, the generation of a specific microenvironment and an address to which material can be sent. It is therefore often quite a surprise for many to learn that a broad range of bacteria also have specialised cytoplasmic structures or organelles, the most common of which are called bacterial microcompartments (BMCs). Although BMCs are not lipid-bound they are however surrounded by a semi-permeable proteinaceous shell to generate polygonal structures that encase a specific metabolic process. There are two basic types of BMCs reflecting their participation in anabolic (carboxysomes) or catabolic (metabolosomes) processes. The anabolic carboxysomes are involved in carbon fixation, utilising an encapsulated carbonic anhydrase and ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO). The catabolic metabolosomes are often associated with the breakdown of mucosal-derived metabolites such as fucose, 1,2-propanediol, choline and ethanolamine and contain multistep pathways that involve the conversion of the metabolite into an aldehyde and its subsequent disproportionation into an alcohol and acid. The best studied metabolosome is the propanediol utilisation (Pdu) BMC. Using a multidisciplinary approach we have redesigned the Pdu BMC and demonstrated how new functionality can be engineered within the compartment. Our deconstruction of the BMC into its component pieces has also allowed us to develop ways of generating cytoplasmic scaffolds onto which metabolic enzymes can be attached. Collectively these approaches can be used to generate improved metabolic activity within the cell.

---

### IL03

#### Host Microbiome Interactions in Health and Disease

E. Elinav\*<sup>1</sup>

<sup>1</sup>Weizman Institute of Science, Department of Immunology, Rehovot, Israel

The mammalian intestine contains trillions of microbes, a community that is dominated by members of the domain Bacteria but also includes members of Archaea, Eukarya, and viruses. The vast repertoire of this microbiome functions in ways that benefit the host. The mucosal immune system

co-evolves with the microbiota beginning at birth, acquiring the capacity to tolerate components of the community while maintaining the capacity to respond to invading pathogens. The gut microbiota is shaped and regulated by multiple factors including our genomic composition, the local intestinal niche and multiple environmental factors including our nutritional repertoire and bio-geographical location. Moreover, it has been recently highlighted that dysregulation of these genetic or environmental factors leads to aberrant host-microbiome interactions, ultimately predisposing to pathologies ranging from chronic inflammation, obesity, the metabolic syndrome and even cancer. We have identified various possible mechanisms participating in the reciprocal regulation between the host and the intestinal microbial ecosystem, and demonstrate that disruption of these factors, in mice and humans, lead to dysbiosis and susceptibility to common multi-factorial disease. Understanding the molecular basis of host-microbiome interactions may lead to development of new microbiome-targeting treatments.

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#### IL04

##### Sensory and regulatory RNSs shaping host-pathogen interactions of *Yersinia*

P. Dersch<sup>\*1</sup>

<sup>1</sup>Helmholtz Centre for Infection Research (HZI), Department Molecular Infection Biology, Braunschweig, Germany

No abstract has been submitted.

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#### Short Lecture

##### Biotechnology 1

16 April 2018 • 08:00–10:00

#### BTV01

##### Directing the carbon flow for optimal production of either single cell oil or gluconic acid in *Cryptococcus podzolicus* and *Trichosporon porosum*

X. Qian<sup>1</sup>, M. Jiang<sup>2</sup>, K. Ochsenreither<sup>\*1</sup>

<sup>1</sup>Karlsruhe Institute of Technology, Technical Biology, Karlsruhe, Germany

<sup>2</sup>Nanjing Tech University, College of Biotechnology and Pharmaceutical Engineering, Nanjing, China

**Introduction:** The newly identified single cell oil (SCO) producing yeast species *Cryptococcus podzolicus* and *Trichosporon porosum* were isolated from beat bog samples. During fermentation under SCO producing conditions gluconic acid was formed extracellularly by both isolates as a second product in considerable amounts (Schulze et al. 2014) and is therefore competing for substrate availability.

**Objectives:** Due to the simultaneous formation of both products and the resulting competition for carbon source, yields and concentrations of both products are lowered. The objectives of this study are therefore to identify parameters which inhibit the synthesis of one product in order to optimize the production of the second product in both yeast species.

**Materials & methods:** Both yeast species were cultivated in a mineral-salt medium using small-scale bioreactors. Different nitrogen sources as well as varying glucose concentrations, addition of vitamins and enhanced aeration were tested for their influence on lipid production and composition as well as gluconic acid production. Products and substrate concentrations were determined via GC and HPLC.

**Results:** By using a complex nitrogen source, e.g. peptone, instead of ammonium-salts, single cell oil production is favored and gluconic acid production is delayed. Using this approach production phases of both products can be clearly separated for both yeast species. Furthermore, high glucose concentrations and high aeration rates have an impact on gluconic acid production.

**Conclusion:** By changing process parameters and medium composition, carbon flow can be directed to one of the products in order to optimize yield and final product concentration.

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#### BTV02

##### Cyclic triterpenoid production with tailored

##### *Saccharomyces cerevisiae*

B. E. Ebert<sup>\*1</sup>, E. Czarnotta<sup>1</sup>, K. Walter<sup>1</sup>, A. Lewandowski<sup>2</sup>, C. Knuf<sup>3</sup>, S. A. Jacobsen<sup>3</sup>, T. Polakowski<sup>2</sup>, J. Maury<sup>3</sup>, C. Lang<sup>2</sup>, J. Förster<sup>3</sup>, L. M. Blank<sup>1</sup>

<sup>1</sup>iAMB -Institute of Applied Microbiology, ABBt - Aachen Biology and Biotechnology, RWTH Aachen University, Aachen, Germany

<sup>2</sup>Organobalance GmbH, Berlin, Germany

<sup>3</sup>Novo Nordisk Foundation Center for Biosustainability, Lyngby, Denmark

Triterpenoids are secondary plant metabolites derived from squalene and consist of six isoprene units (C<sub>30</sub>). Many of them or their synthetic derivatives are currently being investigated as medicinal products for various diseases. The cyclic triterpenoid betulinic acid is of special interest for the pharmaceutical and nutritional industry as it has antiretroviral, antimalarial, and anti-inflammatory properties and has potential as an anticancer agent. Despite their obvious industrial potential, the application is often hindered by their low abundance in natural plant sources. This poses challenges in a biosustainable production of such compounds due to wasteful and costly product purification.

Here, we present a novel biotechnological process for the production of betulinic acid using tailored *Saccharomyces cerevisiae* strains. The multi-scale optimization of this microbial process included:

- pathway engineering by determination of optimal gene combination and dosage,
- compartment engineering to increase the reaction space of the betulinic acid pathway, and
- strain engineering by implementation of different push, pull and block strategies.

In parallel we developed the fermentation process and were able to boost the performance of the engineered yeast by optimization of medium composition, cultivation conditions, carbon source and mode of fermentation operation in lab scale bioreactors. Product purification was achieved by a one-step extraction with acetone.

The final process was evaluated in terms of economic and ecological efficiency and rated to be competitive with existing plant extraction procedures with potential for further performance improvement.

### BTV03

#### Type and capacity of glucose transport influences succinate yield and productivity in two-phase cultivations of *E. coli* K 12

L. Kyselova<sup>1</sup>, D. Kreitmayer<sup>1</sup>, A. Kremling<sup>1</sup>, K. Bettenbrock<sup>\*1</sup>  
<sup>1</sup>Max Planck Institute for Dynamics of Complex Technical Systems, Experimental Systemsbiology, Magdeburg, Germany

Succinate is an important bio-based product. Typical strategies to enhance succinate production in *E. coli* are k.o. of alternative fermentation products and overexpression of enzymes catalyzing reactions from pyruvate or PEP to oxaloacetate. Another important k.o. candidate is the Glc-PTS, the major glucose uptake system in *E. coli*. The main drawback is, that the resulting mutants are strongly impaired in anaerobic growth, demanding a two-stage production strategy. We constructed a set of strains all unable to produce acetate, lactate and ethanol, but differing in glucose uptake. The initial strain, KBM151101, has the native Glc-PTS. This strain was compared to an isogenic ptsG- strain, SB2, and to a ptsG- strain, expressing the glucose facilitator, Gif, from *Z. mobilis*, KBM1673. These strains were analyzed in two-stage cultivations with respect to growth, succinate production and production of further by-products. The experimental analyses were complemented by flux-balance analysis. The results demonstrate that k.o. of ptsG drastically enhanced succinate yield but at the same time resulted in very low productivity, due to low glucose uptake. Expression of the PTS-independent Gif in this strain, unexpectedly reduced yield as well as productivity. Based on FBA data, we hypothesized that fluxes from phosphoenolpyruvate to oxaloacetate catalyzed by the ATP gaining PEP carboxykinase have to be enhanced in order to improve this strain. The data hint to a significant expression of this enzyme in SB2, that is reduced by enhancing glucose uptake. This was experimentally verified. The data show that careful analysis of the metabolic network and a fine tuning of fluxes and gene expression in central metabolism is necessary to obtain an optimal succinate production strain.

### BTV04

#### Identification of key enzymes for bioalcohol formation in the thermophilic bacterium *Thermoanaerobacter* sp. Strain X514

L. Hitschler<sup>\*1</sup>, J. Roth<sup>1</sup>, M. Basen<sup>1</sup>  
<sup>1</sup>Goethe University Frankfurt, Institute of Molecular Biosciences, Molecular Microbiology & Bioenergetics, Frankfurt a. M., Germany

**Introduction:** *Thermoanaerobacter* species ferment sugars to ethanol, acetate, hydrogen and lactate, with ethanol often being the main product [1]. In addition, we recently found that *Thermoanaerobacter* sp. Strain X514 reduced different carboxylic acids to the corresponding alcohols with sugars as electron donor, as described for a genetically modified strain of the hyperthermophilic archaeon *Pyrococcus furiosus* [2]. The genome of *Thermoanaerobacter* sp. Strain X514 harbors nine genes encoding putative alcohol dehydrogenases and two genes encoding putative aldehyde dehydrogenases [3].

**Objectives:** We aimed to identify, purify and characterize the abundant alcohol dehydrogenase (Adh) responsible involved in bio-alcohol formation in *Thermoanaerobacter* sp. Strain X514.

**Methods:** Strain X514 was grown on glucose, and NADH- and NADPH-dependent acetaldehyde reduction activities were purified from the cell-free extract by a classical sequence of chromatographic steps, identified by MALDI-TOF MS and biochemically characterized.

**Results:** A secondary-alcohol dehydrogenase (AdhB) was purified as the most abundant alcohol dehydrogenase. AdhB displayed high catalytic activities for NADPH-dependent (rather than NADH-dependent) aldehyde and ketone reduction as well as for oxidation of secondary alcohols.

**Conclusion:** The purified NADPH-dependent alcohol dehydrogenase (AdhB) is likely responsible for acetaldehyde reduction to ethanol in *Thermoanaerobacter* sp. Strain X514, and potentially involved in the reduction of other aldehydes. Currently, we are investigating enzymes responsible for the reduction of Acetyl-CoA and of NADP+.

#### References:

- [1] Verbeke TJ *et al.* (2013), *PLoS One* **8**: e59362
- [2] Basen M *et al.* (2014), *PNAS*, **111**: 17618–17623
- [3] Hemme CL *et al.* (2011), *Appl Environ Microbiol* **77**: 7998-8008

### BTV05

#### Improving the production of ethylmalonyl-CoA pathway-derived compounds from methanol

L. Pöschel<sup>\*1</sup>, E. Gehr<sup>1</sup>, F. Sonntag<sup>1</sup>, J. Schrader<sup>1</sup>, L. Schada von Borzyskowski<sup>2</sup>, T. J. Erb<sup>2</sup>, J. A. Vorholt<sup>3</sup>, M. Buchhaupt<sup>1</sup>  
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<sup>2</sup>Max Planck Institute for Terrestrial Microbiology, Marburg, Germany  
<sup>3</sup>ETH Zurich, Institute of Microbiology, Zurich, Switzerland

Methanol is a promising alternate feedstock for biotechnological processes. Its use lowers the cost of downstream processing due to the application in inexpensive minimal media and it does not directly compete with food production. The  $\alpha$ -proteobacterium *Methylobacterium extorquens* as an extensively studied model organism for methylotrophy serves as promising production host for valuable compounds directly from methanol. The ethylmalonyl-CoA pathway (EMCP) as part of its primary metabolism harbors several CoA-esters such as ethylmalonyl-, methylsuccinyl- or mesaconyl-CoA whose free dicarboxylic acid derivatives potentially present promising synthons for chemical industry. The release of these compounds was reached in the past by expression of an unspecific CoA-thioesterase. However, the EMCP cannot be easily manipulated to enhance product levels, since it has an essential function for growth on C1 and C2 compounds. A knockout of any EMCP gene results in a lethal growth phenotype. Therefore, an EMCP bypass, namely a glyoxylate shunt, was introduced to make the EMCP facultative as an anaplerotic pathway and accessible for biotechnological applications. Furthermore, dicarboxylic acid re-import strongly limits the process efficiency. By using the cytotoxic substrate 2,2-difluorosuccinic acid (DFS) we could isolate mutants with a diminished acid uptake rate and identify an involved acid import protein. We could further show the inhibitory effect of DFS on the EMCP with complementation and growth experiments, as well as the consequent influence on EMCP-derived acid production. In summary we demonstrated different ways to enhance the overall dicarboxylic acid production with *M. extorquens* from methanol by modulating the primary carbon flux and by reducing the product re-uptake.

## BTV06

### Carbon-efficient feedstock conversion for the production of bio-succinate and further reduced compounds

P. Weyrauch<sup>1</sup>, M. Roger<sup>2</sup>, A. Arnold<sup>1</sup>, W. Gabrielli<sup>3</sup>, F. Sargent<sup>2</sup>, F. Brown<sup>1</sup>

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Succinate is widely recognised as a platform chemical (production at 30,000 - 50,000 tonnes/annum) which has already received attention within the context of industrial biotechnology as a model for sustainable manufacturing.

However, the yield from traditional sugar-based bioprocesses for succinate production is constrained because a significant amount of carbon present in the bioprocess feedstock is lost as gaseous CO<sub>2</sub>. In order to maximise carbon use efficiency, additional exogenous energy is required in the form of hydrogen gas which can be supplied as a co-substrate alongside glucose and emerging CO<sub>2</sub>.

As part of a previous investigation (Roger *et al.*, *Curr. Biol.*, 2018) we demonstrated efficient cellular processing of both CO<sub>2</sub> and hydrogen gas to generate formate via the reverse reaction of a formate: hydrogen lyase. Furthermore, building on previous work on *Corynebacterium glutamicum* (Litsanov *et al.*, *Appl. Environ. Microbiol.*, 2012) we have demonstrated an efficient glucose to succinate fermentation process in *Escherichia coli*, which when supplemented with formate, can co-utilise CO<sub>2</sub> as a tandem feedstock with minimisation of oxidised by-products (e.g. pyruvate) formation. Combining these important results we seek to advance our technology to deliver an enhanced bioprocess towards the production of succinate with carbon conversion efficiency beyond that possible with glucose and CO<sub>2</sub> alone.

Using this as a model system, we believe this approach is deployable on a wider scale to minimise and eliminate CO<sub>2</sub> arising in other bio-based chemical manufacturing applications. This will provide an opportunity to increase CO<sub>2</sub> abatement even further compared to conventional sugar/biomass derived "green" fermentation processes.

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## BTV07

### Increasing the Space-Time Yield in Lactic Acid Production by Use of Biofilms

L. Cuny<sup>1</sup>, A. Hille-Reichel<sup>2</sup>, P. Oedman<sup>3</sup>, H. Horn<sup>4</sup>

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The application of biofilm technology for the production of both low-cost and valuable compounds allows the white biotechnology to benefit from the advantages of biofilms compared to suspended cultures. In this study, we focus on the production of optically pure lactic acid. Recently, the interest in lactic acid has greatly increased, especially due to the synthesis of polylactic acid, a biodegradable and biocompatible polymer. However, the high costs of lactic acid are responsible for its low competitiveness to petroleum-derived plastics.

For the production of lactic acid, we cultivate a monoseptic biofilm consisting of *Lactobacillus* bacteria with a strong preference for planktonic growth (provided by BASF SE). Biofilm growth is achieved in a glass tube reactor. The biofilm system can be cultivated in a continuous mode and kept monoseptically for at least 3 weeks. It was shown that higher cell densities lead to a significantly increased space-time product yield compared to the planktonic culture. The productivity reached a constant value after only 3 days of cultivation and no decline was observed during the rest of the cultivation period, confirming thus the suitability of the system for long term production. Thus, the use of biofilms proves to be a promising method to increase the productivity and thereby reduce production costs of lactic acid.

The analysis of biofilm performance at different flow conditions revealed that productivity increases with the flow velocity. This is explained by the decreased retention time of the liquid phase in the tube reactor and, thus, a minor pH drop caused by the released lactic acid. At low flow velocities, the pH drops to a value where growth and production are significantly inhibited.

During the cultivation, the biofilm (thickness and structure) was analyzed by Optical Coherence Tomography (OCT), a non-invasive imaging tool that can be applied in-situ during reactor operation without disturbing system or process. At the end of the experiment, the biofilm was additionally visualized by Nuclear Magnetic Resonance (NMR) to investigate the biofilm coverage over the cross-sectional area of the tube reactor.

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## BTV08

### Highly effective biofilm inhibition by the first metagenome-derived AI-2 Quenching enzyme

N. Weiland-Bräuer<sup>1</sup>, M. Kisch<sup>2</sup>, N. Pinnow<sup>1</sup>, A. Liese<sup>2</sup>, R. A. Schmitz<sup>1</sup>

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The majority of bacteria grow as surface-associated consortia in biofilms. In contrast to their planktonic growing counterparts, bacteria within biofilms show increased resistance to many classical antibiotics, and thus represent a major challenge in medicine and industrial processes. One of the underlying mechanisms crucial for biofilm formation, pathogenicity and virulence is cell-cell communication (quorum sensing, QS). Thus, QS is an attractive and most likely effective target for novel anti-biofilm drug design. In this study, we aimed to identify naturally occurring QS interfering activities (quorum quenching, QQ) in metagenomic large-insert libraries using a recently established reporter system and evaluate concerning their biofilm inhibiting potential. Seven metagenomic large-insert libraries were constructed from various habitats and screened for QQ activities using our recently established reporter system resulting in the identification of 142 metagenomic clones interfering with acyl-homoserine lactones (AHLs), and 13 with autoinducer-2 (AI-2). Identified ORFs were expressed in *E. coli*, N-terminally fused to maltose binding protein (MBP) and purified by affinity chromatography. Highest biofilm inhibitory effects were identified for QQ-2 using different biofilm assays (crystal violet, flow cell) Moreover, purified and chemically immobilized QQ-2 inhibited biofilm formation of *K. oxytoca* as well as several clinical *K. pneumoniae* isolates. QQ-2 represents an oxidoreductase most likely reducing the signaling molecules AHL and AI-2 to QS-inactive hydroxy-derivatives. The identified novel QQ-2

protein efficiently inhibits AHL and AI-2 modulated biofilm formation; and thus appears particularly attractive for medical and biotechnological applications.

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## Short Lecture

### Environmental Microbiology and Ecology 1

16 April 2018 • 08:00–10:00

#### EMV01

##### The vector function of microplastics for specific bacterial assemblages depends on environmental conditions

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Microplastics (MP) have been detected in a wide range of marine habitats, but a deeper understanding of the microbial interaction with this anthropogenically introduced substrate is lacking. To explore the role and composition of MP biofilms, we investigated MP-dependent and -independent assemblage factors along an environmental gradient with an *in situ* experiment. Polystyrene (PS), polyethylene (PE) and wooden pellets were incubated for two weeks at 7 stations in the coastal Baltic Sea, the river Warnow, and a waste water treatment plant (WWTP). The developed assemblages as well as the water communities (free-living and particle-attached fractions) were investigated applying high-throughput 16S rRNA gene sequencing. The study showed that different environmental conditions played a major role for the colonization processes, and that plastic-specific bacterial assemblages were solely formed under certain conditions, such as lower nutrient concentration and higher salinity. In comparison with natural surfaces, MP attracted significantly higher numbers of members of the families *Hyphomonadaceae* (esp. *Hyphomonas* sp.) and *Erythrobacteraceae* (esp. *Erythrobacter* sp.). The latter genus is known for the ability to utilize polycyclic aromatic hydrocarbons (PAH), and plastic, particularly PE, has been reported to accumulate PAHs from the environment. Our study establishes that the local environment prevailing shapes the particle colonization, but that MP-specific assemblage factors exist. This highlights the ecological significance of specific MP-associated microbial assemblages in aquatic environments and eminently in plastic accumulation zones.

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#### EMV02

##### Indole – A potential agent for chemical grazing defence in bacteria

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Within the microbial loop in marine environments, interactions between eukaryotic predators and bacterial prey are important drivers of microbial diversity, community

structure, and evolution. To avoid predation, bacteria employ several tactics, such as change of motility patterns, size, shape, or surface properties. However, only few studies have addressed secondary metabolites as chemical grazing defence mechanisms. Indole has been found as a signalling molecule in bacteria, but is a known toxin for eukaryotic organisms. We used 175 strains of closely related *Vibrios* in a screen with *Artemia salina* (brine shrimp) and found that 80% of the strains tested had a lethal effect on the brine shrimps. Additionally, full genome sequence analysis of 680 different *Vibrio* spp., revealed that 72% were able to produce indole, based on the presence of the marker gene *tnaA*. Analyses of culture extracts using Kovac's reagent and confirmed with thin layer chromatography, and H1-NMR analyses showed the abundance of indole. The toxicity of indole was tested to determine the LD<sub>50</sub> of indole in the main types of bacterial predators, e.g. a copepod, a nanoflagellate, and a ciliate species. Additional tests with *Vibrio ordalii* strain 12B09, a strain with high indole production, showed that a nanoflagellate and a ciliate predator showed significantly less growth on the wildtype as compared to a *tnaA* knockout mutant that did not produce indole. In addition, tests found that indole production is proportional to tryptophan concentrations in the environment. These results suggest that indole is not only used as signalling agent, but also plays a major role in chemical grazing defence in different *Vibrio* strains and might have even larger implications for the microbial loop and marine ecosystems.

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#### EMV03

##### Microbial life on a sand grain: from bulk sediment to single grains

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Globally, marine surface sediments constitute a habitat for estimated  $1.7 \times 10^{28}$  prokaryotes. For benthic microbial community analysis, usually, several grams of sediment are processed. In this study, we made the step from bulk sediments to single sand grains to address the microbial community directly in its micro-habitat: the individual bacterial diversity on 17 sand grains was analyzed by 16S rRNA gene sequencing and visualized on sand grains using CARD-FISH.  $10^4$  to  $10^5$  cells were present on grains from 202  $\mu\text{m}$  to 635  $\mu\text{m}$  diameter. Colonization was patchy, with exposed areas largely devoid of any epigrowth (mean cell-cell distance  $4.5 \pm 5.9 \mu\text{m}$ ) and protected areas more densely populated ( $0.5 \pm 0.7 \mu\text{m}$ ). Mean cell-cell distances were 100-fold shorter compared to the water column. In general, growth occurred in monolayers. Each sand grain harbors a highly diverse bacterial community as shown by several thousand species-level OTU<sub>0.97</sub>. Only 4 to 8 single grains are needed to cover 50% of OTU<sub>0.97</sub> richness found in bulk sediment. Although bacterial communities differed between sand grains, a core community accounting for >50% of all cells was present on each sand grain. The communities between sediment grains are more similar than between soil macroaggregates.

## EMV04

### The $\beta$ -hydroxyaspartate pathway: A missing link in the marine global carbon cycle?

L. Schada von Borzyskowski<sup>\*1</sup>, K. Krüger<sup>2</sup>, L. Hermann<sup>3</sup>, B. Pommerenke<sup>1</sup>, P. Claus<sup>1</sup>, N. Socorro Cortina<sup>1</sup>, T. Glatter<sup>1</sup>, U. Maier<sup>4</sup>, E. Bremer<sup>3</sup>, R. Amann<sup>2</sup>, T. J. Erb<sup>1</sup>

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Photosynthetic algae are major contributors to the primary production of the planet, converting gigatons of carbon dioxide into biomass each year. Heterotrophic bacteria can utilize carbon compounds excreted by algae, such as carbohydrates, DMSP, and the photorespiratory metabolite glycolate.

Here we investigated the enzymology, molecular genetics, physiology, and ecology of the  $\beta$ -hydroxyaspartate pathway (BHAP) for the assimilation of glycolate, which was only partially understood so far.

Enzymes of the BHAP were overexpressed and kinetically characterized. Using the model organisms *Paracoccus denitrificans* and *Ruegeria pomeroyi*, growth on glycolate was studied in detail, and the expression and regulation of this pathway was investigated. Comparative (meta)genomics were applied to study the distribution and *in situ* relevance of the BHAP.

We identified the previously unknown gene cluster of the BHAP and assigned functions to the four encoded enzymes that show so far unknown reactivities. The genetic regulator of the pathway was shown to interact with glyoxylate as an effector, and proteomics confirmed strong expression of the pathway enzymes during growth on glycolate or glyoxylate, while deletion mutants of the BHAP were unable to grow on these carbon sources. The BHAP was found to be distributed among terrestrial and especially marine Proteobacteria.

Marine metagenomics data suggest that the BHAP serves as an important route for the assimilation of side products of algal photosynthesis in the ocean. In conclusion, our results point to a missing link in the marine global carbon cycle.

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## EMV05

### Cobaviruses - a phage group infecting marine Rhodobacteraceae is found in highly productive marine regions

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A novel phage group infecting marine Rhodobacteraceae – the Cobaviruses – was investigated using a combination of phage cultivation, functional genomics and database mining. Our aim was to determine genomic characteristics as well as phylogeny, host range, environmental distribution and

potential habitats. Cobaviruses include three new, related phages specific for *Lentibacter* sp. SH36 (vB\_LenP\_VB1, vB\_LenP\_VB2 and vB\_LenP\_VB3), further phage genomes from environmental data sets and the isolates Roseobacter virus SIO1 and Celeribacter phage P12053L. Phylogenetic analysis places them within the new subfamily *Riovirinae*, genus *Siovirus*.

Gene composition and presence of direct terminal repeats (DTRs) indicate a genome replication and packaging strategy similar to that of T7 phage. Several auxiliary metabolic genes (AMGs) present in the genome are involved in nucleotide metabolism and elimination of the stress response of the host. Lysis proceeds via the canonical holin-endolysin pathway. Genetic analysis indicates that the Cobavirus virion has a classical podoviral structure, with exception of the tail fibers, which have similarities with those from myo- and siphoviruses. Cobaviruses and their hosts are distributed worldwide in highly productive marine areas. The known host range of Cobaviruses as a group includes members of the genera *Lentibacter*, *Sulfitobacter* and *Celeribacter* and it potentially extends to other members of the *Rhodobacteraceae*. Phylogenetic analysis of the glutaredoxin and ribonucleotide reductase (RNR) genes indicate that cobaviruses potentially infect bacteria associated with phototrophic and grazing protists, potentially resisting lysis in food vacuoles and thus being "protist resistant bacteria".

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## EMV06

### Turnover rates coupled to transcriptomics reveal dominant microorganisms responsible for cycling iron, sulfide and hydrogen in Kermadec Arc hydrothermal fluids

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<sup>2</sup>University Hamburg, Microbiology & Biotechnology, Hamburg, Germany

Hydrothermal emissions at volcanic arcs likely play a larger role for element cycling in the ocean than those at mid-ocean ridges because metal fluxes are higher and biogeochemically essential metals are released at shallower water depths. Nevertheless, hydrothermal systems at volcanic arcs are much less studied than those from mid-ocean ridges. The vents at the Kermadec Arc provide an excellent opportunity to investigate the role of microbially catalyzed element cycling of hydrothermal fluid-released compounds. The fluids can have very low pH and can be enriched in trace metals, particularly in iron. In contrast to many mid-ocean ridge fluids, these emissions can have relatively low sulfide and hydrogen contents. Based on this knowledge two main questions arise: (i) Is iron (II)- rather than sulfide- or hydrogen-oxidation primarily fueling biomass synthesis and (ii) which microorganisms are responsible for re-cycling these compounds? To investigate these questions we incubated hydrothermal fluids emitting from four actively venting areas in the Kermadec Arc. Hydrothermal emissions were supplemented with iron (II), sulfide or hydrogen and radioactively labeled bicarbonate and incubated for eight hours. Microbial iron (II)-, sulfide- and hydrogen-consumption as well as respective autotrophic CO<sub>2</sub> fixation rates were determined. Additionally, via transcriptome analyses, the microorganisms that were active in these incubations were identified. Under the provided conditions, iron (II) addition lead to the highest autotrophic CO<sub>2</sub> fixation rates in most of the tested fluids. According to the transcriptome data it appears that certain epsilonproteobacterial lineages as well as Thiomicrospira spp. were the key active players in these iron-amended incubations.

## EMV07

### Marine Foams: Ephemeral Hotspots of Microbial Life at the Air-Sea Interface

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The air-sea interface spans two-thirds of the Earth's surface. The sea-surface microlayer (SML), a biofilm-like layer at the boundary between atmosphere and hydrosphere, is known for its unique physicochemical and biological properties compared to subsurface underlying waters (ULW). The SML has important implications for matter and gas exchange processes. Currents, breaking waves and bursting bubbles compress surface-active material in the SML, thus leading to sea foam production. The global foam coverage of oceans is unknown, because its occurrence in the marine environment is patchy with a lifespan limited to hours or days. Foams support trophic networks, however, a comprehensive investigation on the bacterial community inhabiting marine foams, SML and ULW is still missing to date. Here we shed light on this ephemeral habitat by analysing samples from different marine systems (tropical Indian Ocean, North Sea). Our results indicate strong enrichment of bacteria and small autotrophs in foams compared to non-foamy SML and ULW. Using a high-throughput sequencing approach we show that foam, SML and ULW differ in their 16S-rRNA (gene)-based community structure. Particle-attached bacterial communities in the foam were distinct from their free-living counterparts, potentially indicating fast and adaptive colonization of substrate, e.g. by taxa of the *Saprospiraceae* and *Alteromonadaceae*. Increased relative abundances of *Trichodesmium* and *Synechococcus* 16S rRNA indicates enhanced activity in foams and SML of the two taxa and suggest high tolerance towards strong solar radiation experienced at the surface. We conclude that marine foams are neglected hotspots of microbial life at the air-sea interface with distinctive features compared to non-foamy SML.

## EMV08

### Cross-feeding of ammonium from marine methylamine and glycine betaine degrading bacteria to diatoms as a widespread metabolic interaction

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**Background & Question:** Dissolved organic nitrogen (DON) compounds originating from marine organisms are important carbon, energy and nitrogen sources for heterotrophic bacteria in the oceans. The *Rhodobacteraceae* *Donghicola* sp. strain KarMa can degrade the ubiquitous DON compound monomethylamine subsequently providing ammonium to the diatom *Phaeodactylum tricornutum* in co-culture<sup>[1]</sup>. As methylamines (MA) and glycine betaine (GBT) are also excreted by diatoms, associations with bacteria utilising these organic nitrogen compounds may be a selective advantage in the typically nitrogen-limited marine environment.

**Methods:** This hypothesis was investigated with strain KarMa in co-culture with three diatom strains. Furthermore,

six additional *Rhodobacteraceae* type strains harbouring MA and GBT degradation pathways were chosen based on an *in silico* analysis and characterised in co-culture with *P. tricornutum*.

**Results:** Various DON compounds were degraded by strain KarMa in co-cultures and ammonium was provided to diatoms for growth under photoautotrophic and mixotrophic conditions. The composition of the provided DON and the carbon-to-nitrogen ratio influenced growth of both organisms. Ammonium cross-feeding of the additional type strains after DON degradation showed species- and metabolite-specific patterns. As none of the tested bacteria was methylotrophic, bacterial growth was dependent on diatom-released substrates in all MA containing co-cultures indicating mutual interactions.

**Conclusion:** Taken together, cross-feeding of ammonium from DON degrading *Rhodobacteraceae* may represent a widespread and important factor in establishing and stabilizing interactions with photoautotrophic diatoms in the oceans.

## References:

[1] Suleiman M. and Zecher K. *et al.* (2017) *AEM* 82(24): 7113-7122

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## Short Lecture

### Infection Biology 1

16 April 2018 • 08:00–10:00

## IBV01

### Redox-sensing under hypochlorite stress and infection conditions by the Rrf2-family repressor HypR in *Staphylococcus aureus*

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**Introduction:** *Staphylococcus aureus* is a major human pathogen and has to cope with reactive oxygen species (ROS) and HOCl during infections, which requires efficient protection mechanisms to avoid destruction.

**Objectives:** Here, we have investigated the changes in the RNA-seq transcriptome by the strong oxidant NaOCl in *S. aureus* USA300 and identified the novel Rrf2-family redox-sensing regulator HypR as most strongly induced under NaOCl stress (1).

**Materials & Methods:** Using mutational analysis and biochemical methods, we have studied the function and redox-sensing mechanism of HypR under NaOCl stress in *S. aureus*.

**Results:** HypR was characterized as redox-sensing repressor that negatively controls expression of the *hypR-merA* operon and directly senses and responds to NaOCl

and diamide stress by a thiol-based redox switch. Mutational analysis identified Cys33 and the conserved Cys99 as essential for NaOCl-sensing while Cys99 is also important for repressor activity of HypR *in vitro* and *in vivo*. The redox-sensing mechanism of HypR involves Cys33-Cys99 intersubunit disulfide formation by NaOCl stress both *in vitro* and *in vivo*. Moreover, the HypR-controlled flavin disulfide reductase MerA was shown to protect *S. aureus* against NaOCl stress and increased survival in J774A.1 macrophage infection assays.

**Conclusion:** Here, we identified a new member of the widespread Rrf2 family as major redox-sensor of hypochlorite stress in *S. aureus* that uses a thiol-disulfide switch to regulate defense mechanisms against the oxidative burst under infections in *S. aureus*.

#### References:

[1]Loi VV et., al. (2018). Redox-sensing under hypochlorite stress and infection conditions by the Rrf2-family repressor HypR in *Staphylococcus aureus*. *Antioxid Redox Signal.*, In press.

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#### IBV02

##### Targeting the anti-staphylococcal antibody response using a bead-based array approach

T. Meyer<sup>\*1</sup>, S. Michalik<sup>1</sup>, J. Steinke<sup>2</sup>, B. Bröker<sup>2</sup>, U. Völker<sup>1</sup>  
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<sup>2</sup>University Medicine Greifswald, Department of Immunology, Greifswald, Germany

**Introduction:** About 30% of the human population is asymptotically colonized with *Staphylococcus aureus*. However, this commensal can also act as a pathogen causing a wide range of severe conditions. Both carriers and non-carriers display a broad range of anti-staphylococcal antibodies with pronounced inter-individual variations in antibody specificities and titers [Verkaik 2009, JInfectDis]. Studies of bacteremia patients indicate that these antibodies might have protective potential [Stentzel 2016, JProteomics].

**Objective:** The factors shaping the human antibody repertoire against staphylococcal antigens have only partially been explored. To gain a better understanding, the humoral response to a total of 148 heterologously expressed or chemically synthesized antigens was recorded in pooled serum or plasma samples of different patient groups as well as in healthy individuals.

**Material & methods:** Serial dilutions of all samples were incubated with xMAP-beads (Luminex) presenting the antigens in a multiplexed assay. Bound antigen-specific serum antibodies were quantified using detection antibodies against human total IgG, IgA, and IgG4. The complex dataset was quantitatively evaluated with a newly developed analysis pipeline written in R and implemented in a Shiny App.

**Results:** Antibody response values were determined using an advanced iterative data fitting pipeline. The comparison of carrier and non-carrier samples already showed detectable differences whereas samples of patients suffering from severe illnesses (e.g. Cystic fibrosis, Atopic dermatitis) displayed strongly diverging patterns.

**Conclusion:** A comprehensive dataset was generated revealing vast individual and disease-dependent differences

in the antibody repertoire allowing further correlation analyses.

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#### IBV03

##### Studies on the Resistance Mechanism of the Novel Topoisomerase Inhibitor Cystobactamid

K. Cirniski<sup>\*1</sup>, J. Herrmann<sup>1</sup>, S. Hüttel<sup>2,3</sup>, T. Giambatista<sup>3,4</sup>, N. Zaburannyi<sup>1,3</sup>, M. Stadler<sup>2,3</sup>, M. Brönstrup<sup>3,4</sup>, R. Müller<sup>1,3</sup>

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Cystobactamids, a novel compound class isolated from *Cystobacter* spp., display very pronounced activity on Gram-positive and Gram-negative bacteria, including Enterobacteriaceae, non-fermenters, and multidrug-resistant isolates that are the causative agents of some hard-to-treat nosocomial infections. Structure-activity relationship (SAR) studies showed that full spectrum coverage in terms of antimicrobial activity (e.g. cUTI, cIAI and HAP/VAP pathogens) can be achieved by derivatization of the scaffold. Analysis of the biosynthetic gene cluster in *Cystobacter* sp. revealed a resistance gene that encodes a pentapeptide repeat protein – a known resistance factor against topoisomerase poisons. The bacterial gyrase and topoisomerase IV were confirmed as molecular targets of the cystobactamids (IC50 in sub- $\mu$ M range) and the compounds stabilize the topo II cleavage complex which results in DNA double-strand breaks. Although the mode of action (MoA) of cystobactamids is similar to that of fluoroquinolones we only observed minor cross-resistance. Based on the MoA of cystobactamids a very low frequency of resistance (FoR) for the target pathogens (10-10 to 10-11) was expected. Surprisingly, FoR values are in the range of 10-8 for both, *Escherichia coli* and *Pseudomonas aeruginosa*. Intriguingly, resistance mutations rarely occurred in *gyrA* or *parC* genes (target mutations) and instead, all mutants bear disruption mutations in a largely uncharacterized two-component regulatory system (TCS). The mode of resistance (MoR) and the role of the TCS is currently under investigation. As its disruption is described to attenuate virulence mainly due to motility reduction the relatively high FoR might be less important with respect to the potential application as an antibiotic.

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#### IBV04

##### Molecular analysis of regulatory factors and environmental signals controlling secretion systems important for *Yersinia* virulence

V. Knittel<sup>\*1</sup>, P. Sadana<sup>2</sup>, S. Seekircher<sup>1</sup>, A. K. Heroven<sup>1</sup>, A. Scrima<sup>2</sup>, P. Dersch<sup>1</sup>

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*Yersinia pseudotuberculosis* is an enteric pathogen that causes gastrointestinal diseases in humans. Virulence regulation of *Y. pseudotuberculosis* is mainly controlled by the carbon storage regulator system, consisting of the RNA-binding protein CsrA and the small non-coding RNAs CsrB and CsrC. Recently, we discovered that expression of the Type VI secretion system 4 (T6SS4) is regulated by CsrA. Transcription of the T6SS4 operon is strongly upregulated in the absence of CsrA. In addition, we were able to

demonstrate that the T6SS4 regulation occurs via a so far uncharacterized hypothetical CsrA-controlled protein. This protein, named RovC, is conserved in *Y. pseudotuberculosis* and *Y. pestis*. RovC does not exhibit any homology to known proteins or protein domains. It is maximally expressed in stationary growth phase at 25 °C. CsrA represses transcription of *rovC* and furthermore, CsrA regulates RovC expression on the post-transcriptional level by directly binding and stabilizing the *rovC* mRNA transcript.

Overexpression of RovC strongly induces expression of a translational T6SS4"-*lacZ* reporter fusion. Moreover, all genes belonging to the T6SS4 operon are downregulated in a *rovC* mutant strain. Purified RovC protein directly interacts with the promoter region of the T6SS4 operon. The binding region was narrowed down by footprinting to a region close to the -35 and -10 region of the T6SS4 promoter. Additionally, overexpression of RovC in the *Y. pseudotuberculosis* YPIII strain results in secretion of the T6SS4 effector Hcp.

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## IBV05

### Characterization of inner membrane regulated proteolysis of ToxR, depending on cysteine-thiol-oxidation/reduction state and bile salts in *Vibrio cholerae*

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In *Vibrio cholerae*, ToxRS are key regulators for the virulence network regulation. ToxR is located in the inner membrane exposing a transcription factor active cytosolic N"- and a periplasmic located C"-terminal domain. The latter contains two cysteine residues and enables ToxR to form intramolecular disulfide bonds. In this report, ToxR degradation is characterized and divided into DegS dependent and independent proteolysis. A DegS independent proteolysis is determined by starvation and alkaline medium conditions. Whereas DegS dependent ToxR proteolysis responds to an array of different conditions and mutations affecting the oxidation and reduction state of the cysteine thiol groups of ToxR. Interestingly, in the presence of bile salt ToxR proteolysis is shut off independently of the route of proteolysis. Studies on a *toxS* knockout mutant reveal that ToxR is less stable in the presence or absence of sodium-deoxycholate, and the bile depending stimulation of ToxR transcriptional factor activity is mainly facilitated in the presence of ToxS. Therefore, we postulate two conditions for ToxR activation: One addresses ToxR levels regulated by proteolysis due to starvation and alkaline pH or reduction of cysteine thiol groups by the the DegS-pathway. The second activation state focuses on ToxRS complex formation, and further activation of ToxR transcription factor activity. Since both conditions are significantly influenced by sodium-deoxycholate, our data overall suggest a comprehensive bile sensory function for the ToxRS complex.

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## IBV06

### A Genome Database for *Clostridioides difficile* implemented in EnteroBase

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EnteroBase is an open source and publicly accessible, strain-based database for bacterial genome sequences, with daily automatic retrieval of newly published short-read data, subsequent assembly and allele calling (<http://enterobase.warwick.ac.uk/>). Within EnteroBase, we implemented a database for *Clostridioides difficile* genome sequences, which currently (as of October 2017) holds 6,752 assembled genomes from *C. difficile* isolates from at least 27 countries. Genomic phylogenetic relations can be assessed on the basis of allelic profiles for ribosomal multilocus sequence typing (rMLST; synchronized with PubMLST), core genome MLST (cgMLST), and whole genome MLST (wgMLST) schemes, graphically depicted in minimum spanning and neighbor joining trees. We used rMLST to classify *C. difficile* isolates into clonal lineages, which largely corresponded to PCR ribotypes in a prospective study. Hence, an isolate's PCR ribotype could be predicted from its genome sequence, based on phylogenetic analysis. We applied cgMLST to trace spatial spread within and between healthcare institutions, to investigate outbreaks of *C. difficile* infections and within-patient diversity for discriminating recurrence from reinfection. We found that the discriminatory power of cgMLST was similar to that of cgSNP analysis, and that pairwise distances from the two methods were strongly correlated. However, cgMLST is less computationally expensive and more easily standardized than cgSNP analysis. An advantage of EnteroBase is that previously published *C. difficile* genome sequences can directly be interpreted in a global context, which greatly assists in identifying outbreak sources and routes of epidemic spread. The web-based user interface of EnteroBase readily allows application by non-bioinformaticians.

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## IBV07

### Panoramic view on *Staphylococcus aureus* biofilm physiology

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**Introduction:** *Staphylococcus aureus* is well known for its capacity to form biofilms on host tissues and implants, which often leads to chronic infections in patients suffering from osteomyelitis, endocarditis or cystic fibrosis. Despite that up to 80 % of human bacterial infections are biofilm-associated and Staphylococci are one of the leading causes of this type of infection, little is known about *S. aureus* biofilm physiology.

**Objectives:** Consequently, we aimed to compare protein and metabolic profiles of planktonic and biofilm cells using *state-of-the-art* omics technologies to shed light on molecular key factors during *S. aureus* biofilm formation.

**Material & methods:** To this end, we established a flow system suited for highly reproducible cultivation of biofilms and subsequent multi-omics analyses. This system allowed us to analyse the intra- and extracellular biofilm proteome and to determine concentrations of 39 extracellular metabolites.

**Results:** Our proteome analysis revealed that fermentation, nitrate respiration, capsule biosynthesis, osmotic stress response, cardiolipin biosynthesis as well as the production of toxins and proteases are playing important roles during biofilm growth. Most of these data could be confirmed by phenotypic assays. Moreover, extracellular proteome analysis uncovered an accumulation of proteins with a high pI value, which were shown to play an important role in cell aggregation during biofilm formation.

**Conclusion:** Here we present the first study combining GeLC-MS/MS and metabolic footprint analysis of *S. aureus* biofilms in a flow system. Our integrated multi-omics data contribute to a better understanding of biofilm-associated *S. aureus* infections - an essential prerequisite for the development of novel antimicrobial therapies.

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## IBV08

### Aminoglycoside resistance in *Pseudomonas aeruginosa* - The role of active efflux in the clinic

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Hospital acquired infections due to multi-drug resistant pathogens are one of the major challenges for public health and modern medicine today. It is therefore important to understand antibiotic resistance mechanisms in order to develop novel antibiotic substances and optimize the treatment of infected patients. In *Pseudomonas aeruginosa* (PA), the MexXY efflux pump assumed to be a major determinant of aminoglycoside resistance and is often found to be upregulated in clinical isolates.

In this study, we investigated the impact of MexXY upregulation on aminoglycoside resistance in a set of >400 clinical PA isolates.

Efflux pump expression data of each isolate was correlated with its respective susceptibility against the aminoglycoside antibiotic tobramycin. Furthermore, the frequency and nature of mutations within the substrate specificity-providing pump component *mexY* and its negative regulator *mexZ* were investigated. Additionally, we developed an *in vivo* fitness assay using *Galleria mellonella* and investigated the fitness impact of a *mexXY* knockout.

Generally, expression levels of the efflux pump correlated with protein abundance. However, we could not detect any correlation between efflux pump expression and the respective susceptibility phenotype. A number of sequence variations in *mexY* were detected, explaining the observed contradiction of expression and susceptibility phenotype in those isolates. Moreover, a *mexXY* mutant exhibited increased fitness in the *in vivo* competition assay.

Given the absent correlation between resistance and efflux pump expression, and a fitness advantage of an efflux pump loss *in vivo*, we speculate that the PA isolates are adapting to an additional condition in the chronically infected host besides antibiotic stress.

## Short Lecture Microbial Cell Biology 1 16 April 2018 • 08:00–10:00

### MCBV01

#### Novel factors directing cell division in the stalked budding alpha-proteobacterium *Hyphomonas neptunium*

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Many well-studied bacterial model organisms have relatively simple cell shapes, such as cocci or rods, and divide via binary fission. However, the bacterial world shows a huge diversity in morphology. This is particularly true for the alpha-proteobacteria, which include various species with a more complex, cell cycle-dependent morphology. Their representative *Hyphomonas neptunium* starts off as a flagellated ovococoid swarmer cell that grows a stalk from its cell body after loss of its flagellum. The tip of this stalk then swells up to generate a daughter cell in a budding type of cell division. This division is highly asymmetric, as the constriction takes place right at the budneck, giving rise to an ovococoid daughter cell and a stalked mother cell that can immediately produce new offspring.

In order to identify novel factors potentially involved in this unusual mechanism of proliferation, we compared the genomes of 12 strains of stalked budding bacteria and identified 81 genes that are present in all (or 11/12) of these genomes, but not in the genomes of 4 organisms that divide via binary fission. Out of these 81 genes, 62 genes have an uncharacterized function. We decided to construct single-knockout mutants of these genes and investigate their phenotypes. In case genes appeared to be essential, as no deletion mutants could be obtained, the phenotype of depletion strains was studied. The objective of this screening is to identify genes involved in the growth and division of *Hyphomonas* species and related stalked budding alpha-proteobacteria. In future research, the functions of the respective gene products will be studied in-depth in order to obtain a deeper understanding of the mechanism underlying this intriguing way of dividing a bacterial cell.

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### MCBV02

#### Cell length-dependent transition in MinD-dynamics drives a switch to asymmetric division and determines the site of complete chromosome segregation in polyploid *Vibrio parahaemolyticus* swarmer cells

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*Vibrio parahaemolyticus* exists as either swimmer or swarmer cells, specialized for growth in liquid and on solid environments, respectively. The swarmer cell population is heterogeneous and consists of both long and short swarmer cells. We show that swarmer cells divide but the placement of the division site is cell length-dependent; short swarmers divide symmetrically at mid-cell, while long swarmers switch to an asymmetric placement of the divisions site along the long axis of the cell. Swarmer cells are polyploid for both chromosome 1 and 2 and display a clear correlation between swarmer cell length and chromosome count with increasing copy number for each chromosome with increasing cell length. The origins of both chromosomes are clearly segregated and positioned equidistantly along the long axis of the cell, which ensures that independent of the division

pattern, each swarmer daughter cell inherits at least one copy of each chromosome at cell division. Despite a clear segregation of *ori* regions, complete chromosome segregation only occurs at the site of Z-ring formation in an FtsK dependent manner. Our data indicate that the switch to asymmetric division is regulated by a cell length-dependent transition in the localization-dynamics of the division regulator MinD from a pole-to-pole oscillation in short swimmers to a multi-node standing-wave oscillation in long swimmers. Asymmetric division contributes to swarmer-population heterogeneity, as both progeny cells have initiated the swarm-program but possess distinct capabilities: a swimming proficient short cell and a swimming deficient long cell.

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#### MCBV03

##### Everything flows and nothing abides – The regulatory role of the Min system in *Bacillus subtilis*

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Rod-shaped bacteria like *Escherichia coli* or *Bacillus subtilis* divide at the geometric middle via binary fission, aided by mechanisms that ensure a tight spatiotemporal control of divisome placement. One of these systems, termed Min system, is found in many bacteria. Interestingly, the Min system is dynamically oscillating in *Escherichia coli*, but was thought to be static in *B. subtilis*. In *B. subtilis* the Min system is composed of MinCDJ and a topological determinant DivIVA. DivIVA was assumed to stably localize to the poles and recruit MinCDJ, creating a fixed gradient decreasing towards midcell, with MinC actively inhibiting initiation of division anywhere but midcell. Here, we present new data on the unexpected dynamics of the *B. subtilis* Min system.

To avoid overexpression artifacts, we constructed strains where the individual Min components are encoded as functional fluorescent fusion constructs in their native genomic loci. Utilizing these strains, we characterized dynamics of the proteins via fluorescent recovery after photobleaching (FRAP) studies. Surprisingly, all Min components were found to be highly dynamic.

Combining the spatiotemporal localization data with photo-activated localization microscopy (PALM) and individual protein quantities determined via in-gel fluorescence, we were able to provide key data for biophysical modelling of the time-resolved localization and interaction between the Min components. Modelling and in vivo data support our hypothesis that the *B. subtilis* Min system dynamics lead to a recruitment to midcell positions, where ongoing cell division takes place. Thus, the Min system in *B. subtilis* is not a protection system for aberrant division at cell poles, but rather a cell cycle regulator.

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#### MCBV04

##### Identifying the biological role of a protein specific to prokaryotes located in bacterial functional membrane microdomains

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Bacterial membranes contain protein machineries that orchestrate signal transduction, protein trafficking, and cytoskeleton rearrangement. Membrane components are distributed heterogeneously, and bacteria thus organize

nanoscale platforms termed functional membrane microdomains (FMM, also called bacterial lipid rafts), which in certain aspects resemble the so-called lipid rafts of eukaryotic cells. In the human pathogen *Staphylococcus aureus*, FMM organization requires membrane carotenoid aggregation and co-localization with the scaffold protein flotillin, a homologue of the eukaryotic raft-associated flotillin. In all bacterial genomes, the flotillin gene is cotranscribed with another gene that encodes a protein of unknown function, termed NfeD. Here we used the *S. aureus* bacterial model to study the role of the NfeD protein in FMM formation and integrity. We found that NfeD is associated with flotillin and is located exclusively in FMM. Using a number of biochemical and cell biology approaches, we show interaction of these two proteins in large oligomeric complexes, crucial for correct subcellular flotillin distribution and oligomerization. Absence of NfeD negatively affects flotillin function, leading to severe defects in FMM-associated cellular processes including virulence. This protein shows putative protease activity and has a C-terminal binding domain, possibly for signaling molecules that might be responsible for activating the protein. In light of these findings, we currently work on the hypothesis that this unknown protein participates in FMM protein quality control, by checking correct oligomerization of FMM-associated protein complexes.

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#### MCBV05

##### Bacterial microcompartment construction using components of the propanediol utilisation metabolosome from thermophilic *Geobacillus* spp.

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**Question:** Bacterial microcompartments (BMCs) are sub-cellular proteinaceous organelles which appear to sequester highly reactive pathway intermediates such as the propanediol utilising metabolosome (Pdu). The ability to control the assembly of BMCs and their biochemical cargo offers biotechnological opportunities, not yet achieved in thermophiles.

**Methods:** The pdu operon in the genome of thermophilic *G. thermoglucosidasius* NCIMB 11955 was examined. The five genes, pduA, pduBB", pduJ, pduK and pduN, encoding proteins that form the shell of BMCs were amplified. Initially, the empty BMCs were assembled by expression of the pduABB"JKN proteins in *Bacillus subtilis* 168CA. Circular structures with clear boundaries were visualised by TEM in thin-sectioned cells. Superfolder GFP (sfGFP) fused to the Propanediol utilisation protein, PduP, was incorporated into the lumen of BMCs.

**Results:** Given the *Bacillus*-based observations, the pduABB"JKN and pduP-sfgfp were placed under the control of a maltose inducible promoter for simultaneous co-expression in *Geobacillus* spp. The fragments were cloned into shuttle vectors capable of conjugative transfer from *Escherichia coli* S17-1 to *Geobacillus* spp and transferred into *Geobacillus* strains lacking the pdu operon, such as *G. subterraneus*.

**Conclusions:** These results show for the first time the generation of synthetic protein compartments derived from components of the Pdu organelle of thermophilic *G. thermoglucosidasius* and targeting of enzymes to their lumen. The engineering of BMCs with tagged sfGFP in *G. subterraneus* is currently in progress.

## MCBV06

### Cooperative alginate production in the freshwater phototrophic consortium "*Chlorochromatium aggregatum*"

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Phototrophic consortia are highly regular multicellular associations in which a central motile Betaproteobacterium is surrounded by numerous cells of green sulfur bacteria, the so called epibionts. They divide in a highly coordinated way and display a rapid signal transfer between the epibionts and the central bacterium suggesting a tight interaction between the partners. The molecular basis of this interaction was studied in the consortium "*C. aggregatum*" by analysing several unique genes of the epibiont which are absent from its non-symbiotic relatives. One of these genes, Cag\_1919, contains a RTX domain and shows genomic characteristic of an alginate lyase which processes the last step in alginate synthesis. To study this first evidence of alginate production in freshwater bacteria, consortia were incubated with an exogenous alginate lyase. The complete disaggregation of consortia suggests that alginate is involved in the multicellularity of consortia. A thiobarbituric acid assay and microTOF mass spectrometry confirmed that heterologously expressed Cag\_1919 possesses the capability to degrade alginate. However, the epibiont only contains the alginate lyase itself. Genome analysis revealed that the central bacterium possesses an almost complete alginate synthesis pathway, only missing the final alginate lyase step. dStorm super-resolution microscopy and high resolution immunogold labelling localized the epibiont lyase at contact sites between the two symbiotic partners. In conclusion, the epibiont transfers a protein necessary for the completion of alginate synthesis to its heterotrophic partner, employing a RTX module known from pathogens of eukaryotes. This cooperative synthesis pathway is involved in maintaining the multicellularity of "*C. aggregatum*".

## MCBV07

### Building a filament for all conditions - How spatial flagellin organization affects flagellar function

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Many bacteria are motile by means of a single helical flagellum at the cell pole which allows forward and backward swimming in liquid media. However, many natural bacterial habitats consist of structured environments, such as sediments, water-saturated soils, or tissues, where bacteria run the risk of getting stuck.

We monitored swimming of the sediment isolate *Shewanella putrefaciens* with fluorescently labeled flagellar filaments under conditions mimicking its natural habitats. We found that when cells get stuck they can resort to a novel movement mechanism: the flagellum wraps around the cell body in a spiral-like fashion which enables the cells to escape by a screw-like backward motion\*.

The polar flagellar filament of *S. putrefaciens* is composed of two different flagellins, FlaA and FlaB. Selective fluorescent

labeling revealed that the two flagellins are spatially organized: while FlaB forms the major part of the filament, FlaA is predominantly incorporated at the base of the filament close to the cell pole. This arrangement relies on a simple regulatory mechanism resulting in consecutive production and export of FlaA and FlaB. Filaments exclusively formed by FlaA or FlaB showed different helix characteristics. Cells with FlaA- or FlaB-only filaments exhibited significant differences with respect to subpopulation distributions of speed and turning angle. Most notably, a FlaA-only filament would never show screw formation. The results strongly indicate that the wild-type set-up with two distinct spatially arranged flagellins stabilizes the flagellar filament and that this benefits flagella-mediated movement over a wide range of conditions.

## References:

[1] Kühn, M. J. *et al.* (2017) Bacteria exploit a polymorphic instability of the flagellar filament to escape from traps. *PNAS*

## MCBV08

### A cyclic-di-GMP phosphodiesterase and its interaction partners constitute essential components of the rhizobial elongasome and divisome

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During cell elongation many rod-shaped bacteria insert new peptidoglycan (PG) along the lateral wall in a dispersed fashion, supported by the actin-like protein MreB, while the tubulin-like FtsZ governs cell division.  $\alpha$ -Proteobacterial Rhizobiales lack MreB and elongate at the new cell pole, but scaffolding or regulatory proteins driving unipolar cell wall biogenesis are largely unknown. We identified factors controlling polar growth and septation in the symbiotic  $\alpha$ -proteobacterium *Sinorhizobium meliloti*. Using fluorescence microscopy, genetic analysis, muopeptide profiling, protein biochemistry methods and bacterial two-hybrid analysis, we discovered that the membrane-anchored cyclic-di-GMP phosphodiesterase RgsP is an essential protein that dynamically localizes to the sites of PG biosynthesis at the new cell pole and the septum. RgsP is composed of 7TMR-DISM, PAS, GGDEF and EAL domains. It either directly or indirectly engages in protein-protein interactions with at least seven previously uncharacterized essential membrane-anchored or periplasmic proteins, which localize to the growing pole, septum, or both. These include the essential PG-binding LytM domain protein RgsM, possibly involved in cleavage of peptide bridges in PG. Depletion of Rgs proteins resulted in growth inhibition and abnormal PG muopeptide composition. RgsP and RgsM orthologs from related  $\alpha$ -rhizobial species were able to complement the corresponding *S. meliloti* gene depletion phenotypes. Rgs proteins are candidates for the regulators of unipolar growth and the scaffold that guides PG biosynthesis in Rhizobiales.

## Short Lecture Fungal Biology 1 16 April 2018 • 08:00–10:00

### FBV01

#### Generation of an arginine-tRNA-adapted *Saccharomyces cerevisiae* strain for effective expression of *Aspergillus fumigatus* proteins

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Heterologous gene expression using model systems becomes increasingly important in proteomic research, but is often hindered by the codon usage bias. The fungus *Aspergillus fumigatus* has become a model for airborne pathogenic fungi and a better knowledge of protein function and interactions is crucial to understand disease progression. Its codon usage revealed considerable differences when compared to the model organism *Saccharomyces cerevisiae*, impeding previous attempts for heterologous gene expression.

In this study, we engineered *S. cerevisiae* strains with modified levels of rare tRNA, which led to a significant increase of the translation of heterologous target mRNA molecules. An artificial codon nonsense mutation at the 5' end of the  $\beta$ -galactosidase reporter gene in a stop codon read-through assay was used to demonstrate the generation of functional nonsense suppressor tRNA<sup>Gln<sup>S</sup>CUA</sup>. To show that higher tRNA levels resulted in increased protein production, a copper-based survival assay was performed. Increased transcription of tRNA<sup>Arg<sup>CCG</sup></sup> molecules, recognizing rare codons in a modified *CUP1* gene, led to higher copper resistance. Genome integration of a tRNA expression cassette yielded arginine-tRNA-adapted *S. cerevisiae* strains with increased tRNA levels for tRNA<sup>Arg<sup>CCG</sup></sup>, tRNA<sup>Arg<sup>GCC</sup></sup> and tRNA<sup>Arg<sup>UCC</sup></sup>.

Such strains drastically improved the monitoring of protein interactions from *A. fumigatus* bait and prey sequences in yeast two-hybrid experiments. In the future, the principle to overcome limited recombinant protein expression by the adaptation of tRNA molecules instead of codons will provide new designer yeast cells for efficient protein production and genome-wide protein/protein interaction analyses.

### FBV02

#### Novel players of unconventional secretion in the smut fungus *Ustilago maydis*

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The corn smut fungus *Ustilago maydis* exports chitinase Cts1 via an unconventional secretion pathway. Yeast like cells of this eukaryotic model grow by budding. In this process, two septa are formed that seal off mother and

daughter cell. Upon daughter cell formation, Cts1 localises to the fragmentation zone delimited by the septa and hence, passes the cytoplasmic membrane by an unknown mechanism. Proteins controlling the formation of the secondary septum are essential for this localisation. In the fragmentation zone, Cts1 participates in degradation of the cell wall leading to physical cell separation and its own release. To further characterize the unconventional secretion pathway, we developed and applied a novel genetic screen based on UV mutagenesis to identify components essential for Cts1 secretion. Three mutants with strongly reduced Cts1 secretion were identified. Remarkably, all mutants expressed truncated versions of one particular protein, termed Jps1 (jammed in protein secretion screen 1). Localisation studies demonstrated that Jps1 co-localises with Cts1 in the fragmentation zone of dividing yeast cells indicating that the proteins might interact. While loss of Jps1 leads to cytoplasmic accumulation of Cts1 and strongly reduced unconventional secretion, deletion of the chitinase does not disturb Jps1 localisation. Hence, a specific factor involved in unconventional export of Cts1 has been identified. Currently, we focus on the molecular characterization of Jps1 and its connection to unconventional Cts1 secretion.

### FBV03

#### The cell dialog mechanism mediating vegetative cell fusion in fungi is conserved in the plant pathogen *Botrytis cinerea*

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In 1888, H. M. Ward described for the first time very accurately fusion of two hyphae in *Botrytis spp.* In 2009, the molecular mechanism behind this cell fusion process was elucidated in the red bread mold *Neurospora crassa*. In this fungus, cell-cell communication is based in a "cell dialog" mechanism, in which cells take turns in signal sending and receiving. These physiological states are defined by the alternating membrane recruitment of either the MAP kinase MAK-2 (while the cell receives the signal) or the protein SO (while the cell sends the signal).

In recent years, cell fusion has been reported for many other fungi occupying diverse ecological niches, for example: the plant-endophyte *Epichloë festucae*, the plant-pathogen *Fusarium oxysporum*, or the zombie-ant fungus *Ophiocordyceps unilateralis*, among many others. However, the mechanisms mediating cell fusion in these organisms have not been studied, and the question whether the "cell dialog" process is conserved remained unanswered.

In this study, we show that the MAK-2/SO homologous of *Botrytis* (BMP-1/BcSO) oscillate in a similar manner during fusion-related cell-cell communication. Surprisingly, we found that *N. crassa* and *B. cinerea* germlings even undergo mutual attraction and establish physical contact, although complete fusion events were not detected.

Together, these data indicate that the cell-cell communication mechanism mediating cell fusion is conserved in *B. cinerea* and that different fungal species share a common molecular language.

## FBV04

### Phosphorylation-dependent regulation of fungal signaling pathways

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**Introduction:** The filamentous fungus *Sordaria macrospora* is an excellent genetic system to study biological processes in eukaryotes such as multicellular development. As a fundamental process, we investigate the formation of fruiting bodies, which is controlled by various signaling pathways, including the NADPH oxidases (NOX) complex, the pheromone response MAP kinase pathway and the highly conserved striatin-interacting phosphatase and kinase (STRIPAK) complex. In *S. macrospora*, this complex is involved in sexual development, hyphal fusion and vegetative growth and comprises the core components protein phosphatase 2A (PP2A) and the membrane protein PRO22.

**Objectives:** We are interested in characterizing specific phosphorylation sites of target proteins which are potentially dephosphorylated by the STRIPAK complex to get a detailed insight into the regulatory mechanism of this complex.

**Materials & methods:** On the basis of proteomic and phosphoproteomic analysis of STRIPAK deletion strains, we generated phospho-deficient and phospho-mimetic variants of target proteins in order to unravel the function of various phosphorylation sites.

**Results:** The phosphoproteomic analysis showed that the regulator of NADPH oxidases, the scaffold protein of the pheromone response pathway and a member of the p21-activated kinase (PAK) family are putative phospho-targets of the STRIPAK complex. The mutation of specific phosphorylation sites revealed their role during the sexual development of *S. macrospora*.

**Conclusion:** Our data suggest that the STRIPAK complex is critical for the regulation of signaling networks by protein phosphorylation/dephosphorylation. Further experiments will increase our knowledge about the mechanistic function of STRIPAK in eukaryotic biological processes.

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## FBV05

### Schizophyllum pheromone response and dikaryotic development

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For sexual reproduction in tetrapolar basidiomycetes, pheromone recognition and response are necessary. We have previously analyzed components involved in this process, establishing labeled nuclei (histone 2B fused to GFP), microtubule and actin staining by immunofluorescence and LifeAct, dissecting the mating cascade of events, the involvement of Ras, and the specific two-gene encoded dynein in *Schizophyllum*. The activation of Ras via G-protein-coupled receptors (GPCR) signaling pathways. Interrelation between Ras signaling and phosphatidylinositol signaling by inositol monophosphatase, a key enzyme in inositol production, has been observed. Constitutively active Ras1 leads to repression of inositol monophosphatase transcription and as a consequence alters inositol phosphates profile. Less abundance of the cytoskeleton

protein actin-1 in the wild type and constitutive active Ras1 mutant on the proteomic level could be linked to a less dynamics of actin cytoskeleton verified by Lifeact-GFP strain after LiCl treatment. The regulator of G-protein signaling, Thn1, plays a key role in coordinating sesquiterpene production, pheromone response and sexual development. The gene *thn1* is transcriptionally regulated in response to mating with a role in clamp cell development and transcriptional down-regulation of *hyd6* and *sc3* hydrophobin genes, and it negatively regulates cAMP signaling and secondary metabolism. Disruption of *thn1* affects dikaryotization by reducing clamp fusion and development with predominant non-fused pseudoclamps. Thn1 was also shown to be necessary for the biosynthesis of sesquiterpenes and an altered spectrum of sesquiterpenes in  $\Delta thn1$  strains is linked to transcriptional up-regulation of biosynthesis genes.

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## FBV06

### RNA editing leads to proteome diversification during fungal sexual development

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RNA editing is the selective insertion, deletion, or substitution of nucleotides, leading to sequence changes that could alternatively be directly encoded by the DNA. It is conserved in all domains of life and occurs in diverse RNA species. Adenosine (A) to inosine (I) RNA editing is common in metazoa and was recently detected in filamentous fungi. During translation, I is interpreted as guanosine (G), effectively leading to A-to-G changes in amino acid codons. RNA-seq revealed that RNA editing is linked to the sexual phase in the model ascomycete *Sordaria macrospora*. Editing of protein-coding transcripts leads to putative amino acid changes in 361 "edited in fruiting body formation" (EFD) proteins, 47 of which show an extended C-terminus due to editing of their stop codon to a tryptophan codon (stop-loss). Using a mass spectrometry approach, we identified peptides from C-terminal EFD protein extensions specifically in protein extracts from perithecia, but not in protein extracts from complete mycelia. Synthetic peptides were used to quantify DNA-encoded and extended protein isoforms. This approach revealed that the abundance of extended proteins increases in late sexual development. Deletion of *efd* genes and complementation analysis with never edited and pre-edited alleles revealed functions for *efd* alleles in ascosporeogenesis. Further, we show that C-terminal extension of proteins leads to localization changes due to additional targeting signals. Taken together, A-to-I RNA editing occurs specifically during late sexual development in *S. macrospora* and could be a means for proteome diversification during ascosporeogenesis, a process that requires massive cellular reorganization.

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## FBV07

### Exploiting fungi for secondary metabolites

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With their diverse structures, biosyntheses and biological activities, fungal metabolites have attracted chemists and biologists for many years. Moreover, fungal secondary metabolites have resulted in the development of

indispensable therapeutics, as exemplified by penicillin, lovastatin, fingolimod and cyclosporine.<sup>1</sup>

In the past years, we have studied neglected groups of both Basidiomycota and Ascomycota for the production of novel metabolites, relying on extensive field work, phylogenetic pre-selection methods, state-of-the-art methods for fermentation, downstream processing and structure elucidation. These projects have resulted in the discovery of several novel bioactive compounds from rare and new species, using a bioassay-guided isolation procedure.

Specifically, a new species of the pleurotoid genus *Hohenbuehelia* sp., which readily produces fruitbodies and can trap nematodes in culture, yielded various novel pleurotin derivatives.<sup>2</sup> Novel cuparenic-mevalonic acid conjugates were found from a new species of the genus *Deconica*.<sup>3</sup> Moreover, chlorinated gymnopalynes A and B with an acetylenyl side chain were isolated from a new *Gymnopus* species.<sup>4</sup>

From *Hypoxylon rickii* we isolated over 20 novel metabolites from six different chemical classes from a single fermentation.<sup>5</sup>

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#### FBV08

##### **Transcriptome analysis of the unrelated fungal $\beta$ -lactam producers *Acremonium chrysogenum* and *Penicillium chrysogenum* reveal Velvets important role during conventional strain improvement.**

D. Terfehr<sup>\*1</sup>, T. A. Dahlmann<sup>1</sup>, U. Kück<sup>1</sup>

<sup>1</sup>*Ruhr-University Bochum, General and Molecular Botany, Bochum, Germany*

**Introduction:** *Acremonium chrysogenum* and *Penicillium chrysogenum* are the industrial producers of the  $\beta$ -lactam antibiotics cephalosporin C and penicillin G, which are used worldwide for the treatment of bacterial infections. Wild-type isolates from these taxonomically unrelated fungi were improved in conventional mutagenesis programs to reach economically relevant titers of  $\beta$ -lactam antibiotics. However, there is still a lack of knowledge how the increased  $\beta$ -lactam production was achieved.

**Objectives:** We used RNA-seq analyses of wild-type and industrial strains of both species to address the question whether both fungi have undergone similar expressional changes during strain improvement. Furthermore, we investigated two mutants lacking Velvet, a global regulator of fungal secondary metabolism.

**Methods:** RNA-seq, identification of secondary metabolite clusters, expression quantification, and functional categorization.

**Results:** Improved strains of both fungi show common expressional adaptations, namely the upregulation of pathways supplying precursors and energy for  $\beta$ -lactam production and shutting down not required pathways or cellular functions. Furthermore, we elucidated Velvets regulatory network in the improved strains, containing approximately 50% of all secondary metabolite clusters. Most importantly, strain improvement and Velvet affect the expression of a large set of genes in a similar manner in both improved industrial fungi.

**Conclusion:** The major finding of our comparative transcriptome analysis is that strain improvement programs in two unrelated fungal  $\beta$ -lactam antibiotic producers affect similar metabolic pathways and cellular functions. Furthermore, we assign Velvet an important role during strain improvement in both organisms

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## Short Lecture Biochemistry & Secondary Metabolomics 16 April 2018 • 08:00–10:00

### BSMV01

#### **Exposing the dioxygen-binding mode of a bacterial flavin-dependent oxygenase**

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The ubiquitous flavin-dependent enzymes commonly facilitate redox reactions such as the dehydrogenation or oxygenation of organic substrates. Recently, the non-canonical flavoenzyme EncM, which catalyzes a key oxidative rearrangement in the biosynthesis of the bacterial polyketide antibiotic enterocin, was shown to employ a flavin-N5-oxide as oxygenating species in place of the common flavin-C4a-peroxide. Yet, the molecular basis and structural requirements promoting the enzymatic formation of the flavin-N5-oxide via reaction of reduced flavin with O<sub>2</sub> remain unknown. Here we employ O<sub>2</sub>-pressurized X-ray crystallography among other techniques to elucidate the spatial positioning of O<sub>2</sub> in the surroundings of the EncM flavin cofactor and provide a rationale for the generation of the flavin-N5-oxide. We show that EncM features a highly-defined cavity at the *re*-side of the flavin, in which O<sub>2</sub> is situated in close proximity to N5, consistent with the geometric and mechanistic requirements for generation of a transient flavin-N5-peroxide adduct that affords flavin-N5-oxide upon elimination of the distal oxygen atom. While the reaction of flavoproteins with oxygen has been intensively investigated for decades, this study for the first time unambiguously elucidates the spatial arrangement between O<sub>2</sub> and the flavin cofactor required for formation of a covalent flavin-oxygen adduct. Our work thus illustrates how O<sub>2</sub> reactivity can be harnessed in an enzymatic environment and provides crucial knowledge for future rational design and bioengineering of flavoproteins.

## BSMV02

### Plasmid Replication Associated Single-Strand Specific Methyltransferases.

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<sup>3</sup>CNRS Gif-sur-Yvette, France

Recent advances in Next Generation SMRT sequencing technology allows not only the sequence and assembly of a variety of genomes, but also the detection of the epigenetic status of the sequenced DNA including detection of 6mA, 4mC and oxidized forms of 5mC. The 48% of organisms harbor active Type II MTases with no apparent cognate restriction enzyme. These "orphan" MTases exhibit patterns of incomplete methylation that distinguish them from RM system MTases. The genome and methylome analysis of total DNA from two pathogenic strains, *Burkholderia cenocepacia* J2315 and *E. coli* O104:H4 has revealed the presence of two unusual MTases not previously characterized. Both were plasmid-encoded by ORFs in pBCA072 for *B. cenocepacia* J2315 and pESBL for *E. coli* O104:H4. In this report we present data on the biological and biochemical properties of these two very closely-related MTases. They both result in single-stranded, almost non-specific m6A modification, within the motif SAB (where S = C or G and B = C, G or T). This methylation is partial and only detected on plasmid DNA. We have called these enzymes M.BceJIII and M.EcoGIX respectively. The genetic and biochemical studies suggested that the activity of these enzymes are associated with plasmid replication and depended on the origin of replication. Moreover, we demonstrated that these enzymes work as a complex with DNA polymerase I during plasmid replication. It is possible they control plasmid and phage replication by discriminating DNA polymerase I-dependent and non-dependent plasmids origins. Also we suggest that the base flipping inherent to DNA modification may allow the MTase to perform a DNA helicase function and thereby help to control the rate of DNA polymerization to prevent excessive recombination.

## BSMV03

### Structure of the 1.7 MDa Hydrazine Dehydrogenase Complex from Anammox Bacteria

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<sup>2</sup>Max Planck Institute of Biophysics, Department of Structural Biology, Frankfurt a. M., Germany  
<sup>3</sup>Radboud University, Department of Microbiology, Institute for Water and Wetland Research, Nijmegen, Netherlands  
<sup>4</sup>Delft University of Technology, Department of Biotechnology, Delft, Netherlands

Anaerobic ammonium-oxidizing (anammox) bacteria use the oxidation power of nitrite (NO<sub>2</sub><sup>-</sup>) to oxidize ammonium (NH<sub>4</sub><sup>+</sup>) to the unusual intermediate hydrazine (N<sub>2</sub>H<sub>4</sub>), which is finally oxidized to N<sub>2</sub> in a reaction catalyzed by the octaheme c-type cytochrome hydrazine dehydrogenase (HDH). Like other members of this family, HDH forms covalently crosslinked trimers (α<sub>3</sub>) which have been shown to associate into higher oligomers in solution namely 24mers (α<sub>3</sub>)<sub>8</sub> and 30mers (α<sub>3</sub>)<sub>10</sub> with molecular masses of 1.6 and 2.0 MDa, respectively (Maalcke, 2016). We determined the crystal structure of a 24meric HDH complex from the anammox organism *Kuenenia stuttgartiensis* at 2.8 Å resolution. This complex is composed of eight conically shaped HDH trimers that are positioned at the corners of a cube pointing their apexes outwards. This assembly enables the interactions of 24

hemes from each trimer to form an astonishing network of 192 hemes. In addition, 12 molecules of a ~10 kDa protein identified as the gene product of *kustc1130* were found at each edge of the cubic assembly between two neighboring HDH trimers. This complex has an overall molecular mass of 1.7 MDa. This work was complemented by structural studies of both 24mer and 30mer HDH assemblies by cryo-electron microscopy (cryo-EM). Various biophysical methods showed that oligomerization of HDH trimers to both 24mers and 30mers was promoted at high ionic strength (>150 mM). Moreover, the presence of *kustc1130* preferably induced the formation of the 24mer assembly independent of ionic strength. HDH was found to be most active with its binding partner at 100-150 mM ionic strength. We therefore propose that the 24mer HDH assembly with 12 molecules of its binding partner is the physiologically relevant oligomeric state of HDH.

## BSMV04

### A modified pathway for leucine degradation in bacteria and archaea

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<sup>2</sup>Christian-Albrechts University of Kiel, Institut für Allgemeine Mikrobiologie, Kiel, Germany

Many microorganisms, both bacteria and archaea, are capable to catabolize amino acids, e. g. leucine. So far, only one pathway for aerobic catabolism of leucine is known. It begins with its deamination and following oxidative decarboxylation yielding isovaleryl-CoA. Its oxidation leads to 3-methylcrotonyl-CoA, which is then carboxylated to 3-methylglutaconyl-CoA. The 3-methylglutaconyl-CoA hydratase catalyzes its hydration to (S)-3-hydroxymethylglutaryl-CoA [(S)-3-HMG-CoA], which is then cleaved to acetyl-CoA and acetoacetate by (S)-3-HMG-CoA lyase. Here we introduce a modified pathway of leucine degradation, which differs from the conventional one in the last two steps: the hydration of 3-methylglutaconyl-CoA to 3-HMG-CoA is catalyzed by an (R)-specific enoyl-CoA hydratase, whereas (R)-3-HMG-CoA cleavage is catalyzed by a citrate lyase β-subunit-like (CitE-like) protein. This pathway functions in most haloarchaea (e.g., *Haloarcula marismortui* and *Haloferax mediterranei*) as well as in some actinobacteria (e.g., *Mycobacterium tuberculosis*). Being different from the classical pathway in its key enzymes and intermediate, this modified pathway is elusive for bioinformatic analysis. The key enzymes of the modified pathway are highly specific for (R)-stereoisomer of the substrate. Our study demonstrates that variety of stereoisomers should be considered in the studies of natural and, potentially, in design of synthetic metabolic networks.

## BSMV05

### Adaptation of the filamentous fungus *Aspergillus nidulans* to low temperature stress

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<sup>3</sup>Friedrich Schiller University Jena, Department of Microbiology and Molecular Biology, Jena, Germany

Natural products can serve as mediators of biological communication and as valuable sources for therapeutic compounds. The genes involved in the production of these

compounds are usually organised in clusters. To elucidate the variety of natural compounds produced by an organism different stress factors can be applied, such as sharp temperature shifts, to induce the expression of silent gene clusters. To explore the range of bioactive compounds formed at low temperature stress, we studied the transcriptome and proteome profile of *A. nidulans* at 10 °C and 37 °C.

The low temperature stress response was investigated via comparative gel-based (DIGE) and gel-free (LC-MS/MS) proteomic approaches. Genes involved in the categories of cold stress protection, cell development and biosynthesis of natural products were up-regulated. Moreover, the specific formation of natural products at low temperature was confirmed at the metabolite level by LC-MS/MS. On the genetic and protein level our analyses revealed the induction of factors, which regulate the sexual cycle in *A. nidulans*. Further on, the sporogenic factor PsiA was found, which regulates the balance between asexual and sexual spore development. Some of the identified secondary metabolites like asperuranone have been reported to trigger morphological differentiation and to exhibit anti-proliferative activity in human cell lines. Additional induced secondary metabolites show biological activity against gram-positive bacteria and fungi.

In summary, our study demonstrates that low temperature stress induces the production of a variety of secondary metabolites in *A. nidulans*. This approach represents a strategy to exploit the potential of filamentous fungi as sources of secondary metabolites.

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#### BSMV06

##### What we can learn from microaerophilic bacteria - comparative genome survey of $\epsilon$ -proteobacteria uncovers hidden biosynthetic potential

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**Introduction:** Natural products of microbial origin have been a rich source of compounds for drug discovery. In particular, actinomycetes and myxobacteria are prime producers of industrially important natural products. However, studies of such well-investigated organisms have led to high rediscovery rate of already known natural product classes. Nevertheless, global genome mining efforts implied the general abundance of secondary metabolite gene clusters within all bacterial phyla. Motivated by these findings, we performed a detailed comparative genome survey of bacteria belonging to the  $\epsilon$ -proteobacteria, a bacterial phylum that has so far been overlooked in terms of secondary metabolite production.

**Objectives:** Genome-analysis revealed unique putative secondary metabolite clusters within microaerophilic bacteria.

**Materials and Methods:** We applied a comparative bioinformatic genome analysis and identified a so far uncharacterized NRPS-PKS biosynthetic hybrid cluster. After verification of gene expression by RT-PCR, the identification of the respective secondary metabolite was accomplished using a comparative metabolomic and NMR-guided

identification strategy. The structure proposal was verified by a modular total synthesis and the bioactivity of different derivatives was tested.

**Results:** The identified secondary metabolite is unusual as it is the first hybrid natural product produced by microaerophilic/anaerobic bacteria; it contains a very rare modified amino acid and shows selective activity against certain pharmacological important proteases. Using synthesized derivatives, we performed the structure-activity studies, which indicate to specific mode of action.

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#### BSMV07

##### Approaches to find New Antibiotics from Novel Indonesian Actinomycetes

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Indonesia, as a "biodiversity hotspot", harbours unknown microbial species, which may produce novel antibiotics. Our project aims to identify new antibiotics from Indonesian actinomycetes. Two Javanese strains (4100 and 4103) have been pre-characterised and their genome has been sequenced. To isolate new compounds, we pursue two approaches.

**Regulator-driven approach:** The SARP (*Streptomyces* Antibiotic Regulatory Protein)-type regulator PapR2 is a promising candidate to activate the expression of silent gene clusters in actinomycetes. Overexpression of *papR2* in *S. lividans* leads to the activation of the silent undecylprodigiosin biosynthetic cluster. To activate silent clusters, strain 4100 was transformed with the vector pRM4/papR2. Genome mining with the cluster finding tool antiSMASH identified two SARP homologous sequences and five SARP-binding motifs. Mass spectrometry revealed a mass pattern unknown so far. The production of the corresponding compound must be induced by PapR2.

**Genome-mining-driven approach:** Strain 4103 produces a substance active against gram+ bacteria. In a *Bacillus subtilis* promoter-based reporter assay 4103 turned out to induce a promoter, which reacts on DNA synthesis targeting substances. In the genome of strain 4103 antiSMASH detected a phosphonate cluster, among others. Further bioinformatics screening showed that this cluster contains core genes involved in DNA repair and nucleotide biosynthesis. These genes may be part of a DNA repair function as a self-resistant mechanism. Bioactivity tests against the phosphonate-sensitive strain WM6242 confirm the production of a phosphonate. The expression of the essential gene *pepM* under production conditions could be confirmed via RT-PCR. So 4103 produces a DNA damaging phosphonate antibiotic.

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#### BSMV08

##### The marine $\gamma$ -proteobacterium *Microbulbifer* sp. HZ11 brominates *Pseudomonas aeruginosa* derived alkyl quinolones

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**Question:** Marine organisms are a promising source for new natural compounds. The marine Gram-negative bacterium *Microbulbifer* strain HZ11 harbors a gene cluster similar to

the alkyl quinolone (AQ) biosynthetic operon of *Pseudomonas aeruginosa*. AQs such as 2-heptyl-3-hydroxy-4(1H)-quinolone (PQS) and 2-heptyl-4(1H)-quinolone (HHQ) serve as virulence inducing signal molecules for *P. aeruginosa*.

**Objectives:** We aim at the identification of AQ-type compounds resulting from *de novo* biosynthesis or AQ-modifying reactions of *Microbulbifer* sp. HZ11. In order to pave the way for the production of new bio-active compounds we attempt to characterize the enzymes involved.

**Methods:** Alkyl quinolones and derivatives were synthesized enzymatically or chemically. *Microbulbifer* cultures were screened for new AQs and for AQ modifying activities. Compounds were identified by LC/LC-MS, and the effect of new AQs on bacterial growth was determined.

**Results:** Supplementation of *Microbulbifer* cultures with HHQ resulted in the formation of PQS and an unknown compound which was identified as a brominated AQ. Pentyl and nonyl derivatives of HHQ as well as the antibiotic compound 2-heptyl-4-hydroxyquinoline *N*-oxide (HQNO) were brominated as well, while this was not the case for PQS. Brominated AQs were less inhibitory for growth of *Microbulbifer* strain HZ11 than the parent compounds.

**Conclusion:** Bromination might comprise a mechanism to detoxify AQs produced in marine polymicrobial communities, likely contributing to the fitness of *Microbulbifer* strain HZ11. The underlying halogenase might be exploited for biotechnological approaches and is also a promising tool for the attenuation of AQ-mediated virulence in *P. aeruginosa*.

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## Short Lecture

### Archaea

16 April 2018 • 08:00–10:00

#### ARV01

##### How do coal degrading methanogens make biogas? Unravelling central metabolism of *Methermicoccus shengliensis*

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Methane is the second most important greenhouse gas on earth. The main source of methane emission into the atmosphere are methanogenic archaea emphasizing the importance of those organisms for the global carbon cycle. Although methanogens have been studied for more than 111 years [1], a novel methanogenic pathway was recently discovered: the thermophilic methanogen *Methermicoccus (M.) shengliensis* is able to use a large variety of methoxylated aromatic compounds as substrates for methane generation [2-4]. Despite the significance and novelty of this unique archaeon a detailed analysis of its metabolism is still missing. Here, we used transcriptomic and proteomic methods to investigate the response to growth on methoxylated aromatics along with enzymological characterization of new methyltransferase enzymes. The transcriptomic analysis revealed a gene cluster highly expressed under growth on the methoxylated compound trimethoxybenzoate. The encoded enzymes are most likely essential for methoxytrophic methanogenesis. Four genes were chosen for heterologous expression in *E. coli* and subsequent purification by affinity chromatography.

Unravelling the ability of this methanogen to convert methoxylated compounds to methane is indispensable for future applications of *M. shengliensis* in coal bed methane production or biodegradation of oil.

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#### ARV02

##### Structure and function of the archaeal chemotaxis protein CheY

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Motility is an important feature of microorganisms and provides an efficient strategy to respond to environmental changes. Bacteria and archaea have developed fundamentally different rotary motors enabling their motility, termed flagellum and archaellum respectively. Bacterial motility along chemical gradients, chemotaxis, critically relies on the response regulator CheY. When phosphorylated CheY inverts the rotational direction of the flagellum via a switch complex at the base of the motor. Some archaea, like euryarchaea, have received the chemotaxis system via horizontal gene transfer.

The structural difference between archaellum and flagellum and the presence of functional CheY in archaea raises the question how the CheY protein changed to allow communication with the archaeal motility machinery.

We solved the crystal structure of archaeal CheY and show that it shares the overall structure and mechanism of magnesium-dependent phosphorylation with its bacterial counterpart. However, bacterial and archaeal CheY differ in the electrostatic potential of their  $\alpha 4$  helix. In bacteria, the  $\alpha 4$  helix is important for interaction with the flagellar switch complex, a structure that is absent in archaea. We demonstrated that phosphorylation dependent activation, and conserved residues in the archaeal CheY  $\alpha 4$  helix, are important for interaction with the archaeal specific adaptor protein, CheF. This protein forms a bridge between the chemotaxis system and the archaeal motility machinery.

Conclusively, archaeal CheY proteins conserved the central mechanistic features between bacteria and archaea, but differ in the  $\alpha 4$  helix to allow binding to an archaellum specific interaction partner.

### ARV03

#### Acetate metabolism in *Haloferax volcanii*: Characterization of an acetate transporter and of enzymes involved in acetate activation and gluconeogenesis

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*Haloferax volcanii* is an aerobic haloarchaeon that grows on acetate as the sole carbon and energy source. The genes and proteins involved in the transport and activation of acetate, in anaplerosis and in gluconeogenesis (PEP-synthesis) during growth on acetate were analyzed applying transcript analyses, biochemical characterizations of enzymes and growth experiments with respective deletion mutants. (i) An acetate transporter of the sodium-solute-symporter family (SSF) was identified and characterized. Kinetics of acetate transport were analyzed using <sup>14</sup>C-labeled acetate. The functional involvement of the transporter was proven in transport experiments with a  $\Delta$ ssf-deletion mutant. (ii) Two paralogous AMP-forming acetyl-CoA synthetases (ACS) involved in acetate activation have been identified and characterized. (iii) The functional involvement of the glyoxylate cycle as an anaplerotic sequence was concluded from growth experiments with an isocitrate lyase knockout mutant. (iv) Enzymes involved in gluconeogenesis from acetate, namely two malic enzymes and PEP-synthetase, were identified and characterized. Together, this is the first report - based on transport experiments - of a functional acetate transporter in the domain of archaea and a comprehensive study of enzymes involved in acetate activation and gluconeogenesis in *H. volcanii*.

### ARV04

#### Requirement of methanogenesis for CO-dependent growth of *Methanosarcina acetivorans*

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Methanogenesis is a unique energy metabolism carried out by members of the domain *Archaea*. While most methanogens employ only a single methanogenic pathway, *Methanosarcina* (*M.*) species are more metabolically versatile. *M. acetivorans* cannot use hydrogen and carbon dioxide as growth substrates, but is able to grow on methylated compounds (e.g. methanol), acetate or carbon monoxide (CO). During growth on CO, substantial amounts of acetate are produced, which is generated through the acetyl-CoA pathway and could, thus, be coupled to energy conservation by substrate-level phosphorylation. This raises the question if *M. acetivorans* can grow on CO without the need to produce methane. In order to address this question, the operon encoding the energy-converting *N*<sup>5</sup>-methyltetrahydroscarinapterin (H<sub>4</sub>SPT):coenzyme M (HS-CoM) methyltransferase (MTR), which couples the reversible methyl-group transfer from H<sub>4</sub>SPT to HS-CoM to translocation of sodium ions, and, thus, serves as a chemiosmotic coupling site, was deleted from the chromosome. The mutant still needed a methyl-group donor (e.g. methanol or trimethylamine) to grow with CO, even though only in small, noncatabolic amounts. During experimentation with the *mtr* mutant strain, several suppressor strains were selected, which can grow on CO alone. One of the suppressor strains, MKOmrSF, grew on CO similar to the wild type and the *mtr* mutant (on CO +

methanol) in terms of growth rate, cell yield, and metabolites produced, except no significant amounts of methane could be detected. Genome sequencing of MKOmrSF revealed a  $\pm$  200 kbp chromosomal lesion, which responds to ca. 3% of the genome. The question of how the organism can bypass the previously assumed unconditional requirement of methane formation will be discussed.

### ARV05

#### Regulation of the *mtsDFH*-genes from *Methanosarcina acetivorans* as a paradigm of archaeal signal transduction

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*Methanosarcina acetivorans* is an obligate anaerobic archaeon, which is capable to use a wide range of substrates for methanogenesis. In order to metabolise methyl sulfides, the three corrinoid/methyltransferase fusion proteins MtsD, MtsF and MtsH are important. These are transcriptionally and post-transcriptionally regulated by the availability of dimethyl sulfide. The regulators MsrC, MsrF and MsrG are involved in this transcriptional regulation. Genetic studies indicate the regulators' function as activators [1]. Beside the mentioned regulators, the sensor kinase MsmS is involved in the regulation of the methyl transferase MtsF. MsmS is a multi-domain protein and regulates its autophosphorylation via the redox state of the covalently bound heme cofactor [2]. The genome of *M. acetivorans* codes for two additional homologous sensor kinases, which are all encoded in a genomic region close to the Msr regulators and the Mts methyl transferases. Therefore, it has been suggested that the sensor kinases and the regulators are part of a two-component regulatory system.

In order to investigate whether MsmS and MsrG belong to one signal transduction pathway, protein-protein interaction studies using *in vivo* crosslinking experiments were performed. First results indeed suggest that MsmS and MsrG interact with each other. Using electrophoretic mobility shift assays we furthermore were able to show that the regulator MsrG specifically binds to two potential sites in the promoter region of *mtsH*. Apart from the identification of a specific MsrG binding site, future studies aim at exploring the signal transduction within this system to get a better understanding of archaeal signal transduction.

#### References:

[1] Bose A. et al. (2009) Microbiol. 1.

[2] Molitor B. et al. (2013) J. Biol. Chem. 25.

### ARV06

#### Characterization of the putative Radical S-adenosyl-L-methionine methyltransferase responsible for the unique C-5-methylation of arginine in the methyl-coenzyme M reductase.

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Biological CH<sub>4</sub> formation by methanogenic archaea is a globally important process in the biogeochemical carbon cycle. About one billion tons of CH<sub>4</sub> are released each year, with one third escaping into the atmosphere. Methyl-coenzyme M reductase (MCR) plays the key role in this

process by catalyzing the last and CH<sub>4</sub>-releasing step. MCR converts CH<sub>3</sub>-coenzyme M and coenzyme B to a heterodisulfide with the release of CH<sub>4</sub>. Crystal structures of different MCRs revealed the presence of several posttranslational amino acid modifications. Among these, a 5-C-(S)-CH<sub>3</sub>-Arg is located in the substrate binding tunnel of McrA. The methyltransferase that introduces the CH<sub>3</sub> group at the inert sp<sup>3</sup>-hybridized C-5 position of Arg is unknown.

In our work, we identified a Radical SAM methyltransferase responsible for the methylation of Arg. Subsequent to genome analysis and multiple sequence alignments we created a ko strain by deleting the candidate gene *ma4551* (methanogenesis marker 10) in *M. acetivorans* WWM1. This led to the production of an active MCR lacking the methylation of the respective Arg. Growth experiments with the wt and the ko strain demonstrated that the methylated Arg is important for MCR stability under stress conditions. The thermal stability of the purified MCR variant lacking the methylation is impaired in comparison to the wt enzyme. The melting points (T<sub>m</sub>) were determined with the nanoDSF method. For the MCR from the wt a T<sub>m</sub> of 82.6±0.2 °C was determined. In contrast, the T<sub>m</sub> of MCR from the ko strain was at 74.6±0.1 °C.

In the future, the newly identified Radical SAM methyltransferase Ma4551 from *M. acetivorans* will be produced and purified in order to further characterize it. An enzyme activity assay will be established to study the methylation of Arg *in vitro*.

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#### ARV07

##### Unraveling the role of 2-keto-3-deoxy(-6-phospho)-gluconate aldolase in hexose and pentose metabolism of *Sulfolobus acidocaldarius* by mutational analyses

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*S. acidocaldarius* degrades hexoses (D-glucose and D-galactose) via the branched ED pathway while pentose (L-arabinose and D-xylose) have been shown to be degraded simultaneously via the Weimberg and Dahms pathways in a 1:1 ratio. In both branches of the ED pathway as well as in the Dahms pathway, the 2-keto-3-deoxy (-6-phospho)-gluconate aldolase (KD(P)GA) plays a key role catalyzing the cleavage of the 2-keto-3-deoxy-hexanoate and -pentanoate derivatives. Notably, bioinformatic studies indicated that only one KD(P)GA homologue exists in *S. acidocaldarius*, which is encoded by the gene *Saci\_0225*. The aldolase has already been characterized, but no *in vivo* functional analysis by gene deletion has been performed so far. Here, we constructed an aldolase deletion mutant of *S. acidocaldarius* MW001 and compared its growth phenotype on hexose and pentose with the wild type. The aldolase deletion completely abolished growth on D-glucose/dextrin, which is in agreement with the previous findings. However, the growth of the mutant on pentoses was surprisingly not influenced indicating that the aldolase dependent Dahms pathway is not involved and thus dispensable for pentose degradation in *S. acidocaldarius* MW001. These findings were confirmed by the deletion of the key enzyme of the Weimberg pathway, the 2-keto-3-deoxy-arabinoate/xylonate dehydratase (KDAD/KDXD). This gene deletion resulted in a complete growth deficiency of the mutant on pentose sugars. Thus, the aldolase dependent Dahms pathway could not substitute the aldolase independent Weimberg pathway. In summary, the results confirmed the key function of KD(P)GA in the

branched ED pathway and showed that *S. acidocaldarius* MW001 strictly rely on the Weimberg pathway for pentose degradation.

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#### ARV08

##### CRISPRi as a tool to repress gene expression in archaea

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At the moment no methods are available to knock down genes in archaea and only a handful of regulatable promoters are accessible. This situation severely hampers the study of essential genes in particular and genetic analysis of complex gene networks in general.

CRISPR-Cas systems can be repurposed as effective tools for gene regulation in bacteria and eukarya, using the type II specific protein Cas9 (1, 2). Archaea do not possess type II systems and known bacterial Cas9 proteins might not be active due to extreme growth conditions. So we take advantage of the endogenous type I-B system of *H. volcanii* to repress gene expression.

A short crRNA guides a multi-Cas-protein effector complex (Cascade) to bind foreign DNA and then the nuclease Cas3 is recruited and catalyses DNA degradation. In a *cas3* deletion strain, Cascade with crRNAs matching a gene of interest will bind to the target DNA. If crRNAs bind to the promoter or coding region, transcription initiation or elongation will be blocked, repressing expression of the respective gene (3, 4). Just recently we established CRISPRi for archaea in the model organism *H. volcanii* using the endogenous type I-B system (5).

We show that CRISPRi can be applied successfully as a gene regulatory tool in *Haloflex* in a plethora of contexts. We demonstrate effective knockdown of a plasmid-borne gene, as well as chromosomal genes, which can be monocistronic or part of a polycistronic operons. We also successfully achieved repression of essential genes.

#### References:

- [1] Doudna and Charpentier, 2014. *Science*, 346(6213):1258096
- [2] Qi et al., 2013. *Cell*, 152(5):1173-83
- [3] Rath et al., 2015. *Nucleic Acids Res*, 43(1):237-46
- [4] Luo et al., 2015. *Nucleic Acids Res*, 43(1):674-81
- [5] Stachler and Marchfelder A, 2016. *JBC*, 2016, vol.291, no. 29, pp. 15226-15242

## Hans-Günter-Schlegel Lecture

### 16 April 2018 • 11:30–12:15

IL05

#### Lipoproteins of Gram-Positive bacteria: Key players in immune response and commensalism

F. Götz\*<sup>1</sup>

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Bacterial lipoproteins (Lpp) belong to the class of lipid-anchored proteins that are in Gram-negative bacteria anchored both in the cytoplasmic and the outer membrane and in Gram-positive bacteria only in the cytoplasmic membrane. In contrast to Gram-negative bacteria, lipoprotein maturation and processing is not vital in Gram-positive bacteria, however, they play an important physiological role, in nutrient and ion acquisition allowing particularly pathogenic species to better survive in the host. In Gram-positive bacteria Lpp represent the major protein group of the surfacome. They also represent important MAMPs (microbe-associated molecular patterns) by alerting our immune system via interaction with TLR2 (Toll-like receptor 2). More recently it has been shown that the lipid structure of Lpp has a profound influence on the intensity of our immune response. In commensal staphylococcal species Lpp carry a long-chain N-acyl group, while non-commensal species carry only N-acetylated lipid moiety. While the non-commensal species and their isolated Lpp induce a fulminant immune reaction the commensal species rather lulls our immune system. These findings confirm our hypothesis that successful pathogenic bacteria but also harmless commensal bacteria can only survive in the host when they manage to escape or evade the immune defense system. There are two main strategies of bacteria to circumvent the immune system. One is directed against phagocytes including inhibition of chemotaxis or phagocytosis or colonization of phagocytes. The other strategy is directed against the innate and adaptive immunity such as avoiding to evoke an immune response.

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## Plenary Lecture

### Plant-Microbe-Interaction

### 16 April 2018 • 13:30–14:30

IL06

#### How plant pathogenic bacteria manipulate the plant

U. Bonas\*<sup>1</sup>

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Pathogenicity of most Gram-negative plant-pathogenic bacteria depends on the plant-inducible type III secretion (T3S) system which translocates effector proteins (T3Es) into the plant cell cytosol. We study the interaction between *Xanthomonas campestris* pv. *vesicatoria* (Xcv) and its host plants pepper and tomato. The bacteria enter the plant tissue through stomata or wounds and stay locally in the intercellular spaces of the plant tissue. In susceptible plants, T3Es interfere with host cell processes to the benefit of the pathogen and allow bacterial multiplication. In resistant plants, single resistance genes mediate specific recognition of a particular T3Es often resulting in the induction of a hypersensitive response (HR), a rapid and localized programmed cell death halting pathogen ingress. Xcv injects more than 35 different T3Es into the plant cell, termed Avr (avirulence protein) or Xop (Xanthomonas outer protein). Among the T3Es from Xcv are plant immunity suppressors, cell death inducers, a ubiquitin ligase, , the transcription

factor AvrBs3 (also termed "TALE") and proteins of unknown function. Selected T3Es will be discussed.

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IL07

#### Microbiome research for biotech and health solutions

G. Berg\*<sup>1</sup>

<sup>1</sup>Institute of Environmental Biotechnology, Graz, Austria

The host-associated microbiome is a crucial factor for fitness and health of the holobiont. Although this fact is known more than 100 years, multi omics technologies revolutionized this field of research. Deep insights and novel findings now allow specific applications in biotechnology. For example, the new perspectives influence plant protection approaches such as biocontrol in agriculture [1]. The development of new tools may impact i) the detection of new bio-resources for biocontrol and plant growth promotion, ii) the optimization of fermentation and formulation processes for biologicals, iii) stabilization of the biocontrol effect under field conditions and iv) risk assessment studies for biocontrol agents. Examples are presented and discussed for the applications mentioned above, as well as an illustration of the global importance of next-generation bio-products as a sustainable alternative for agriculture. Moreover, the central role of the plant microbiome and their beneficial inhabitants for human and environmental health will be discussed [2]. Finally, multi omics can be used to identify new bioactive metabolites, e.g. antibiotics, enzymes and volatiles, for many biotechnological purposes from the diverse world of host-associated microbiomes [3].

#### References:

[1] Berg & Raaijmakers. Saving seed microbiomes. ISME J. 2018

[2] Berg *et al.* Plant microbial diversity is suggested as the key to future biocontrol and health trends. FEMS Microbiol Ecol. 2017

[3] Müller *et al.* Bioprospecting plant-associated microbiomes. J Biotechnol. 2016

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## ePoster Session 1

### Microbial Cell Biology I (MCBP)

### 16 April 2018 • 14:30–17:30

MCBP351

#### Functional membrane microdomain dynamics in *Bacillus subtilis*

R. Wagner\*<sup>1,2</sup>, D. Lopez<sup>1,2</sup>

<sup>1</sup>Spanish National Center for Biotechnology, Madrid, Spain

<sup>2</sup>University of Wuerzburg, Institute for Molecular Infection Biology, Wuerzburg, Germany

The cell membrane, reported as the fluid mosaic model (Singer & Nicholson, 1972), was initially thought to be a homogenous mixture of lipids and proteins. This model has been revisited based on the existence of diverse membrane lipid species that, given their physicochemical properties, laterally segregate and form membrane microdomains. As they have affinity for specific membrane lipids, membrane proteins organize laterally, leading to the formation of microdomains with a specific lipid-protein composition. Assembly of membrane microdomains, a feature traditionally associated exclusively with eukaryotic cells (e.g., membrane or lipid rafts), is now acknowledged to take place in bacterial membranes. Bacterial membranes organize functional

membrane microdomains (FMM), which in certain functional and structural aspects resemble the lipid rafts of eukaryotic cells. FMM act as platforms that promote efficient interaction and oligomerization of protein partners. The FMM are very dynamic structures that move within the cell membrane. The molecular mechanisms that underlie this FMM dynamics, as well as its biological significance, are yet to be understood. We use physiological and genetic studies through live-cell imaging, coupled to a number of biochemical approaches, to determine the mechanisms that orchestrate FMM dynamics. In the bacterial model *Bacillus subtilis*, we explore how FMM dynamics might be influenced by different cell structures, and found that bacterial cell envelope integrity is indispensable. We propose a model in which the cell envelope and its associated cellular structures play an important role in FMM dynamics.

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#### MCBP352

##### **The Missing Cyanobacterial Inorganic Carbon Sensor; The PII-like signaling protein SbtB integrates the cAMP sensing to modulate the inorganic carbon response**

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<sup>3</sup>University of Rostock, Plant Physiology, Rostock, Germany

The PII superfamily consists of widespread signal transduction proteins found in all domains of life. In addition to canonical PII proteins involved in C/N sensing, structurally similar PII-like proteins evolved to fulfill diverse, yet poorly understood cellular roles<sup>1</sup>. Cyanobacteria evolved highly specialized carbon concentrating mechanism (CCM) to cope with limiting atmospheric CO<sub>2</sub> levels, augmenting intracellular inorganic carbon (Ci) levels to ensure efficient CO<sub>2</sub>-fixation<sup>2</sup>. The sodium-dependent bicarbonate transporter SbtA is highly expressed under Ci limitation together with the conserved uncharacterized PII-like SbtB protein. Here, we provide the first structural, biochemical and physiological characterization of this unique PII-like protein from *Synechocystis* sp. PCC6803. SbtB can bind a variety of adenine nucleotides; structures revealed different complexes with cAMP, ADP and AMP, explaining the plasticity of effector binding. The nucleotide-binding pocket was identified to be located between the subunit clefts of SbtB, perfectly matching the structures of canonical PII proteins. This clearly indicates that proteins of the PII superfamily arose evolutionary from a common ancestor, whose structurally conserved nucleotide binding pocket has evolved to sense different adenyl nucleotides fulfilling various signaling functions. Moreover, physiological and biochemical characterization of wild-type and mutant cells provide evidence for involvement of SbtB in low Ci acclimation. Our results suggest that SbtB acts as Ci sensor protein via integrating the energy state of the cell and cAMP binding, highlighting an evolutionary conserved role for cAMP in signaling the cellular carbon status.

#### References:

[1] FEBS J. 283:425-437 (2016).

[2] Life, 5:348-371 (2015).

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#### MCBP353

##### **Characterisation of the Hydrogenase Chaperone HycH of the *Escherichia coli* Formate Hydrogenlyase (FHL) Complex**

P. Skorupa<sup>\*1</sup>, S. König<sup>2</sup>, U. Lindenstrauß<sup>1</sup>, C. Pinske<sup>1</sup>

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*Escherichia coli* is able to produce molecular hydrogen under fermentative conditions. This reaction is catalysed by the seven-subunit, membrane-associated formate hydrogenlyase (FHL) complex. The substrate formate is oxidized by the formate dehydrogenase H subunit of the cytoplasmically-oriented complex. The electrons are subsequently transferred by three FeS-proteins to a [NiFe]-hydrogenase (HycE), which generates hydrogen<sup>1,2</sup>. These five subunits are attached to the membrane by two membrane-integral subunits. Recently, we could identify HycH as a chaperone for HycE prior to its attachment to the complex<sup>3</sup>. Our aim is to understand FHL complex assembly and the requirements of the proteins for optimal function. We are currently mapping the HycH interaction with HycE to identify the role of this interaction. By using classical bacterial two-hybrid assays and molecular tools, we have studied the protein-protein interactions important in complex maturation. Furthermore, we are using biophysical methods such as DLS and SAXS to study the oligomeric state of HycH and its interaction with HycE. The oligomeric state of HycH varies between being a homodimer up to a homodecamer. Moreover, we could identify further interaction partners of HycH. The molecular envelope of HycE and HycH both alone and in complex has been generated from the SAXS data. Together, these methodologies will help us to understand the FHL assembly and will be of great benefit for the biotechnological exploitation of the hydrogen-producing capability.

#### References:

[1] Sauter, M., Böhm, R., Böck, A., *Mol Microbiol*, **1992**, *6*, 1523–1532

[2] Pinske, C., Sawers R.G., *EcoSalPlus*, **2016**, *7*, 1-32

[3] Lindenstrauß, U., Skorupa, P., McDowall, J.S., Sargent, F., Pinske, C., *Biochem. Journal*, **2017**, *474*, 2937-2950

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#### MCBP354

##### **Heterogeneity of the biofilm regulator CsgD and the extracellular matrix components curli and cellulose production in *E. coli* macrocolony biofilms**

T. K. L. Nguyen<sup>\*1</sup>, D. O. Serra<sup>1</sup>, G. Klauck<sup>1</sup>, R. Hengge<sup>1</sup>

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The development of elaborate morphological patterns in *E. coli* K-12 macrocolonies relies on a precise spatial distribution of flagella, amyloid curli fibres and the exopolysaccharide cellulose: While vegetatively growing cells, entangled by flagella, are located in the bottom layer (i.e. close to the nutrient-providing agar), starving stationary-phase cells producing matrix (i.e. curli and cellulose) can be found in the top layer.

Using fluorescence and SEM microscopy, we found that curli and cellulose form a dense and relatively homogenous nanocomposite in the upper stationary-phase layer that surrounds essentially all cells. Just below this layer, a more heterogeneous zone contains matrix-free and more

elongated cells located immediately adjacent to cells which have switched to stationary-phase physiology and produce mainly cellulose. These matrix producing cells form vertically arranged and cellulose-sheathed pillar-like structures or are embedded within a loose horizontal matrix network.

Consistent with these observations, the overall expression of *csgD*, which encodes the biofilm regulator essential for curli and cellulose synthesis, is heterogeneous in the lower stationary-phase layer and homogenous in the contiguous upper zone. By tracing down cellular lineages, we also show that cells form clonal cell clusters strongly expressing *csgD* where curli and cellulose surround them as a dense nanocomposite. Originating from these, clonal lines of *csgD<sup>ON</sup>* cells form the vertical pillar-like structures which at their lower ends connect to matrix-encased cells of the loose horizontal network.

Finally, we show that this transition area not only exhibits strong heterogeneity when it comes to the production of matrix components, but also in cellular growth behaviour.

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### MCBP355

#### Minor pilins & PilY1 proteins in type IV pili-dependent motility in *Myxococcus xanthus*

A. Treuner-Lange<sup>\*1</sup>, Y. W. Chang<sup>1,2</sup>, M. Herfurth<sup>1</sup>, G. Jensen<sup>2</sup>, L. Søgaard-Andersen<sup>1</sup>

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<sup>2</sup>California Institute of Technology, Pasadena, CA, United States

Type IVa pili are filamentous cell surface structures observed in many bacteria. They pull cells forward by extending, adhering to surfaces, and then retracting. We elucidated the architecture of the T4P machine of *M. xanthus* comprising an outer membrane pore, four interconnected ring structures in the periplasm and cytoplasm, a cytoplasmic disc and dome, and a periplasmic stem [1].

We found, that in addition to the 10 core proteins of the T4P machine, minor pilins and PilY1 are encoded in the genome of *M. xanthus*. To analyze the function of these proteins we systematically deleted minor pilin and/or *pilY1* genes in the genome and analyzed for T4P-dependent motility and T4P formation in the mutants.

Using ECT on intact cells of a nine-fold minor pilin mutant as well as the triple *pilY1* mutant, we only detected non-piliated T4P machines and these empty machines lacked a short periplasmic stem structure that is present in non-piliated T4P machines in wild-type and connects to the inner membrane.

We conclude (1) that minor pilins and PilY1 proteins are essential for T4P formation, (2) that the short stem functions as a priming complex for T4P assembly, (3) that the major pilin PilA, the minor pilins and PilY1 proteins are part of this complex and (4) that this complex is an integral part of the T4P machine.

We are currently using Bacterial 2 hybrid interaction analyses to verify protein-protein interactions of PilA, minor pilins and PilY1 proteins. We will also report on our progress to localize the PilY1 proteins using immunogold-labelling as well as fluorescence microscopy.

#### References:

[1] Chang Y-W, Rettberg L, Treuner-Lange A, Iwasa J,

Søgaard-Andersen L, Jensen G J (2016) Structure of the type IVa pilus machine in vivo. *Science* 351:aad2001

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### MCBP356

#### Characterization of polyphosphate granules of *Ralstonia eutropha*

H. Rosigkeit<sup>\*1</sup>, C. Frank<sup>1</sup>, D. Jendrossek<sup>1</sup>

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Polyphosphate (polyP) was detected in all species that have been looked at (1). In bacteria, polyP is present in form of insoluble, globular inclusions of ≈50 to 200nm. It has the role of a reservoir for P and cations (Ca, Mg) but additional functions are assigned to polyP such as a stress mediator or source for NTPs. Only little is known on the molecular structure and composition of polyP granules. We recently characterized polyP granules of the β-proteobacterium *Ralstonia eutropha*. Remarkably, *R. eutropha* has two type 1 polyP kinases (PPK1a and PPK1b) and five type 2 polyP kinases (PPK2a-PPK2e) (2). Four polyP kinases (PPK1a, PPK2c, PPK2d and PPK2e) and two additional proteins (PptA, PptB) were identified as being associated with polyP granules *in vivo* pointing to a complex macromolecular structure of polyP. However, no evidence was found for the presence of a membrane that surrounds polyP granules as it is known for polyP-containing acidocalcisomes of *A. tumefaciens*, *R. rubrum* or acidocalcisomes of lower eukaryotes (3).

The polyP-attached phosphatases PptA and PptB have a "conserved histidine alpha helical [CHAD]-domain" (4). In this study, we started to address the function of PptA. To this end we developed a tool to quantify the amount of accumulated polyP via extraction of polyP, conversion to monomeric phosphate (Pi) with exo-polyphosphatase and quantification of released Pi using a malachite green assay. Furthermore, we describe the properties of purified PptA and show the effect of *pptA* deletion.

#### References:

[1] Rao NN, Gómez-García MR, Kornberg A. (2009). *Ann Rev Biochem.* 78:605-647.

[2] Tumlirsch T, Sznajder A, Jendrossek D. (2015). *AEM* 81:8277–8293.

[3] Docampo R, Huang G. (2016). *Curr Opin Cell Biol.* 41:66-72.

[4] Tumlirsch T, Jendrossek D. (2017). *AEM* 83:e03399-16-14.

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### MCBP357

#### Influence of the Network of Ferredoxin-like proteins on Hydrogen Metabolism in *Escherichia coli*.

C. Pinske<sup>\*1</sup>

<sup>1</sup>Martin-Luther University Halle-Wittenberg, General Microbiology, Halle a. d. S., Germany

Ferredoxins are iron-sulfur (FeS) cluster-carrying proteins that mediate electron transfer in microbial cells and they are ubiquitously distributed. *E. coli* has more than 20 of these proteins encoded in its genome. The fold of these proteins is well conserved. Members of this super-family of electron-transfer proteins can serve various purposes, including acting as small-subunits of respiratory complexes or electron donors during FeS-synthesis. One member, HydN, has been

associated with a role in the activity of the FeS-containing molybdoselenoprotein formate dehydrogenase H (FdhH), which is part of the hydrogen-producing formate hydrogenlyase complex<sup>1,2</sup>. However, the functions of most ferredoxin-like proteins have yet to be established.

This current work aims to investigate the influence of ferredoxin-like proteins on the hydrogen production of *E. coli*. By monitoring bacterial two hybrid interactions, a network of these ferredoxin-like proteins was identified and the corresponding single and multiple deletion strains created. These strains were characterised with regard to their hydrogen production and formate dehydrogenase activities. A novel member of this protein family was identified to be required for full FdhH activity. Other respiratory proteins with a similar cofactor composition as FdhH were not influenced. The FdhH protein amount and migration pattern were unaltered in the deletion strain leading to the hypothesis that these proteins either form a pool of alternative subunits for FdhH or are specifically required to modify the FdhH cofactors.

#### References:

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#### MCBP358

##### Characterization of acidocalcisomes of *Agrobacterium tumefaciens*

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Acidocalcisomes are well-characterized membrane-enclosed acidic organelles in lower eukaryotes. They contain large amounts of inorganic polyphosphate together with cations such as calcium or magnesium [1]. Only two prokaryotic species, *Rhodospirillum rubrum* and *Agrobacterium tumefaciens*, have been described so far, that can also form acidocalcisomes [2, 3]. The most common bacteria species produce polyphosphate (volutin) granules, but these structures are not surrounded by a membrane and therefore represent a more simple form of inclusions. In this study, we describe the similarities and dissimilarities between acidocalcisomes and polyphosphate granules at the example of *A. tumefaciens* and *Ralstonia eutropha* [4]. To this end we biochemically characterized acidocalcisomes and polyphosphate granules isolated from both species. Furthermore, we determined the *in vivo* localization of fusion of fluorescent proteins with acidocalcisome-specific proteins and with polyphosphate granule-associated proteins (proton translocating pyrophosphatase, [HppA] and polyphosphate kinase [PPK], respectively) and analyzed the effect of *hppA* and/or *ppk* gene deletion in both species.

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#### MCBP359

##### Characterization of a serine/threonine kinase, VPA1044 in *Vibrio parahaemolyticus*

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Bacteria possess several groups of kinases which are usually categorized according to the residue on the substrate that the gamma-phosphate from ATP gets transferred to. One group of kinases are the serine/ threonine kinases which possesses a catalytic domain similar to those found in Eukarya, hence earning them the name "eukaryotic-like" kinases. There has been growing evidence in the last decade that has shed light on the role of these kinases in several important processes including bacterial cell division, virulence and morphogenesis, to name a few. Our general aim was to take a closer look at the known Ser/Thr kinases in *Vibrio parahaemolyticus*. *V. parahaemolyticus* has a dimorphic lifestyle, allowing it to swim in aqueous environments and swarm efficiently upon encountering solid surfaces/ highly viscous environments. We selected one of the known Ser/Thr kinases, namely VPA1044 for further characterization. To get a general idea of the targets it might regulate, we performed an initial proteomics screen on the mutant compared with wild type. Among the proteins significantly regulated, a large portion were found to be involved in cell wall and cell envelope maintenance. This prompted us to test the sensitivity of the kinase to several cell wall degrading agents. VPA1044 was found to be extremely essential for conferring resistance to Polymyxin B, a commercially available antibiotic against Gram negative infections. Furthermore, we also found that the kinase was important for proper swarming behavior. A closer look at the genetic neighborhood of *vpa1044* leads us to propose a phosphorelay mechanism, involving crosstalk with a two-component histidine kinase/response regulator, responsible for the phenotypes we observe.

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#### MCBP360

##### Architecture of *Vibrio parahaemolyticus* swarm-colonies

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*Vibrio parahaemolyticus* is a marine bacterium recognized as the leading cause of human sea-food born gastroenteritis. *V. parahaemolyticus* can form two distinct cell-types: a short swimmer cell and a highly elongated swarmer cell, optimized for life in liquid and on solid surfaces, respectively. Upon surface contact, swimmer cells are able to differentiate into swarmer cells, which exists within the population of swarmer colony. The overall architecture and the organization of cells within swarm colonies, remains an open question. To characterize the swarming colony architecture we performed transcriptomic analysis of cells from distinct regions within the swarmer colony. We show that swarmer colonies are organized into distinct regions of specific cell types. Fully differentiated swarmer cells are present only in the peripheral swarm flares, while the center of the colony contains swimmer proficient cells with a distinct proteome. Furthermore, fluorescence microscopy analyses using a double labelling reporter strain, where sfGFP was fused to a promoter of a gene up-regulated in the periphery of a swarming colony (*vpa1548*) and mCherry was fused to a promoter of a gene up-regulated in the center of a swarming colony (*vp1343*), further confirmed that swarming colonies

consist of specific regions made up of differentially distinct cells, resulting in the distinct architecture of swarm-colonies. We argue that having always a population of non-swarmers within the swarming colony would be an elegant strategy to ensure a continuous release of cells from the swarmer colony that are immediately capable of exploring their surroundings in the search of new beneficial environments.

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#### MCBP361

##### Biosynthesis of cell wall glycopolymers in *Streptomyces coelicolor* A3(2)

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The Gram-positive soil bacterium *Streptomyces coelicolor* A3(2) undergoes a complex life cycle with differentiation in substrate mycelium, aerial mycelium and spores that are protected by a thick spore envelope. Sporulation septation and synthesis of the thickened spore wall are directed by the *Streptomyces* Spore Wall Synthesizing Complex<sup>1</sup> SSSC, a multi-protein complex including MreBCD and other proteins involved in synthesis of peptidoglycan and cell wall glycopolymers (CWG).

In this study, we aim to elucidate the role of CWGs in the life cycle of *S. coelicolor* and to characterize their biosynthesis. Two distinct CWGs, known as the Kdn-containing teichulosonic acid and polydiglycosylphosphate (PDP) have been detected in *S. coelicolor*<sup>2</sup>. The *S. coelicolor* genome encodes six putative CWG-polymerases containing a TagF-like glycerophosphotransferase domain and eleven putative CWG-transferases of the LytR-CpsA-Psr (LCP) family<sup>3</sup>. To identify their function, we started to delete all genes, characterized the mutant phenotypes and analyzed the cell wall composition of vegetative mycelium and spores.

Thus we could show that SCO2578 (PdtA) is a PDP-transferase, which is crucial for proper PG-incorporation at the hyphal tips under stress conditions and required to ensure the integrity of the spore envelope<sup>3</sup>.

SCO2997 was identified as a spore wall specific PDP polymerase for the synthesis of elongated PDPs, which are involved in the proper assembly of the hydrophobic rodlet layer on the spore surfaces.

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#### MCBP362

##### Effect of Eukaryotic-Type Serine/Threonine Protein Kinases (eSTPKs) on Differentiation and Spore Wall Synthesis of *Streptomyces coelicolor* A3(2)

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Streptomycetes are soil living bacteria of the Actinobacteria phylum. In contrast to most other bacteria which divide by binary fission, *Streptomyces* grows by apical tip extension forming a multiple branching mycelium. During morphological differentiation the aerial hyphae are transformed into spore chains by the simultaneous formation of dozens of sporulation septa. Septation and synthesis of the thickened spore wall involves the MreBCD proteins, which direct a rod-shaped morphology in other bacteria by positioning lateral wall synthesis.

Screening of a *S. coelicolor* genomic library by bacterial two-hybrid analyses identified MreBCD interaction partners and led to the concept of the *Streptomyces* spore wall synthesizing complex (SSSC). Interestingly, the SSSC also included PkaI, a putative eukaryotic-type serine/threonine protein kinase, which is encoded within a cluster of five independently transcribed eSTPK genes (SCO4775-4779).

The role of eSTPKs in bacteria and their influence on morphological differentiation and cell wall biosynthesis are not well characterized. However, the delay in sporulation and the presence of aberrant spores in NLΔSCO4775-SCO4779 mutants suggests a regulation of the SSSC by protein phosphorylation. The specific phosphorylation of MreC and PBP2 by PkaI could be demonstrated in coexpression experiments<sup>[1]</sup>.

In our project we want to elucidate the role of eSTPKs/phosphatases on the activity of SSSC proteins to understand whether protein phosphorylation is the key to control and coordinate differentiation in *Streptomyces*.

#### References:

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#### MCBP363

##### Regulatory mechanisms of swimming motility in *Shewanella putrefaciens*

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Although the flagellum provides one of the most widespread means of bacterial motility, many regulatory aspects of the -flagellum and its expression and assembly remain elusive. *Shewanella putrefaciens* possesses two distinct flagellar systems encoded by two separate gene clusters enabling us to study the specificity of flagellar proteins. The polar -flagellum is responsible for the main propulsion of the cell and responds to the chemotaxis system while the lateral flagellar system improves spreading efficiency by increasing directional persistence and establishes only in a subpopulation of cells during planktonic growth in complex media. However, the mechanisms regulating the formation and operation of the lateral flagellum are unknown. We were able to show that the lateral system assembles and functions independently of the polar system and its chemotaxis system. Expression of lateral flagellar genes seem to rely, in

response to environmental cues, on external activators. We were able to identify and characterize several proteins involved in cyclic-di-GMP signaling, which affect lateral - flagellar gene expression and function. The identified phosphodiesterase PdeB might be capable of direct sensing extracellular nutrients and modulating the intracellular c-di-GMP level in response to the nutrient situation, which in turn activates the expression of the lateral flagellar genes and secures the function of the polar flagellum. Another protein, FlgZ, functions, depending on the intracellular c-di-GMP level as a specific brake for the lateral flagellum. The second messenger molecule c-di-GMP thereby affects swimming motility of *Shewanella putrefaciens* at the level of transcriptional control as well as motor function.

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#### MCBP364

##### Chromosome organization in *Corynebacterium glutamicum*

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<sup>1</sup>Ludwig-Maximilians-University Munich, Fakultät Biologie, Planegg-Martinsried, Germany

Bacterial ParABS systems ensure reliable chromosome segregation prior to cell division. Thereby, ParB proteins bind *parS* sequences located close to the origin of replication. The ATPase ParA binds DNA nonspecifically and dissociates upon ParB interaction, thus establishing a concentration gradient that allows directed sister chromosomes segregation. Besides this, most bacteria harbor SMC/ScpAB complexes, which mediate nucleoid condensation via DNA entrapping.

Since mechanisms of chromosome organization have been poorly characterized in actinobacteria we aim to identify DNA binding sites of SMC/ScpAB in presence and absence of ParB and *parS* and to understand dependencies of cellular ParAB localization on each other and on *parS* in *Corynebacterium glutamicum*.

In order to study the impact of *parS* on ParAB localization microscopically, *parS* sites were mutated subsequently and combined with allelic replacements of Par proteins by fluorescently tagged versions. Moreover, chromosomal SMC loading as well as its loading dependency on *parS* and ParB were analyzed via chromatin immunoprecipitations (ChIP-seq) and fluorescence microscopy in relevant mutant strains.

We show that one of ten *parS* sites is sufficient for chromosome segregation. Absence of *parS* impacts on specific ParB-DNA binding and restricts directed ParA localization to cell poles. In turn, polar ParA-enrichment depends on the presence of ParB protein. We identified putative SMC loading in proximity to the *parS* clusters, which is at least partially dependent on *parS* and ParB. Altogether our data give further insights into the coordination of DNA segregation via the ParABS system, indicate mutual influence of ParB and the SMC/ScpAB complex in *C. glutamicum* and suggest differences to known systems.

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#### MCBP365

##### Polar localisation dynamics of the SRP-GTPase FlhF in *Shewanella putrefaciens*

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The bacterial flagellum is a complex microstructure that, for a correct and functional assembly, requires strict regulation, on both a translational and posttranslational level. An important factor required for initiation of flagellar localisation and assembly in many  $\gamma$ -proteobacteria is the SRP-GTPase FlhF. In our model bacteria *Shewanella putrefaciens*, which possess both one lateral and one polar flagellar system, FlhF is only responsible for the polar flagellum synthesis and accordingly only localises polarly. The exact mechanism behind this strict localisation of FlhF is largely unknown, as are also the factor or factors, which are responsible and required for polar FlhF localisation. We observed, that FlhF does not localise in a polarly manner anymore, when the entire gene cluster encoding the components of the polar flagellum was deleted. We therefore analysed the localisation behaviour of fluorophore-tagged FlhF in various *Shewanella putrefaciens* mutants carrying specific, flagellar assembly affecting deletions. The effect of these deletions on flagellar assembly was additionally evaluated via swimming assays. While ruling out many flagellar components as responsible and required for FlhF localisation, it was possible to determine, that the alternative sigma factor 28, FlhA, seems to play a role in the polar localisation of FlhF, as its deletion leads to decreased polar localisation activity of FlhF. Thus, it is possible to conclude, that the polar localisation of FlhF in our model organism depends on the presence of both FlhA and another factor or factors encoded in the polar gene cluster.

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#### MCBP366

##### Examination of the cell wall structure of a vancomycin resistant *Staphylococcus aureus* strain

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*Staphylococcus aureus*, especially MRSA, is a dominant cause of nosocomial disease around the world. The drug of choice against MRSA is vancomycin. Although rare in hospital settings, the number of vancomycin intermediate resistant (VISA) strains rose significantly in recent years. Here we focus on the biochemical alterations in the cell wall of a laboratory VISA strain to elucidate mechanisms for vancomycin resistance. *S. aureus* VC40 is a highly resistant VISA strain (MIC 64  $\mu\text{g/mL}$ ), generated by serial passage of *S. aureus* RN4220 $\Delta$ mutS in the presence of vancomycin. The strain has a thickened cell wall, a characteristic for most VISA strains, and a significantly lower peptidoglycan crosslinking than the respective controls determined by UPLC-MS. The decreased percentage of crosslinking leads to an increase in false target sites for vancomycin. The strain VC40 also has a diminished negative cell wall charge measured by cytochrome C assay. This decreases the first interaction of the positively charged vancomycin molecule with the cell envelope. Interestingly, *S. aureus* VC40 showed upregulation of multiple autolysins in qRT-PCR, because of a mutation in the regulatory gene *walk*, that hinders its autophosphorylation. Autolysins are important for cell division and their activity is inhibited by teichoic acids. Therefore the enzymes hydrolyze the cell wall septa which are teichoic acid free. In order to test the influence of teichoic acids on vancomycin resistance, a MIC assay in the presence of tunicamycin was performed. So far, the inhibition of teichoic acid biosynthesis might have an effect on vancomycin resistance in the strain. In conclusion, these results elucidate mechanisms for vancomycin resistance in laboratory and clinical *S. aureus*.

## MCBP367

### Identification of a novel cell division protein in *Listeria monocytogenes*

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**Introduction:** The late cell division protein GpsB is required for cytokinesis and cell wall biosynthesis of the human pathogen *Listeria monocytogenes*. These effects are explained by an interaction of GpsB with the major penicillin binding protein PBP A1. We noticed that a *L. monocytogenes*  $\Delta$ *gpsB* mutant rapidly acquires mutations suppressing the  $\Delta$ *gpsB* phenotype. Genome sequencing of selected *gpsB* suppressor mutants led to the identification of hitherto uncharacterized genes putatively associated with GpsB function.

**Objectives:** The aim of this study was to characterize the phenotype of mutant strains lacking those genes that were mutated in selected *gpsB* suppressor strains.

**Material & Methods:** Strains lacking *gpsB* suppressor genes were constructed in wild type and  $\Delta$ *gpsB* mutant backgrounds. Growth and antibiotic susceptibility of these strains was analyzed. Effects on cell division and cellular morphology of these strains were analyzed by fluorescence microscopy. Epistasis experiments were performed to examine whether the *gpsB* suppressor genes can be linked to already known ways of  $\Delta$ *gpsB* phenotype suppression.

**Results:** One out of three analyzed *gpsB* suppressor genes turned out to affect cell division. Our results show that the way of suppression exerted by this gene is different from previously known mechanisms of suppression. The function of this gene was previously associated with DNA repair, likely explained by a misannotation.

**Conclusion:** We have identified a new gene associated with GpsB function in *L. monocytogenes* that clearly affects cell division of this important pathogen. Recent results of this ongoing project will be presented on this poster.

## MCBP368

### A new cell cycle regulator in *Hyphomonas neptunium*

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*C. crescentus*, a model organism for bacterial cell cycle studies, divides into two cell types with distinct morphologies and physiology. The DNA-binding response regulator CtrA was identified as a master regulator driving this differentiation process. CtrA is regulated by the CckA-ChpT phosphorelay, and in turn controlled by another phosphorelay, involving the histidine kinase DivJ, the phosphatase PleC, and the single-domain response regulator DivK. The complex signaling network associated with CtrA is highly conserved in  $\alpha$ -proteobacteria. A close relative of *C. crescentus*, *Hyphomonas neptunium* is a newly introduced model organism that divides by the formation of buds at the end of its stalk. Here, we investigate the role of CtrA in the regulation of processes such as DNA segregation, morphogenesis and cell division. Moreover, we dissect the mechanisms controlling CtrA activity and present a potential new component of the CtrA-regulatory phosphorelay in this species.

## MCBP369

### The denitrification machinery super-complex of *Pseudomonas aeruginosa*

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Biochemical pathways, signal transduction cascades, and membrane-attached multiprotein complexes taking part in energy generation rely on fine-tuned, specific and frequently transient protein interactions. Interatomic techniques represent the current state-of-the-art approach to decipher protein-protein interactions at the whole cellular level. Our group endeavored to scrutinize the protein-protein interactions occurring during denitrification in *Pseudomonas aeruginosa*. Denitrification is one of the most prominent, anaerobic, ecologically highly relevant, alternative electron acceptor systems. Four different enzyme complexes Nar, Nir, Nor and Nos receive electrons from various electron donors via quinones from intermediate complexes or directly from primary dehydrogenases to catalyze the following reduction steps: NO<sub>3</sub><sup>-</sup>, NO<sub>2</sub><sup>-</sup>, NO, N<sub>2</sub>O, N<sub>2</sub>. The membrane associated nitric-oxide reductase subunits (NorCB) and the flavo-protein NosR were genetically identified as membrane based assembly platform and used as baits for the elucidation of the denitrification complex. Bait proteins were subjected to affinity chromatography and the interaction partners were further identified through LC-MS/MS. A supra-complex consisting of all enzymes of denitrification, their maturation and cofactor delivery machinery, the electron donor system, ATP synthase, the protein translocating SEC system, and several citric acid cycle enzymes was identified. Furthermore, we performed double immunogold labeling and transmission electron microscopy (TEM) for *in vivo* visualization of co-localized interacting proteins as a second independent experimental approach for the confirmation of obtained results.

## ePoster Session 1

### Membranes and Transport (MTP)

16 April 2018 • 14:30–17:30

#### MTP328

##### Resistance against lantibiotics: the BceAB type transporter NsrFP of *S. agalactiae*

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The need for new antibiotic compounds is raising. Antimicrobial peptides are excellent candidates to fulfill this function. The subclass of lantibiotics contain unusual amino acids and lanthionine rings, which ensure their high stability and high potency. They are active in the nanomolar range and exhibit two main modes of action mainly against Gram-positive bacteria: Binding to the precursor of lipid II as well as pore formation within the bacterial membrane, which leads to immediate cell death. Commercial usage is, however hampered by the presence of genes in human pathogenic strains which, when expressed, confer resistance against such lantibiotics. The human pathogen *Streptococcus agalactiae* COH1, is for example resistant against lantibiotics due to the *nsr*-operon (Khosa et al. 2013) encoding a two-component system NsrRK, the nisin resistance protein (NSR) and an BceAB type ABC transporter NsrFP (Reiners et al. 2017). Interestingly, the genes appear to be evolutionary conserved in human pathogenic bacteria.

Here we shows an expanded substrate specificity of NsrFP not only against lantibiotics, like a 16-fold of resistance

against nisin and 12.5-fold of resistance against gallidermin (Reiners et al. 2017), but also against several known antibiotics, like vancomycin and penicillin. Interestingly, a remarkable fold of resistance of 240 was detected for bacitracin, which itself inhibits the membrane transport of the lipid carrier bactoprenol. Our studies implicate that NsrFP mediates resistance against several antibiotics with diverse membrane targets suggesting that the NsrFP is transporting central components of the Gram-positive membrane.

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### MTP329

#### Expression and functional characterization of cation transporters in *Vibrio natriegens*

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*Vibrio natriegens* is a halophilic, non-pathogenic bacterium. Interestingly, *V. natriegens* has the fastest growth rate of any known non-pathogenic organism with a doubling time of < 10 minutes at optimal growth conditions [1]. In comparison, *E. coli* has a doubling time of 20 minutes. Consequently, *V. natriegens* is a very interesting organism which could become a tool not only for genetic research but also for biotechnological applications. The productivity of industrial fermentation processes is essentially limited by the biomass specific substrate consumption rate (qS) of the applied microbial production system. Since qS depends on the growth rate, *V. natriegens* could be a novel candidate for future biotechnological processes [2]. In this study we demonstrate that *V. natriegens* is a suitable host for the expression of large membrane protein complexes in their active state. First, we could demonstrate by western blotting and immune detection that the multi subunit cation/H<sup>+</sup> antiporter MRP from *V. cholerae* can be expressed in *V. natriegens*. Furthermore, the antiporter activity of the heterologous expressed enzyme could be observed in growth experiments with *V. natriegens*. Second, we could demonstrate that *V. natriegens* is able to express another membrane protein complex, namely the Na<sup>+</sup>-NQR from *V. cholerae*. Here, we also could detect the heterologously expressed enzyme in isolated *V. natriegens* membranes and crude extracts by western blotting and immune detection. Furthermore, the NADH:quinone oxidoreduction activity of membranes was inhibited by Ag<sup>+</sup>, a specific inhibitor of the NQR.

#### References:

[1] Weinstock, Heseck, Wilson, Gibson. Nature Methods 13: 849-855.

[2] Hoffart, Grenz, Lange, Nitschel, Müller, Schwentner, Feith, Lenfers-Lücker, Takors, Blombach. AEM.01614-17.

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### MTP330

#### Adoption of a microbial transmembrane function links corals to betaine biogeochemistry in the ocean

D. Ngugi\*<sup>1</sup>, M. Ziegler<sup>1</sup>, C. Duarte<sup>1</sup>, C. Voolstra<sup>1</sup>

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Glycine betaine (GB) is one of the most common compatible solutes in nature, which is found in all three domains of life. In the tissues of diverse marine invertebrates, GB reaches concentrations (33–215 mM) orders of magnitude higher than in the ambient seawater (up to 25 µM) and predominates over other organic osmolytes, yet the genetic mechanisms governing the preferential accretion of GB

remain uncharacterized – neither the pathway for synthesis nor degradation has been documented to date. Here we present data showing that the genetic blueprint of cnidarians encodes the capacity to metabolize GB. We found that all corals possess the machinery for synthesizing GB via choline oxidation and glycine methylation pathways and for degrading GB. Importantly, our data demonstrates that cnidarians belong to a few (marine) eukaryotes possessing homologs of the sodium-driven high-affinity GB carrier found so far only in prokaryotes, which mediate the uptake of extracellular GB. In turn, this suggests that coral reefs are a potent sink for microbial-derived betaines in the global ocean. The finding that this transmembrane function is an ancestral genetic facet of all sequenced cnidarians and their endosymbionts and the fact that the intrinsic GB metabolic pathway is highly transcribed *in hospite* explains how coral reefs retain resources in oligotrophic seas and has implications for the biogeochemistry of GB in the ocean.

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### MTP331

#### Natural transformation and type IV pilus biogenesis in *Thermus thermophilus*: Characterization of an unusual motor ATPase and a unique secretin complex

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**Introduction:** Uptake of DNA by *Thermus thermophilus* requires a macromolecular transport machinery that spans the entire cell periphery. Some of the subunits play a dual role in natural transformation and biogenesis of type IV pili (T4P). The machinery is highly dynamic and powered by the motor ATPase PilF that pushes the pilus through the outer membrane, sheathed by the secretin complex PilQ.

**Objectives:** We aimed to elucidate the structure of PilQ and functions of PilF domains.

**Materials & methods:** Methods used are: site directed mutagenesis, affinity chromatography based protein purification, single particle cryo-EM.

**Results:** PilF is a hexameric T4P polymerization ATPase with an unusual N-terminus encoding three general secretory domains (GSPII). Deletion of GSPIIA/B had no effect on pilus biogenesis of *T. thermophilus*, however GSPIIA is important for pilus functions. A *pilFΔGSPIIC* mutant was strongly impaired in piliation but hypertransformable. The secretin PilQ forms homooligomers with 6 staggered rings. Cryo-EM analyses of PilQ complexes revealed the structure at a resolution of ~7 Å. Most important, a hitherto unknown crown-like structure located on top of the secretin was observed. This crown is apparently not part of PilQ and important for T4P functionality and natural transformation [1].

**Conclusion:** The DNA translocator of *T. thermophilus* is "crowned" by a novel, so far unknown protein. The GSPII domains in the motor ATPase PilF, seem to confer specificity to the system.

#### References:

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### MTP332

#### Functional analysis of the bacterial dynamin-like protein DynA

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Dynamins are a family of large, multidomain GTPases involved in key cellular processes in eukaryotes, including vesicle trafficking and organelle division. The GTP hydrolysis cycle of dynamin translates to a radical conformational change in the protein structure, which forces the underlying lipid layer into an energetically unstable conformation that promotes membrane rearrangements. Additionally, many bacterial genomes encode dynamin-like proteins, but the biological function of these proteins has remained largely enigmatic. In recent years, our group has reported that the dynamin-like protein DynA from *Bacillus subtilis* mediates nucleotide-independent membrane tethering in vitro and contributes to the innate immunity of bacteria against membrane stress and phage infection. However, so far it is unclear how the molecular mechanism and the role of GTP hydrolysis link membrane remodeling to stress response.

On a structural level, membrane full fusion results in the unification of the lipid and protein components and the intermixing of the volumes. Here we employed content mixing and lipid mixing assays in reconstituted systems to study if the dynamin-like protein DynA from *Bacillus subtilis* could induce membrane full fusion, and further test the possibility that GTP hydrolysis of DynA acts on the fusion-through-hemifusion pathway. Our results based on fluorescence resonance energy transfer (FRET) indicated that DynA could induce aqueous content mixing even in absence of GTP. And FRET efficiencies of lipid mixing and content mixing could keep increasing in a long period. And surprisingly, sudden digestion of protein mediated an instantly rise of content exchange, supporting the assumption that disassembly of DynA is the fundamental power for fusion-through-hemifusion.

### MTP333

#### Spatial dynamics of bacterial phosphotransferase systems in *Corynebacterium glutamicum*

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*Corynebacterium glutamicum* grows on a variety of carbohydrates. Several sugars such as glucose and fructose are taken up and phosphorylated by specific, membrane-integral phosphotransferase systems (PTS). Since *C. glutamicum* is widely used as a producer of glutamate and amino acids in industry, information regarding sugar uptake systems and membrane economy are therefore of biotechnological importance.

Here, we analyzed localization of the fructose specific PtsF and the glucose specific PtsG transporters. To this end, the transmembrane subunits of these components were tagged with fluorescent reporters. To avoid artificial expression heterogeneity, fusion constructs were inserted as allelic replacement in the genome of wild type *C. glutamicum*. The strains were grown in minimal medium supplemented with either the specific sugars taken up by each PTS or acetate. Subcellular localization of the Pts proteins was subsequently analyzed by fluorescence microscopy.

PtsG and PtsF form membrane embedded clusters that localize in a punctate pattern within the cell membrane. The size of PTS clusters changes upon presence or absence of the transported substrate. In presence of the correct PTS sugars, the clusters significantly increase in size, supporting the notion that transport complexes associate under these conditions. We next addressed the influence of membrane area on PTS localization. Therefore, L-forms were generated by culturing the cells in MSM/NB osmo-protective medium with D-cycloserine, which inhibits cell wall synthesis. Although L-forms have larger membrane surfaces PtsG/F foci were similar compared to that of rod-shaped cells. This suggests that the cluster size of Pts proteins is intrinsically regulated and not a consequence of membrane space.

### MTP334

#### Respiration with Oxygen and Nitrate by Spores of *Streptomyces coelicolor* Requires a Functional Cytochrome *bc<sub>1</sub>* Complex

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*Streptomyces coelicolor* A3(2) is a filamentously growing, obligately aerobic actinobacterium that uses both a copper-*aa<sub>3</sub>*-type cytochrome *c* oxidase and a cytochrome *bd* oxidase to respire with oxygen. The *aa<sub>3</sub>*-type oxidase receives electrons from a menaquinol:cytochrome *bc<sub>1</sub>* oxidoreductase (*bc<sub>1</sub>* complex), while the cytochrome *bd* oxidase receives its electrons directly from menaquinol. Using defined knock-out mutants we demonstrate that either of these terminal oxidases is capable of allowing the bacterium to grow and complete its developmental cycle, including production of dormant spores. As is characteristic for other actinobacteria, *S. coelicolor* lacks a soluble *c*-type cytochrome and the genes encoding the *bc<sub>1</sub>* complex and the *aa<sub>3</sub>* oxidase are clustered at a single locus. Combined complementation and immunological analyses of key *bc<sub>1</sub>* complex and *aa<sub>3</sub>* oxidase components revealed that the complete locus was required to form a stable respiratory super-complex. Evidence indicates, however, that a further, alternative form of the *bc<sub>1</sub>*-*aa<sub>3</sub>*-super-complex appears to exist in spores. Notably, when oxygen is absent, spores can respire with nitrate as electron acceptor using respiratory nitrate reductase 1 (Nar1), one of three Nar enzymes in the bacterium. Surprisingly, our studies indicate that in spores, Nar1 activity and the capacity to reduce nitrate depends on a functional *bc<sub>1</sub>*-complex. Reduction of nitrate by spores is, however, independent of the cytochrome *bd* oxidase. Together, our findings indicate that either of the terminal oxidases is sufficient to reduce oxygen in both spores and mycelium, but electron delivery from the *bc<sub>1</sub>*-complex is essential for nitrate reduction in spores.

### MTP335

#### Biological role of outer membrane vesicle-associated small proteins of the phytopathogen *Agrobacterium tumefaciens*

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Bacterial extracellular membrane vesicles are considered as novel players in various cell communication systems. Outer membrane vesicles (OMVs) are released from the outer membrane of many Gram-negative bacteria. The cargo of these extracellular nanostructures comprises distinct

biomolecules such as small compounds, genetic material, proteins and peptides, which are involved in intra- and inter-species communication and pathogenicity.

We have recently demonstrated that the phytopathogen *Agrobacterium tumefaciens*, the causative agent of crown gall disease, releases OMVs into the culture supernatant during growth. A proteome study of the OMV fractions obtained under different growth conditions identified about 60 proteins including the two conserved small proteins Atu2614 and Atu8019 with unknown functions. Both proteins contain a predicted N-terminal signal peptidase II sequence with a C-terminal consensus lipobox, a hallmark of lipoproteins. The mature form of Atu2614 is expected to contain 67 aa and comprises a highly hydrophobic domain with several extended glycine-zipper motifs commonly found in pore-forming membrane proteins. Atu8019 (mature size: 32 aa) shares sequence similarities to entericidin antidote/toxin peptides. To elucidate the biological role of these two OMV-located proteins we combine biochemical and mutagenesis approaches.

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### MTP336

#### Negamycin uses multiple routes for crossing of the cytoplasmic membrane

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Bacterial infections and the increase in antibiotic resistance are among the main health issues of today. Discovering and developing new antibiotics against Gram-negative bacteria is particularly challenging. However, this is not due to a lack of suitable targets, but caused by the strict penetration requirements of the Gram-negative cell envelope. Here, we study a natural product antibiotic to better understand the routes that nature has invented for entry into the bacterial cytoplasm.

Negamycin is a pseudo-dipeptide antibiotic with promising activity against Gram-negative and Gram-positive bacteria, including Enterobacteriaceae, *Pseudomonas aeruginosa* and *Staphylococcus aureus* and good efficacy in infection models (1,2). It binds to ribosomes with a novel binding mode, stimulating miscoding and inhibiting ribosome translocation (3). No cross-resistance with other ribosome-binding antibiotics was observed. We investigated the passage of negamycin across the cytoplasmic membrane using *Escherichia coli* as a model.

Our results show that negamycin is able to cross the cytoplasmic membrane by more than one route. We could identify multiple transporters that are involved in the translocation of negamycin across the cytoplasmic membrane. Furthermore, an uptake based on the membrane potential is suggested.

The example of negamycin demonstrates that passage across the bacterial cell envelope can be multi-faceted, even for a single small agent and that for cytoplasmic anti-Gram-negative drugs understanding of entry process and target interaction are equally important.

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### MTP337

#### PlaB, a novel phospholipase A of *Pseudomonas aeruginosa*

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*Pseudomonas aeruginosa* is a well-known Gram-negative pathogen infecting immunocompromised individuals and patients suffering from cystic fibrosis, AIDS and cancer<sup>1</sup>. This bacterium is known to produce various lipolytic and proteolytic enzymes as well as different types of adhesins, which mediate pathogenicity<sup>2</sup>. Although, many virulence associated genes of *P. aeruginosa* have been identified, the function of about 40 % of all genes is still unknown.

Here, we aim to identify, purify and characterize novel virulence factors of *P. aeruginosa* with lipolytic activity. By bioinformatics analysis we have identified several putative lipolytic enzymes, among them PlaB with homology to a known virulence factor of *Legionella pneumophila*<sup>3,4</sup>. The expression of PlaB in *E. coli* yielded a functional enzyme with phospholipase A activity against typical bacterial phospholipids. After co-expression with GroEL/GroES chaperones, PlaB could be purified from the membrane fraction using immobilized metal affinity chromatography. PlaB showed a preference for phospholipids and lysophospholipids with long chain fatty acids without specificity for phospholipid head groups as it hydrolyzed phosphatidyl-choline, -ethanolamine and -glycerol. Activity measurements using a *P. aeruginosa* PlaB transposon mutant showed reduced intracellular phospholipase A activity as compared to the respective wild-type strain. Preliminary biofilm assays suggested a role of PlaB for biofilm formation in *P. aeruginosa*.

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- [2] Jaeger, K.-E. and F. Kovacic (2014) Methods Mol Biol 1149: 111-134.
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### MTP338

#### Fatty acids and phospholipids as regulators of *Pseudomonas aeruginosa* phospholipase PlaF function

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The Gram-negative human pathogen *Pseudomonas aeruginosa* produces many virulence factors, among them are several phospholipases.<sup>1</sup> These contribute to phospholipid homeostasis, damage of host cell membranes and modulation of lipid signaling in eukaryotic cells.<sup>1</sup> PlaF, a phospholipase A of *P. aeruginosa* which is anchored to the cytoplasmic membrane via a single transmembrane (TM) helix, exists in monomeric and dimeric form. PlaF is capable to hydrolyse various phospholipids *in vitro* and *in vivo* releasing medium chain fatty acids.

Biochemical and structural data suggest a regulation of PlaF function through reversible dimerization triggered by fatty acids. Molecular dynamics simulations in a phospholipid bilayer allowed us to propose a mechanism for PlaF activation by monomerisation. Structural rearrangement of monomeric PlaF at the membrane surface results in accessibility of the active site cavity to the membrane phospholipids.

PlaF variants with mutated amino acids in the TM helix and ligand binding site were purified and characterized by activity, thermal unfolding and *in vitro* crosslinking measurements. Mutation V33A did not destabilize PlaF, yet it resulted in enhanced dimerization and reduced activity. Moreover, we have identified a hydrophilic patch, formed through interactions of S29 and T32 of two PlaF molecules, as an important structural feature for regulation of fatty acid inhibition. To test the role of phospholipids for PlaF function, the protein was reconstituted in small unilamellar vesicles (SUV) composed of pure phosphatidyl-choline, -ethanolamine and -glycerol (PC, PE, PG). Activity measurements revealed a negative effect of PG, but positive effects of PE and PC on PlaF activity.

#### References:

[1] Flores-Díaz, M., *et al.* (2016) *Microbiol Mol Rev.* 80:597-628

### MTP339

#### Identification of a potential late intermediate of Tat-dependent protein translocation

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The twin-arginine translocation (Tat) system transports folded proteins across membranes of prokaryotes and plant plastids. In *Escherichia coli*, distinct interactions between the three components TatA, TatB, TatC, and the signal peptide of Tat substrates result in two major complexes that migrate at 440 and 580 kDa referring to blue-native polyacrylamide gel electrophoresis. Both complexes contain multiple copies

of TatB and TatC. They can initiate transport by binding the signal peptide of Tat substrates. While the 440 kDa complex is also formed in the absence of TatA, formation of the 580 kDa complex is promoted by TatA. Substrate can associate to 440 as well as to 580 kDa complexes, resulting in approx. 500 and 600 kDa complexes, respectively. We found a new TatABC complex which is shifted to even higher molecular weights (approx. 660 kDa) by introducing the photo-crosslinker amino acid *p*-benzoyl-L-phenylalanine (*p*BPA) into the first periplasmic loop of TatC. This loop plays a crucial role for TatC-TatC interactions and, accordingly, UV light generated a TatC-TatC crosslink. However, the shifted complex did not depend on the crosslink and was most likely induced by the aromatic properties of the *p*BPA side chain. We thus substituted this position by natural aromatic residues and found that tyrosine had indeed similar effects, although the shifted complex was less abundant and the usual complexes were still predominant. In case of the *p*BPA substitution, the 580 kDa complex can be formed in the absence of TatA, suggesting that this mutation promotes changes that usually depend on TatA. We propose that this substitution stabilizes a late stage intermediate of the Tat translocase in which TatA binding sites are occupied by TatB.

### MTP340

#### Analysis of an unusual thermophilic TatCA system in *Bacillus subtilis*

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The Tat system can transport folded and often cofactor-containing proteins across the cytoplasmic membrane. While Tat systems of Gram-negative bacteria usually consist of TatA, TatB, and TatC, low-GC Gram-positive bacteria generally lack TatB. In these cases, TatA is believed to fulfill functions of TatA and TatB. Tat systems of Gram-positive bacteria are therefore regarded as "minimal" Tat systems. We have analyzed the Tat system encoded by the *tatCA* operon of the thermophilic Gram-positive *Moorella thermoacetica*. The *tatCA* operon is unusual because the order of the genes is reversed in comparison to almost all other Gram-positive bacteria. We could establish a functional *M. thermoacetica* TatCA system in a *Bacillus subtilis* strain lacking all indigenous Tat system components. A natural Tat substrate of *B. subtilis*, the heme-containing protein YwbN, could be demonstrated to be translocated by this heterologous *Moorella* TatCA system. TatA and TatC interacted with each other, but this interaction was rather weak and not as tight as known for TatBC complexes in TatABC systems.

### MTP342

#### Analysis of the role of cation/proton antiporters in *Corynebacterium glutamicum* for pH homeostasis

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Besides protons, Na<sup>+</sup> and K<sup>+</sup> ions are the most important monovalent cations for bacterial cells. Na<sup>+</sup> ions are not only used for sodium-coupled energy conservation and energy transduction but also for solute uptake, activation of intracellular enzymes and pH homeostasis. Potassium represents the most abundant cation in the prokaryotic

cytoplasm and plays a role in the control of the membrane potential, osmotic stress response, and pH homeostasis.

*C. glutamicum* is an important production organism for amino acids production and serves as model organism for related Mycobacteria. In *C. glutamicum* Na<sup>+</sup> ions are mainly taken up by Na<sup>+</sup>/solute symport. For K<sup>+</sup> ions there is only one functional uptake system present, the channel CglK. The export of both cations is brought about by a set of 4 cation/proton antiporters: ChaA, NhaP, Mrp1, and Mrp2. The physiological characterization of *mrp1* and *mrp2* deletion mutants resulted in increased sensitivity to elevated sodium and potassium concentrations, indicating roles in Na<sup>+</sup>/H<sup>+</sup>- and K<sup>+</sup>/H<sup>+</sup>-antiport, respectively. The mutant *C. glutamicum* AQM lacking all four antiporters turned out to be highly sensitive to increased salt concentrations, especially at alkaline pH and during cultivation in complex medium. By the use of the genetically encoded pH sensor protein pHluorin we analysed the internal pH in *C. glutamicum* WT and *C. glutamicum* AQM during cultivation in different media. Internal pH values above 9 were detected transiently for both strains when cultivated at alkaline pH, but recovery to neutral internal pH proceeded faster in *C. glutamicum* WT than in *C. glutamicum* AQM. pH homeostasis of strain AQM was impaired in presence of high salt contents. Important consequences of these results for the physiology of *C. glutamicum* are discussed.

#### MTP343

##### Lon-dependent proteolysis of the signal recognition particle component Ffh in *Escherichia coli*

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Membrane protein transport sustains cellular homeostasis and membrane integrity. In prokaryotes one mechanism for membrane protein trafficking is the cotranslational transport by the signal recognition particle (SRP), which consists of the protein component Ffh and the regulatory 4.5S RNA [1-4]. Ffh contains two functional domains, i. e. the M and NG domain. The M domain recognizes signal sequences of nascent membrane proteins and binds the 4.5S RNA by a helix-turn-helix motif [5,6]. The NG domain harbors a GTPase activity and interacts with the membrane-anchored SRP receptor FtsY [7]. GTP-dependent interaction of SRP and FtsY allows release of nascent membrane proteins to the protein-translocation complex SecYEG [7-9].

We identify that in *E. coli* the cytosolic AAA<sup>+</sup> protease Lon is involved in growth phase-dependent proteolysis of Ffh. In order to determine the recognition mechanism we separately analyzed the stability of the two functional Ffh domains *in vivo*. While the NG domain was stable, the M domain was degraded over the entire growth curve, respectively. Thus, Lon recognizes the M domain. These results reveal an involvement of the AAA<sup>+</sup> protease Lon in regulation of membrane protein transport and membrane integrity.

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#### MTP344

##### Evidence for a protein transport functionality encoded within the *Clostridium difficile* pathogenicity locus PaLoc

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The pathogenicity locus (PaLoc) of *Clostridium difficile* comprises five genes (*tcdR*, *tcdB*, *tcdE*, *tcdA* and *tcdC*). While the proteins TcdB and TcdA represent the main toxins of this pathogen, TcdR and TcdC are involved in the regulation of their production. TcdE is a holin family protein, members of which are usually involved in the transport of cell wall-degrading enzymes (autolysins) for phage-induced lysis. In the past, TcdE has been implied in transport of TcdB and TcdA, but it is unclear whether TcdE rather contributes to toxin release by lytic functions. TcdE can be produced in three isoforms that are initiated from distinct N-terminal ATG codons. When produced in *Escherichia coli*, we found that the longest TcdE isoform resulted in growth arrest. The shorter isoforms strongly and equally induced lysis. This lysis was not dependent on the existence of an autolysin. Importantly, when the *E. coli* strain contained an autolysin, rapid lysis was also observed with the longest TcdE isoform, supporting the view that TcdE is able to contribute to specific protein transport, as do other holins. Growth defects of the longest TcdE isoform alone are likely to be induced by a degradation to the lytic shorter isoform sizes. In conclusion, the long isoform of TcdE is a good candidate for a toxin-specific transporter.

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#### MTP345

##### Outer membrane vesicles of *Dinoroseobacter shibae* – proteome, DNA content and signalling molecules

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The development of outer membrane vesicles (OMV) is a fundamental trait of all living cells. They can contain DNA, signalling molecules, lipids and proteins, and serve cell-cell communication by transporting effector molecules between bacteria and host. This mechanism is especially important for hydrophobic compounds and in vast, dilute environments like the ocean. The production of membrane vesicles at certain growth stages is a striking feature of *D. shibae* cultures. We started to clarify their physiological role. Initial work focused on microscopical observation of the vesicles, their purification and concentration by tangential flow filtration, and the removal of flagella from the sample by density gradient

ultracentrifugation. Purified OMV preparations were analysed by electron microscopy, DNA was extracted for sequencing, and acylated homoserine lactones (AHLs) were analysed by chemical methods and bioassay. The proteome of the OMVs was analysed in comparison with the bulk cytoplasmic proteome and massive enrichment of certain proteins was discovered.

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## ePoster Session 1

### Secondary Metabolomics (SMP)

16 April 2018 • 14:30–17:30

#### SMP421

##### Preliminary investigation of the phytochemical properties of aqueous and ethanolic crude extracts of *Hunteria umbellata* K. (Schum) seeds and its antihypertensive effects on salt induced hypertension in Wistar Rats.

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*Hunteria umbellata* seeds are used ethnomedicinally to treat obesity, pain, swellings, anaemia and as immune booster. The aim of this study was to investigate the preliminary phytochemical properties of aqueous and ethanolic crude extracts of *Hunteria umbellata* seeds and its physiological effects on salt induced hypertension in experimental animals. The phytochemical studies were carried out according to the methods of Association of Official Analytical Chemist (A.O.A.C). Twenty - five (25) female adult Wistar rats were administered with 8 % of NaCl (salt) for 2 weeks and shared into five groups of five animals in each group. The results of the quantitative phytochemical components of aqueous and ethanolic extracts revealed the presence of oxalate, phytate, tannins, flavonoids, saponins, alkaloids, phenols, cyanogenic glycoside and anthraquinones. The blood pressures of the animals were taken before and after salt treatments at a weekly interval. The administration of the standard drug (propranolol) caused a reduction in high systolic blood pressure of the hypertensive experimental animals from 162.00 mmHg to 134.00 mmHg, while the diastolic pressure was observed to fall from 103.00 mmHg to 73.67 mmHg. The administration of aqueous extract of *H. umbellata* seeds caused a reduction of the systolic blood pressure of the hypertensive experimental animals from 159.20 mmHg to 136.25 mmHg, while the diastolic blood pressure was observed to fall from 103.80 mmHg to 80.25 mmHg. The administration of ethanolic extract of *H. umbellata* seeds caused a reduction in systolic blood of the hypertensive experimental animals from 160.20 mmHg to 133.00 mmHg, while the diastolic pressure was recorded to drop from 102.20 mmHg to 76.75 mmHg. The results presented here revealed the effect of

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#### SMP422

##### Rapid reporter strain-based screening approach for the detection of antibacterial natural products

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Bacteria are masters of adaptation, which is reflected by the dramatic increase of antibiotic-resistant bacteria over the

past decades. In order to maintain the lead in the fight against bacterial infections, there is an urgent need to find new antimicrobial compounds with novel mechanisms of action. Natural products remain an excellent source for antimicrobial drug discovery as they have evolved for interaction with biological molecules. Following the classical growth inhibition-based screening approach using producer strains or corresponding supernatants and extracts, it is difficult to determine whether there are multiple distinct antimicrobial substances hidden behind an observed zone of inhibition. Here, a reporter strain panel was developed for efficient screening of pure substances, crude extracts, culture supernatants as well as producer strains themselves. For the generation of the reporter strains, promoters known from *Bacillus subtilis* large-scale transcriptome studies to specifically respond to the presence of different antibiotic stresses were selected [1, 2]. It could be validated that the different promoters were specifically induced upon interference with distinct bacterial metabolic areas including RNA synthesis, DNA structure and synthesis, protein synthesis, fatty acid synthesis or cell envelope integrity. This robust reporter system detects antimicrobial activity while it provides at the same time first insights into the antibiotic mode of action, which allows following up hits more selectively. Furthermore, this approach shows potential for the identification of natural products that have previously been overlooked.

#### References:

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[2] Urban A, *et al.* (2007) DOI 10.1128/AEM.00586-07

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#### SMP423

##### Investigation of new potential protein synthesis inhibitors from uncharacterized Streptomyces of the 'Tübingen strain collection'

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Antibiotics are our most important weapon in the treatment of bacterial infections, including life-threatening hospital infections. Approximately 70% of all known antibiotics are produced by actinomycetes, whereat streptomycetes make up the largest part of it. Over time antibiotic resistances have become a huge major threat to public health and thus it is urgently needed to find new effective antibiotics. The bacterial ribosome is a hot spot for the action of many successful antibiotics. However, not all promising ribosomal binding sites have been therapeutically exploited so far. The aim of this project is to identify/characterize new protein synthesis inhibitors.

In order to find new protein synthesis inhibitors the Tübingen strain collection is currently screened by a *B. subtilis* promoter-based reporter assay in the AG Heike Brötz-Oesterheld. So far, one strain (Tü 6430) turned out to be induce the *yheI* promoter, which reacts upon substances causing translation arrest. Thus, Tü 6430 is a promising strain to produce a protein synthesis inhibitor. It has been grown in different culture media and for different time points to determine the optimal production conditions. Bioassays showed antibiotic activity against Gram-positives and yeast, but not against Gram-negatives. The genome sequence of

Tü 6430 has been analyzed by AntiSMASH. Thereby one huge type1 PKS-arylpolypolyene-NRPS gene cluster (~226 kb) has been identified, which may encode for the antibiotic substance. This biosynthetic gene cluster is currently investigated genetically. Furthermore, Tü 6430 culture extracts were fractionated and the active fraction were analyzed via HPLC/MS. The masses in this purified fraction conform to no known substance in our database. Therefore, Tü 6430 might produce a new compound.

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#### SMP424

##### Conversion of the *Pseudomonas aeruginosa* virulence factor 2-heptyl-4-hydroxyquinoline *N*-oxide by *Mycobacterium abscessus* and *Bacillus subtilis*

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The virulence factor 2-heptyl-4-hydroxyquinoline *N*-oxide (HQNO), produced by the human pathogen *Pseudomonas aeruginosa*, is known to inhibit respiratory electron transfer. Recently, we showed that other microorganisms are capable of converting HQNO. The ubiquitous *Bacillus subtilis*, and *Mycobacterium abscessus*, an emerging human pathogen, detoxify HQNO by specifically glycosylating and methylating the *N*-oxide moiety of HQNO, respectively (1). Since enzymes responsible for HQNO modification are unknown so far, we aimed at characterizing these proteins. Using bioinformatics, the highly homologous proteins YdhE, YjiC, and YojK of *B. subtilis* were identified as potential candidates for HQNO glycosyltransferases. All three proteins, purified from recombinant *Escherichia coli*, showed comparable catalytic efficiencies for HQNO and UDP-glucose at pH 7.5 (about 10<sup>2</sup> (s\*mM)<sup>-1</sup> and 10<sup>1</sup> (s\*mM)<sup>-1</sup>, respectively). The potential methyltransferase was enriched from *M. abscessus* using a multi-step protocol and identified by protein mass spectrometry. The enzyme, a putative class I S-adenosyl-methionine (SAM)-dependent methyltransferase, annotated as hypothetical protein, was expressed in *E. coli*. It methylates HQNO using SAM with a catalytic efficiency of 10<sup>-4</sup> (s\*mM)<sup>-1</sup>. The substrate specificities of the three glycosyltransferases and the methyltransferase towards a series of alkylquinolones and flavonoles are currently being analyzed. The proteins identified in this study are the first enzymes known to detoxify HQNO. This capability could lead to a better fitness of *B. subtilis* and *M. abscessus* when living together with *P. aeruginosa* in polymicrobial environments such as soil, or lungs of patients with cystic fibrosis.

#### References:

[1] Thierbach *et al.*, ACS Chem Biol. 2017 Sep 15,12(9):2305-2312

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#### SMP425

##### Biosynthesis of cyclic peptides with unique chemical properties in fungi

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The secondary metabolites astins are antitumor compounds derived from the Chinese medicinal plant *Aster tataricus*. Structurally, astins are pentapeptides with unusual non-proteinogenic amino acids such as the unique dichloro-

proline which strongly suggest a non-ribosomal peptide synthetase (NRPS) being involved in their biosynthesis. We speculated that the actual producer of astins is a microorganism living in close contact with the plant. A very similar group of compounds, the hepatotoxic cyclochlorotines, are produced by the widespread mold *Talaromyces islandicus*. For both groups, astins and cyclochlorotines, a similar biosynthesis pathway can be assumed.

We aimed to identify the biosynthetic pathways for astins and cyclochlorotines.

Bioinformatic analysis of the genome sequence of *T. islandicus* identified a wealth of biosynthetic gene clusters (BGCs) potentially coding for the synthesis of secondary metabolites (1). We showed by gene silencing that one NRPS, CctN, is responsible for the synthesis of cyclochlorotines (2) and proposed a detailed model for their biosynthesis. The work is the first description of an NRPS gene cluster in the fungal genus *Talaromyces*.

In terms of the astin containing plant *A. tataricus*, several endophytic fungi could be isolated, out of which one produces astins in pure culture. Moreover, our findings suggest that the plant could participate in modifying reactions of the astins. By sequencing and analyzing the genome of the novel endophytic fungus (3), the astin BGC could be identified which enables the depiction of the astin biosynthesis.

#### References:

[1] Schafhauser *et al.*, (2015) J Biotechnol 211:101-2

[2] Schafhauser *et al.*, (2016) Environ Microbiol 18(11):3728-41

[3] Jahn *et al.*,(2017) J. Biotechnol 257:233-39

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#### SMP426

##### An integrated functional genomics approach to unravel the *mode-of-action* of novel anti-infective compounds

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**Introduction:** Due to the worldwide spread of antibiotic resistance, antibacterial agents are urgently needed. Quantitative gel-free proteomics and complementary Omics-technologies have emerged as valuable tools to study the physiology of microbes under antibiotic stress conditions.

**Objectives:** The presented study aims at the generation of a comprehensive antibiotic signature library for *Staphylococcus aureus* that can be used as a template for the comparative analysis of proteome signatures generated by antimicrobial compounds of unknown mode of action. To this end, we applied our signature library of five antibiotics with well-defined different molecular targets to characterize the poorly understood antimicrobial mechanism of telomycin (NP) and a derivative of telomycin (SAR).

**Materials & methods:** *S. aureus* HG001 was exposed to increasing concentrations of various antibiotics to determine

sublethal concentrations in order to map the specific antibiotic stress response. The soluble protein fractions of *S. aureus* HG001, harvested 60 min and up to 360 min after antibiotic treatment were prepared and analysed using a gel-free LC-IMSE approach in combination with the Hi3 method for absolute protein quantification.

**Results:** Each antibiotic showed an individual protein expression profile and drug target specific signature proteins were identified (e.g. cell wall, ribosome, and replication machinery). The proteome analysis of NP or SAR treated *S. aureus* suggests that these molecules interfere with cell division and in parallel induce membrane depolarization.

**Conclusion:** We established a workflow for a comprehensive antibiotic stress proteome signature library of *S. aureus*. Using this strategy, we gained insight into the mode-of-action of NP and SAR.

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#### SMP427

##### The putative PAINs nostotrebin 6 and derivatives from *Nostoc* sp. inhibit the trypanosomal cysteine protease rhodesain

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The trypanosomal cysteine protease rhodesain plays a major role during parasitic infection by *Trypanosoma brucei*. It is regarded as a promising target for drugs against the parasite [1]. Cyanobacteria are known as a rich source of protease inhibitors [2], thus a collection of about 670 cyanobacteria extracts was screened for inhibitory activity against rhodesain [3]. The most promising hit extract from a *Nostoc* sp. strain was fractionated using flash and high-performance liquid chromatography, resulting in the isolation of 14 inhibitory compounds. Structure elucidation by nuclear magnetic resonance (NMR) spectroscopy and high-resolution mass spectrometry revealed one of the compounds to be Nostotrebin 6 [4], while the others are new intriguing structurally related, monomeric, dimeric, or trimeric compounds. Nostotrebin 6 acts as an inhibitor of acetyl- (IC<sub>50</sub> 6 μM) and butyrylcholinesterase (IC<sub>50</sub> 7 μM) [4], exhibits cytotoxicity and pro-apoptotic activity [5] and antibacterial activity [6]. Due to its broad bioactivity spectrum and as, to our knowledge, no structure-activity relationship (SAR) has been established so far, we assume Nostotrebin 6, a polyphenolic compound, to be a pan-assay interference (PAIN) compound.

#### References:

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[2] Breuning A, Degel B, Schulz F, Buchold C, Stempka M, Machon U, Heppner S, Gelhaus C, Leippe M, Leyh M, Kisker C, Rath J, Stich A, Gut J, Rosenthal PJ, Schmuck C, Schirmeister T. Journal of Medicinal Chemistry **2010**; 1951-1963

[3] Zelik P, Lukesova A, Cejka J, Budesinsky M, Havlicek V, Cegan A, Kopecky J. J Enzyme Inhib Med Chem **2010**; 414-420

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#### SMP428

##### The secreted metabolome of *Streptomyces chartreusis* and implications for bacterial chemistry

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*Actinomycetes* are known for producing diverse secondary metabolites. Combining genomics with untargeted data-dependent tandem mass spectrometry and molecular networking, we characterized the secreted metabolome of tunicamycin producer *Streptomyces chartreusis* NRRL 3882. The genome harbors 128 predicted biosynthetic gene clusters. We detected more than 1000 distinct secreted metabolites in culture supernatants, only 22 of which were identified based on standards and public spectral libraries. *S. chartreusis* adapts the secreted metabolome to cultivation conditions. A number of metabolites are produced iron dependently, among them 17 desferrioxamine siderophores aiding in iron acquisition. Eight new members of this long-known compound class are described. A single desferrioxamine synthesis gene cluster was detected in the genome, yet different sets of desferrioxamines are produced in different media. Additionally, a new polyether ionophore, differentially produced by the calcimycin biosynthesis cluster, was discovered. This illustrates that metabolite output of a single biosynthetic machine can be exquisitely regulated not only with regard to product quantity, but also with regard to product range. Compared to chemically defined medium, in complex medium total metabolite abundance was higher, structural diversity greater, and the average molecular weight almost doubled. Tunicamycins for example were only produced in complex medium. Extrapolating from this study, we anticipate that the larger part of bacterial chemistry including chemical structures, ecological functions, and pharmacological potential is yet to be uncovered.

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#### SMP429

##### Fungus-growing Termites – a Prolific Source of new Actinobacteria and Natural Products

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**Introduction:** *Macrotermite* cultivate a mutualistic food fungus (*Termitomyces* sp.) for nourishment in so called "fungus gardens", a nutrient-rich environment prone to exploitation. Symbiotic and associated bacteria are assumed

to play a major role in the defense of the fungus garden by secretion of antimicrobial small molecules.

**Objectives:** We performed a functional, genomic and chemical analysis of Actinobacteria associated with fungus growing termites to study their role as defensive symbionts.

**Methods:** Using different culture-dependent methods, we first isolated Actinobacteria from insect body parts and the fungus comb. Here, we describe their in-depth phylogenetic analysis and assessed their bioactivities using ecological and pharmaceutically relevant bioassay set-ups. Subsequent co-cultivation studies were performed to monitor the changing metabolome and to identify novel metabolites. The produced antimicrobial secondary metabolites were analyzed and characterized using HPLC/LC-HRMS/NMR. We also performed the characterization of new Actinobacteria strains.

**Results:** Amongst various other strains, two *Actinomadura* strains (*Actinomadura* sp. RB29 and RB68), isolated from the termite gut, were defined as new and fully described. *Actinomadura* sp. RB29 produced reddish pigments, which were identified as new highly substituted tropolone alkaloids (rubterolone A-F). Genome mining revealed the putative biosynthetic pathway. Pairing challenging assays of *Actinomadura* sp. RB29 showed also antifungal activity against co-isolated and antagonistic fungi of termites, and the isolation of the antifungal compound is topic of current investigations.

**Conclusion:** Microbial symbionts and commensals are a rich source of new natural products and new Actinobacteria strains.

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#### SMP430

##### Identification of siderophores produced by *Esca*-associated fungi

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*Esca* is one of the most important grapevine trunk diseases causing devastating damage and decay of grapevines worldwide. It is considered to be a disease complex comprising several fungi; e.g. *Phaeoemoniella chlamydospora*, *Phaeoacremonium aleophilum*, *Fomitiporia mediterranea* and *Eutypa lata*. The disease trigger is still not well understood and effective methods to control *Esca* are urgently needed.

Siderophores are chelators produced by many fungal species to acquire extracellular iron. In several plant-pathogen interactions these secondary metabolites were described as virulence factors, whereas no extensive studies of siderophore involvement in *Esca* disease have been reported to date. Therefore siderophore production by *Esca*-associated fungi and gene expression of putative genes involved in biosynthesis were studied.

To induce the production of siderophores the four species were grown individually in an iron-depleted medium. The iron chelators were extracted via XAD-16 from culture filtrates and analyzed by HPLC-MS comparing retention time, UV-Vis and mass spectra with standards.

Several secreted siderophores were detected: *P. chlamydospora* was found to produce ferrichrysin, whereas triacetylfusigen was observed in cultures of *P. aleophilum*. Siderophores of coprogen group were found in cultures of *E. lata* and *F. mediterranea*. Putative gene clusters were identified by fungiSMASH analysis for *P. chlamydospora*, *P. aleophilum* and *E. lata*. Nonribosomal peptide synthetase and L-ornithine-N<sup>5</sup>-monooxygenase genes likely to be involved in siderophore biosynthesis were identified.

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#### SMP431

##### Heterologous expression of two putative NRPS-like coding genes from *Guignardia bidwellii* in *Magnaporthe oryzae*

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*Guignardia bidwellii* is the causal phytopathogenic fungus of black rot in vines posing a massive threat to organic viticulture. Nearly all green expanding tissues of grapevine accompanied are infected by *G. bidwellii* there by leading to crop losses of up to 80%. Originally, the disease was widespread in North America and was introduced to Europe in the 19th century. In Germany, the first symptoms of the disease were progressively observed 2002 in abandoned vineyards, constantly expanding to cultivated vineyards. Increasing temperatures and atmospheric humidity as a result of climate change are believed to potentially increase the threat posed by the pathogenic effect caused by the fungus. The knowledge concerning about the molecular mechanisms of the host/pathogen-interaction is still rather limited. To date a genetic manipulation of *G. bidwellii* has not been successfully established in order to generate "loss-of-function" mutants and investigate the importance of secondary metabolites in more detail. The phytotoxic dioxolanones phenguignardic acid and guignardic acid have been identified as potential virulence factors involved in the infection process. Guignardic acid was found in leaf material of *G. bidwellii*-infected *Vitis vinifera*, on the contrary no toxins were detected in uninfected leaf material. Two putative NRPS-like genes have been identified for which gene expression correlates with the production of these metabolites. These two genes have been introduced in *Magnaporthe oryzae* via *Agrobacterium tumefaciens*-mediated transformation in order to express these NRPS-like enzymes in a heterologous manner. Phenguignardic acid and guignardic acid were detected in extracts of the respective *M. oryzae* expression mutants.

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#### SMP432

##### Emission of Volatiles changes due to interaction of *Serratia plymuthica* 4Rx13 and *Bacillus subtilis* B2g

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Bacteria emit enormous amounts of various volatile metabolites. Some of these bacterial volatiles play important roles in inter-organismic communication due to manipulation of other bacteria, fungi and plants. Most of current research was performed on volatiles emitted by isolated and mono-cultivated bacterial species not considering that bacterial phenotype differs when strains are embedded in communities as it is found in nature. The knowledge about volatile emission of bacterial species living in communities, however, is still limited. We show that in a structured, low diversity model community of *Serratia plymuthica* 4Rx13 and

*Bacillus subtilis* B2g that grew under nutrient rich conditions the emission of volatile changed compared to self-paired mono-cultivations. In detail, co-culturing revealed a decreased emission of volatiles at the beginning of growth whereas in the later stage the amount of headspace volatiles was increased. In bioassays *A. thaliana* was stronger inhibited in co-cultivation correlating with the higher amount of volatiles. The result that co-cultivation of bacterial species lead to changes and shifts in the volatile profile and that *A. thaliana* is differentially affected by co-cultivation induced differences of these volatiles demonstrate the importance of considering the effect of microbial communities on volatile emission.

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#### SMP433

##### Proteasome inhibitors from *Nocardia* spp. and their Role in Pathogen Survival

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The proteasome regulates numerous cellular processes, such as mitosis, inflammation, apoptosis and immune responses. Exploitation of the ubiquitin-proteasome degradation machinery is therefore a developed strategy by human bacterial pathogens to evade the host immune system. *Nocardia* spp. are gram positive, filamentous Actinobacteria, that can cause localized and systemic Nocardiosis, mostly in immunosuppressed patients. It was previously shown that the highly virulent *N. cyriacigeorgica* GUH-2 is capable of inducing a small molecule-mediated inhibition of the proteasome [1]. Interestingly, we just recently identified the first two biosynthetic gene clusters of the proteasome inhibitors (PI) of the epoxyketone-family, Epoxomicin and Eponemycin, and a homologous pathway was also found in *N. cyriacigeorgica* GUH-2 [2]. We know raise the question if this orphan gene cluster of *N. cyriacigeorgica* GUH2 encodes for a PI and which role this small molecule plays in the pathogenicity of this strain. Identification and characterisation of the putative PI will be accomplished by RED/ET recombineering mediated gene-cluster knock-outs, as well as heterologous pathway expression of the whole candidate gene-cluster. Metabolite production will be followed by LCMS. For the biological characterization of the PI, we just recently established a proteasome inhibition assay and additionally plan to do various mammalian cell-based assay, e.g. infection experiments with live *N. cyriacigeorgica* GUH-2.

#### References:

[1] Barry, D.P. and B.L. Beaman, *Nocardia asteroides* strain GUH-2 induces proteasome inhibition and apoptotic death of cultured cells. *Research in microbiology*, 2007. 158(1): p. 86-96.

[2] Schorn, M., et al., Genetic basis for the biosynthesis of the pharmaceutically important class of epoxyketone proteasome inhibitors. *ACS chemical biology*, 2013. 9(1): p. 301-309.

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#### SMP434

##### Identification of volatiles mediating interactions between marine bacteria

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Bacteria are prolific producers of volatile compounds, but the function of the often complex bouquet of compounds in the ecology of bacteria is largely unknown. To address the impact of bacterial volatiles on other bacteria we investigated single and dual cultures of a subset of marine bacteria originating from the same area of the Pacific Ocean.

Bacteria were grown on A1 medium and the volatiles emitted by agar plate cultures of twelve strains belonging to the genera *Pseudoalteromonas*, *Vibrio*, *Shimia*, *Ruegeria*, *Labrenzia*, *Bacillus*, *Alteromonas*, *Salinispora* and *Erythrobacter* were trapped by closed loop stripping analysis (CLSA). Elution of the compounds was followed by detailed gas chromatography/mass spectrometry (GC/MS) analyses. More than 200 compounds were identified, consisting of compounds belonging to various compound classes such as aromatic compounds, sulfur compounds, pyrazines, terpenes, and fatty acid metabolites, as well as nitrogen containing compounds. In a second set of experiments various combinations of two bacteria were grown on divided agar plates that allowed only volatiles to diffuse freely between the two cultures. In several bacterial combinations with one *Salinispora* strain total or partial suppression of growth of one of the cultures was observed. Combinations with observable effects were selected for a third row of experiments. The bacterial combinations were then grown on two different agar plates in a single container. This time the combined whole volatile bouquet of the two cultures was analyzed by CLSA and GC/MS. Comparison with the bouquets of the individually grown bacteria revealed up- and down regulation of several volatiles, e. g. previously unknown oximes. The results of these analyses will be reported on the poster.

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### ePoster Session 1

#### Microbiota and Eukaryotic Microbes (MBP, EuP)

16 April 2018 • 14:30–17:30

#### MBP404

##### Establishing of a robust metaproteomic pipeline to characterize respiratory and intestinal swine microbiota during bacto-viral co-infections

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**Introduction:** Recent studies indicate a clear correlation between structure and function of the human microbiota, progression of infections and consequently also health status and fitness of the host. Therefore, a comprehensive analysis of (co-)infections must also include a detailed characterization of the host microbiota colonizing different niches, such as the respiratory or gastrointestinal tract.

**Objectives:** We aim to elucidate the dynamic response of the commensal microbiome of the gastrointestinal as well as the respiratory tract of swine prior and after co-infection with Influenza A and *Streptococcus* sp.. A detailed characterization of structure and function of the microbiome during bacto-viral-co-infections will allow identifying molecular markers on the proteome level.

**Materials & Methods:** Feces and lung lavage samples were subjected to different protein extraction protocols. The protein extracts were separated via 1D SDS PAGE and

peptides, derived by trypsin- digestion, were analyzed by state-of-the-art mass-spectrometry.

**Results:** Homogenization of the fecal samples is one of the major challenges for establishing a sample preparation pipeline with high quality outcome. Until now, best results were achieved by mechanical homogenization of samples frozen in liquid nitrogen. Following homogenization several protocols have been tested for optimal protein isolation out of feces. First results indicate that the microbiome of the swine gastrointestinal tract is mainly composed of Firmicutes, Bacteroidetes and Proteobacteria.

**Conclusion:** The amount of reliably identified proteins strongly depends on the employed protocol. Thus, optimized and standardized protocol will be key to obtain high-quality and reproducible microbiome metaproteomics data.

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#### MBP406

##### Effect of a high fat diet on the bile acid metabolism of bacteria in the murine gut

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High fat diet was shown to alter the murine gut microbiome in several studies, but the reasons for this are not fully understood. As high fat diet increases bile acid concentrations in the gut, it is argued that this shift is a major driver of the alterations of the gut microbiome. Bile acids are amphipathic molecules responsible for emulsifying fat, enabling lipid digestion and uptake. They are toxic to bacteria primarily by causing membrane damage. However bacteria can also modify bile acids as matter of detoxifying or for obtaining energy and nutrients. The products of these modifications are called secondary bile acids and can only be produced by bacteria, which harbor bile salt hydrolases. Genes encoding bile salt hydrolases have been found in *C. perfringens*, *Lactobacillus plantarum*, *La. johnsonii*, *Bi. longum*, *Bi. bifidum*, *Bi. adolescentis*, and *Listeria monocytogenes*. Secondary bile acids may contribute to the pathogenesis of colon cancer, gallstones, and other gastrointestinal diseases. Therefore, we investigated how high fat diet is influencing the secondary bile acid forming bacterial community and their metabolism. We used a metagenomics approach in combination with targeted metabolomics to assess bile acid composition and to connect bacterial gene abundances to changes in the bile acid composition.

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#### MBP407

##### Preliminary analysis of the bacterial microbiota on worn spectacles using next generation sequencing of 16S rRNA genes

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**Background:** Surfaces with regular contact to the human body are usually contaminated with a broad variety of microorganisms and might hence be considered as fomites [Egert et al., 2015]. Although very widespread in the population, little is known about the microbial contamination of spectacles and their hygienic relevance in both clinical and

non-clinical environments. Previous cultivation-based analyses from our group suggested a spectacle bacteriota strongly dominated by staphylococci. Here, we investigated the composition of the bacterial microbiota of worn spectacles for the first time using a culture-independent next generation sequencing approach.

**Methods & Results:** 30 spectacles from university staff and students were swab-sampled at three sampling sites (nosepads, glasses and earclips) and analyzed by 16S rRNA gene sequencing on an Illumina MiSeq platform. Only 20 samples from 12 different spectacles (11 earclips, 7 nosepads, 2 glasses) yielded sufficient high quality sequences for complete downstream analyses. In these samples, 26 phyla and 686 genera of bacteria were detected. *Actinobacteria* (78%), *Firmicutes* (17%) and *Proteobacteria* (5%) were the dominant phyla. At genus level, *Propionibacterium* (76%), *Staphylococcus* (15%) and *Corynebacterium* (1.5%) were the most abundant groups, accompanied by many, probably yet-uncultured taxa.

**Conclusions:** Our study provides first insight into the cultivation-independent composition of the bacteriota on worn spectacles. It appears to be dominated by aerobic and anaerobic bacteria from human skin and epithelia. Further improvement of the analysis protocol will be needed to increase the share of spectacles that yield sufficient sequences for molecular community analyses.

#### References:

[1] Egert, M. et al. *Folia Microbiol* (2015) 60: 159

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#### MBP408

##### Illumina MiSeq and Ion Torrent-based search for human intestinal bacteria with a potential role in Parkinson's disease

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**Introduction:** We aim to identify gut bacteria specifically associated with Parkinson's disease (PD) as a basis to investigate their potential role in this particular neurodegenerative disease in more detail.

**Methods:** The stool bacteriota of 39 PD patients and 25 age-matched controls<sup>1</sup> was comprehensively analyzed using Illumina MiSeq- and Ion Torrent-based sequencing of the V4 and V5 variable region of PCR-amplified 16S rRNA genes. Bioinformatics were executed with QIIME and R using the Phyloseq package.

**Results:** Based on preliminary analyses conducted with the Illumina (~ 6 million sequences) and the Ion Torrent dataset (~13 million sequences) so far, we could confirm several trends recently reported in other sequencing studies on the intestinal PD microbiota, such as a significant decrease of the genus *Faecalibacterium*<sup>2</sup> in PD patients. In addition, we observed hitherto unreported changes in minor abundant groups, such as a reduced number of *Atopobium*-related sequences. In general, the Illumina-based dataset showed a

higher number of genera differing significantly between PD and control patients than the Ion Torrent-based dataset.

**Conclusion:** Our data will increase the body of knowledge regarding the role of intestinal bacterial groups in the course of PD. In a long term perspective, they might also serve as a basis for probiotic treatments to alleviate intestinal symptoms associated with PD, such as constipation.

#### References:

[1] Unger, M. M. et al. (2016): *Short chain fatty acids and gut microbiota differ between patients with Parkinson's disease and age-matched controls*. *Parkinsonism Relat. Disord.* 32: 66-72.

[2] Hill-Burns, E. M. et al. (2017): *Parkinson's disease and Parkinson's disease medications have distinct signatures of the gut microbiome*. *Mov. Disord.* 32: 739-749.

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#### MBP409

##### Influence of environmental factors on the bacterial microbiota of domestic washing machines

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**Background:** Washing machines are central hygiene tools in domestic environments. Facilitated by recent, sustainability-driven washing trends (low temperature washing, bleach-free liquid detergents), microorganisms find optimal living conditions here: humidity, warmth, nutrients and rarely cleaned surfaces. The microbiota in washing machines could cause staining, corrosion, machine and laundry odor, and might thus negatively affect laundry hygiene.

**Methods & Results:** The bacterial microbiota of 21 in-use domestic washing machines was sampled at different sites (detergent drawer, door seal, sump, and washing solution) and analyzed by 16S rRNA gene pyrosequencing. Over all samples, 26 phyla, 583 genera and 7161 species OTUs were detected. *Proteobacteria* (86.0%), *Actinobacteria* (5.4%), *Firmicutes* (3.0 %) and *Bacteroidetes* (2.8%) were the dominant phyla. At genus level, *Pseudomonas*, *Acinetobacter* and *Moraxella* were relatively most abundant, the latter of which are known to comprise well-known odor producers. The highest diversity was determined for the detergent drawer on all taxonomic levels, followed by sump, washing solution and door seal. Correlation analysis with data from a user survey revealed that microbial diversity is also driven by the number of wash cycles per month at  $\geq 60^\circ\text{C}$ . It remains to be elucidated in more detail, what this means in terms of machine and laundry hygiene.

**Conclusions:** Domestic washing machines are characterized by a diverse and site-dependent bacterial microbiota, which appears to be influenced by the washing temperature. Our data will help to better understand the hygienic relevance of the washing machine microbiota and to develop targeted hygiene strategies to further improve laundry hygiene.

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#### MBP410

##### Small differences may turn the scale: How carbonate effects the growth of *Prevotella copri* in contrast to *Bacteroides* spp.

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The human gut microbiome can be distinguished into three distinct enterotypes that represent specific states of the microbial community and are dominated by *Prevotella* spp., *Bacteroides* spp. or *Ruminococcus* spp., respectively<sup>1</sup>. Especially, the first two enterotypes have become of emerging interest not only because of their high prevalence in the human population, but also because of their antagonistic relationship<sup>2</sup>. The reason for the repression of *Bacteroides* spp. by *Prevotella* spp. and vice versa is still unknown, though different types of diet seem to support the growth of a particular genus. However, both genera exhibit similar metabolic features and substrate spectra, making it difficult to identify the underlying mechanism of the competition.

Bioinformatic, biochemical and cultural methods were used to analyze the central carbon and energy metabolism of *Prevotella copri* and *Bacteroides vulgatus* as representative members of the genera. Interestingly, we found a striking difference in the basic biochemical features of these organisms. While both species convert glucose via the Embden-Meyerhoff-Parnas pathway and perform fumarate respiration, *P. copri* is unlike *B. vulgatus* not able to further convert succinate to propionate. Because this reaction balances the  $\text{CO}_2/\text{HCO}_3^-$  need of the central metabolism, its nonexistence in *P. copri* creates a high net requirement for  $\text{CO}_2/\text{HCO}_3^-$  making the organism strictly  $\text{CO}_2$ -dependent. In contrast, *B. vulgatus* is able to grow under  $\text{CO}_2$ -limited conditions. Therefore,  $\text{CO}_2/\text{HCO}_3^-$  concentrations may be a crucial but rarely mentioned element in the development of the enterotypes of the human gut.

#### References:

[1] Arumugam *et al.* (2011). *Nature* 473, 174-180.

[2] Ley (2016). *Nat Rev Gastroenterol Hepat* 13, 69-70.

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#### MBP411

##### Full-length 16S rRNA Sequencing - New Perspectives in Microbiome Research

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**Introduction:** Based on their ubiquitous presence, evolutionary stability and changes in its variable sequences, genes of the small ribosomal subunit are the most common used phylogenetic marker. Nevertheless, 16S rRNA analyses are mostly performed by short amplicon sequencing for the ribosomal gene of the small subunit (SSU) and are, thus, limited.

**Objectives:** The aim of this study is to analyse stool samples using full-length SSU rRNA (similar to Karst *et al.* (2016)). This will allow a deeper insight in species and strains present of all domains, i.e., bacteria, archaea, and eukarya. The latter will include the host, but also fungi and, if present, worms and protozoa of the digestive tract.

**Materials & Methods:** RNA was isolated from stool samples and SSU rRNA was extracted. Adapters with unique molecular barcodes were ligated to the 16S rRNA molecules, which were then reversed transcribed and amplified. The library was split and either processed by using the Illumina Nextera Kit (to sequence pieces of the full-length 16S rRNA) or circularized to physically link the adapters in close proximity (necessary to assemble the correct pieces sequenced to full-length, originating from the same parent molecule).

**Results:** The generation of full-length SSU rRNA sequences enable the assessment of complete communities in the gut. Additionally, the method allows a higher resolution for species determination.

**Conclusion:** In conclusion, applying SSU rRNA full-length sequencing on stool samples will expand our knowledge about the organismal diversity in the gut.

#### References:

[1] Karst SM, Dueholm MS, Mcllroy SJ, Kirkegaard RH, Nielsen PH, Albertsen M. Thousands of primer-free, high-quality, full-length SSU rRNA sequences from all domains of life. bioRxiv. 2016.

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#### MBP412

##### Plant microbiome stability upon pathogen attack: lessons from the Brassicaceae smut fungus *Thecaphora thlaspeos*.

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Plants are colonized and interact with a huge variety of microorganisms comprising opportunistic, plant-beneficial and pathogenic microbes. Among them, fungal pathogenic endophytes are able to balance their virulence leading to systemic host colonization over very long timescales without major induction of plant defenses or interference with plant development. However, how endophytes manage to establish and maintain such sustained systemic infections and what determines the type of interaction and host specificity remains largely unknown. Here, we studied *Thecaphora thlaspeos*, a distant relative of the well-studied grass smut *Ustilago maydis* and the only known smut fungus that adapted to Brassicaceae hosts including the model species *Arabidopsis thaliana*. We performed a comparative analysis of microbiome composition, nutrient content and glucosinolate levels in wild *Arabidopsis thaliana* populations in the presence and absence of a *T. thlaspeos* infection. This revealed a markedly low profile of *T. thlaspeos* during infection of *A. thaliana* in the field compared to healthy plants. Remarkably, *T. thlaspeos*-infected and healthy plants did not differ in microbiome and glucosinolate content and composition, respectively. This suggests *T. thlaspeos*, in contrast to other plant pathogens such as *Albugo laibachii*, to pursue a strategy of stealth pathogenesis, that facilitates our long-term goal to establish *T. thlaspeos* as a synthetic endophyte to study plant-microbe and microbe-microbe interactions.

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#### MBP413

##### A small intestine model allows to assess the interaction of probiotics with the intestinal microbiota

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**Introduction:** The project "Good Bacteria and Bioactives in Industry" (BMBF funded alliance GOBI) Health-GI aims to develop *Lactobacillus* strains that prevent chronic gut inflammation.

**Objectives:** The small intestine is the target of choice for probiotics, being the main site for immunological perception and food digestion. However, small intestine microbiota models are lacking to date. We established a human *in vitro* small intestine model, and tested the impact of probiotic lactobacilli (strains *L. rhamnosus* GG and LAB-1) upon the microbiota.

**Material & Methods:** The physiological and enzymatic conditions of mouth, stomach and small intestine were mimicked. Saliva and fecal samples from healthy volunteers were inoculated in the oral and intestinal stages of the model, respectively. A medium simulated a healthy diet. The bacterial composition was determined by selective agar plating, qPCR and 16S rRNA gene sequencing.

**Results:** In the oral phase, Proteobacteria and Firmicutes dominated. In the gastric stage Proteobacteria represented most of the low abundant microbiota. In the small intestinal stage, Firmicutes, Bacteroides and Proteobacteria were the main phyla present. Probiotics survived up to the intestinal stage only in the presence of the healthy diet medium, and represented temporarily a large proportion of the intestinal microbiota. The stimulation of *F. prausnitzii* by probiotics was not significant, but a reduction of Streptococcaceae, Halomonadaceae, Pseudomonadaceae was observed.

**Conclusion:** A small intestinal model for investigating the effect of probiotics on the microbiota was established. This allows for the first time to directly follow the survival of probiotic during stomach and intestine passage and their impact on the intestinal microbiota.

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#### MBP414

##### Characterization of the diversity and community composition of the gut microbiota of *Hermetia illucens* larvae and its effects on substrate associated bacterial communities

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*Hermetia illucens*, commonly known as black soldier fly (BSF), is one of the most promising insects for future application to recycle organic waste in combination with the production of high quality proteins as feed for agricultural animals. The gut microbiota plays a key role in the digestion efficiency that includes the deactivation of potential

pathogenic bacteria of organic waste by the production of antimicrobial compounds and the production of high quality proteins. A first feeding experiment was performed to learn more about the dynamics of the gut microbiota and their effect on the microbiota of the employed feed substrates. Both were investigated at different growth stages by 16S rRNA gene amplicon based Illumina MiSeq sequencing. The analysis showed that the bacterial community of the gut and the employed substrate was mainly composed of four different phyla, *Actinobacteria*, *Firmicutes*, *Bacteroidetes*, and *Proteobacteria*, with differences in relative abundances in each growth stage. Non-metric multidimensional scaling of bacterial community patterns indicated that the gut microbiota and the substrate associated bacterial communities differed among each other and changed over time. Especially the much more diverse bacterial assemblages present in the organic substrates were strongly affected by the BSF activity. Many bacterial genera containing potential pathogens, as *Staphylococcus* spp. or *Acinetobacter* spp., were strongly decreased. In contrast, the gut microbiota was less diverse and BSF larvae specific. Further feeding experiments will be performed to study the activity of the gut microbiota in more detail and to optimize the antimicrobial and digestion efficiency of BSF larvae by the enrichment of a highly active gut microbiota.

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#### MBP415

##### Trimethylamine-producing bacteria in the gut

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Trimethylamine (TMA), a bacterial metabolite primarily derived from dietary quaternary amines such as choline, is converted to trimethylamine-*N*-oxide (TMAO) by liver enzymes in the host. This oxidised product is associated with the development of atherosclerosis and severe cardiovascular diseases. So far, only little information on the identity of TMA producers in the gut is available due to their low abundance and the lack of specific methods for their detection and enumeration. We recently established a diagnostic framework consisting of databases for key genes mediating TMA formation and gene-targeted assays for quantification (qPCR) and characterisation (Illumina sequencing of key genes of TMA production). Applying our techniques on human faecal samples (n=50) we gave first insights into this functional guild, uncovering potential TMA producers in all samples. Although, concentrations were generally low (<1 % of total bacterial community), some samples exhibited high gene abundances. In order to obtain detailed knowledge on parameters governing TMA production, in particular diet, and revealing the dynamics of respective bacteria, we performed a longitudinal dietary intervention study in ileo-caecal fistulated Göttingen Minipigs fed a choline rich diet. Increased urinary TMA levels along with shifts in composition of communities encoding the choline TMA lyase (*cutC*) in both chyme and faecal samples were observed. Additional analysis of intestinal TMA-producing communities of carnivorous, omnivorous and herbivorous *Mammalia* from various taxonomies (n=89) showed respective bacteria being omnipresent in the gut. Our results give crucial insights into the ecology of TMA producers.

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#### MBP416

##### Exploiting termite nest microbiomes for antibiotic discovery by using an ultra-high throughput Microfluidics/FACS driven pipeline combined with a microtiter plate based cultivation strategy

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Infections with multi-resistant Gram negative pathogens are a major threat to our health system. In order to serve the needs in antibiotics development we selected untapped bioresources and implemented high throughput approaches suitable for the discovery of strains producing antibiotics with anti-Gram negative activity.

Our approaches rely on the hypothesis that termite associated bacteria are likely to produce potent antibiotics to defend their hosts against entomopathogenic microorganisms. Termite nests and guts harbor suitable, highly diverse microbiomes in which bacterial taxa are present known to potentially produce natural compounds. In a first step the diversity of *Coptotermes* species nest microbiomes was assessed carefully by using 16S rDNA amplicon sequencing on the Illumina MiSeq platform and nest material was selected to retrieve viable cells by using Nycodenz density gradient centrifugation. In order to analyze the diversity of the culturable termite nest microbiome, bacterial cells were either distributed in 384-well plates (approach 1) or encapsulated in small spheric agarose beads by an high throughput microfluidics technique (approach 2). Cultures obtained from approach 1 were scaled-up in 96-well Duetz-systems for characterization of diversity and for rapid supernatant screening using the bioluminescence-labeled *E. coli* pFU166. The generated droplets of approach 2 simultaneously received a small population of GFP-tagged Gram negative screening cells and were sorted for low fluorescence using FACS. After elimination of redundancy we performed a fast scale-up of active strains.

Implementation of this pipeline allows us to prioritize antibiotics producing strains in a ultra-high throughput fashion and by cultivation of broad diversity in our approaches.

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#### MBP417

##### The correspondence of the nasal microbiome and olfactory function – or: The nose-brain axis

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**Background and question.** The loss of smell is an incisive event, either caused by mechanical impact or subtly during the process of ageing. Besides effects on psychological, social and behavioral performance and thus affecting the quality of life tremendously, the loss of the sense of smell induces a reorganization in the functional network structure of the human brain.

The ability to smell is mediated by olfactory sensory neurons in the ceiling of the nose, the olfactory mucosa. This area is also inhabited by numerous microorganisms. In general, the

human body is associated with trillions of microorganisms (the microbiome) which appear to have tremendous effects on health, disease, behavior and other aspects of human life. The microbial community is capable to communicate with the human body cells and affects functions of human tissues and even brain.

This project aims to decipher the role of the nasal microbiome in olfactory function, dysfunction and regain.

**Methods:** Neuroimaging biomarkers and olfactory function data are correlated with microbiome measures such as diversity, abundance and functional data (metatranscriptomics).

**Results:** In a completed pilot study, we have shown that the microbial community composition in the olfactory mucosa mirrors the capability to smell. Volunteers with impaired smelling capacity showed an increase of specific (anaerobic) microbial groups, such as butyrate producing microorganisms.

**Conclusion:** Understanding the microbial community in the olfactory mucosa increases therapeutic opportunities, and possibly allows monitoring and predicting smell therapy success in future.

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#### MBP418

##### Worlds apart - transcriptome profiles of key oral microbes in the periodontal pocket compared to single laboratory culture reflect synergistic interactions

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Periodontitis is a worldwide prevalent oral disease which results from dysbiosis of the periodontal microbiome. Some of the most active microbial players, e.g. *Porphyromonas gingivalis*, *Treponema denticola* and *Fusobacterium nucleatum*, have extensively been studied in the laboratory, but it is unclear to which extent these findings can be transferred to *in vivo* conditions. Here we show that the transcriptional profiles of *P. gingivalis*, *T. denticola* and *F. nucleatum* in the periodontal niche are distinct from those in single laboratory culture and exhibit functional similarities. GO (gene ontology) term enrichment analysis showed up-regulation of transporters, pathogenicity related traits and hemin/heme uptake mechanisms for all three species *in vivo*. Differential gene expression analysis revealed that cysteine proteases, transporters and hemin/heme-binding proteins were highly up-regulated in the periodontal niche, while genes involved in DNA modification were down-regulated. The data suggest strong interactions between those three species regarding protein degradation, iron up-take, and mobility *in vivo*, explaining their enhanced synergistic pathogenicity. We discovered a strikingly high frequency of Single Nucleotide Polymorphisms (SNPs) *in vivo*. For *F. nucleatum* we discovered a total of 127,729 SNPs in periodontal niche transcripts, which were found in similar frequency in health and disease and covered the entire genome, suggesting continuous evolution in the host. We conclude that metabolic interactions shape gene expression *in vivo*. Great caution is required when inferring pathogenicity of microbes from laboratory data, and microdiversity is an important adaptive trait of natural communities.

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#### MBP419

##### Functional profiling and crystal structures of isothiocyanate hydrolases

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**Introduction/Question:** Plants of the Brassica family, including many of the most important food crops like broccoli and rapeseed, produce toxic isothiocyanates (ITCs) upon tissue damage. The bacterial gene *saxA* was found in cabbage root fly guts, and provides resistance against certain ITCs. ITCs are used as soil insecticides and potential anticancer drugs, so this newly characterized enzyme family might be important in counteracting these applications. The goal of this research was to resolve the structure and to elucidate the substrate range of a novel family of enzymes: isothiocyanate hydrolases.

**Methods:** Seven homologous SaxA proteins from plant and animal associated microbes were heterologously expressed in *E. coli* and purified. Each protein was assayed on GC-MS for its hydrolytic activity on six ITCs, namely methyl-, ethyl-, allyl-, phenyl-, benzyl-, and 2-phenylethyl isothiocyanate. The crystal structures and stoichiometry of two SaxA representatives were resolved using X-ray crystallography and size exclusion chromatography, respectively.

**Results:** Enzymatic characterization of seven phylogenetically related but distinct SaxA proteins exhibited similar activities on six selected isothiocyanates. Notable structural features of SaxA include a hydrophobic active site with two Zn<sup>2+</sup> ions coordinating water/hydroxide, and a flexible cap that is implicated in substrate recognition that covers the active site.

**Conclusion:** Phylogenetic diversity does not determine the substrate specificity of SaxA. SaxA likely forms native dimers with outward-facing active sites that can be covered with a flexible loop that is implicated in substrate recognition. SaxA is a potential mitigator of ITC-based applications in crop loss prevention as well as cancer treatments.

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#### MBP420

##### Evolutionary origin and metabolic roles of termite gut spirochetes: comparative genome analysis of 54 uncultured lineages and the first isolate from cockroach guts

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Spirochetes make up as much as one-half of the microbial community in termite guts and are considered to play important roles in the symbiotic digestion of lignocellulose. Most termite gut spirochetes fall into a monophyletic cluster dubbed "Treponema I" and show up to 25% dissimilarity to the 16S rRNA sequences to other members of the genus *Treponema*. The only isolates are in subcluster Ia and represent sugar-fermenting, free-swimming forms, whereas members of other subclusters are associated with the fiber fraction and may partake directly in the breakdown of lignocellulose. To gain deeper insights into the metabolic roles of the different groups, we have reconstructed the

genomes of 54 uncultured members of the "Treponema I" cluster from the hindgut metagenomes of eight termites. Moreover, we isolated the first representative of the cluster from cockroaches, the closest relatives of termites, using a strategy that combined filtration with deep-agar dilution series. Phylogenetic analysis assigned the isolate a basal position in the entire cluster, which offers important clues about the metabolic capacities of the ancestral form. We are currently conducting a comparative genome analysis of the isolates and the uncultured members of the cluster, which will provide a better understanding of the evolutionary origin of termite gut spirochetes and their diverse roles in the termite gut ecosystem.

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### EuP203

#### Molecular characterization of *Babesia canis* strains detected in naturally infected dogs in Lithuania

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Canine babesiosis caused by *Babesia canis* is an emerging infectious disease in Europe. In Lithuania it has become quite frequent in the last few years, and an increasing number of cases with a wide variety of clinical signs have been recorded throughout the country. Differences in the virulence of *B. canis* are associated with observed genetic heterogeneity among *B. canis* strains. In the present study we aimed to investigate genetic diversity of *B. canis* strains in Lithuania using PCR-RFLP assay and sequence analysis based on the 18S rRNA and *Bc28.1* genes. In total 138 blood samples from dogs suspected of babesiosis were collected in Lithuania during 2016-2017. PCR-RFLP analysis of *B. canis* 18S rRNA gene identified 2 genotypes, from those predominated less virulent 18S rRNA-A (93.3 %) against more virulent 18S rRNA-B (6.66 %) genotype. Three 18S rRNA genotypes of *B. canis* were distinguished by sequence analysis. Two groups of *B. canis* strains were detected by analyzing *Bc28.1* gene and a total of fifteen single nucleotide polymorphisms were identified by sequence analysis. The majority of the *B. canis* strains were of the genotype *Bc28.1*-B (82.1%), followed by genotype *Bc28.1*-A (17.9 %). The distribution of *Bc28.1* genotypes in Lithuania is similar to that obtained in South – West Europe, but different from North – East Europe findings. Our results indicate the presence of different *B. canis* genotypes in Lithuania and provide the basis for study of the relationship between the genetic structure of *B. canis*, their geographical distribution, and the form of the disease in dogs.

#### Acknowledgment:

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## ePoster Session 1

### Fungal Biology (FBP)

16 April 2018 • 14:30–17:30

#### FBP219

Biological control of root-knot nematode *Meloidogyne incognita* infesting Eggplant by the nematode-trapping fungus *Dactylaria brochopaga* and the nematode egg

#### parasitic fungus *Verticillium chlamydosporium* under field conditions.

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Microbial control of root-knot nematode by using the nematode-trapping fungus *Dactylaria brochopaga* & *Verticillium chlamydosporium* as a combined with yeast, molasses and vermiculate is reported under field condition study. The results revealed that the highest percentage reduction in number of nematode larvae per 1kg soil was achieved when applying the fungi *D. brochopaga* & *V. chlamydosporium* together combined with yeast, molasses and vermiculate. Also the highest percentage reduction in number of root-galls per plant (88.7%) was achieved by using the fungi *D. brochopaga* & *V. chlamydosporium* together combined with yeast, molasses and vermiculate. The data revealed that the weight of fruits per bean plant were significantly ( $P \leq 0.05$  and/or  $P \leq 0.01$ ) increased in all nematode-trapping fungus *D. brochopaga* treatments compared to the untreated check treatment.

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#### FBP220

#### The fungal peroxiredoxin Asp f3 is essential for virulence of *Aspergillus fumigatus* and protects against protein oxidation by reactive oxygen species

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The fungus *Aspergillus fumigatus* causes severe and often fatal infections in immunocompromised individuals. Patients at high-risk include those suffering from chronic granulomatous disease, as a defect in NADPH oxidase leads to a reduced capability to produce reactive oxygen species (ROS). Thus, it is assumed that ROS are important for the protection against *A. fumigatus* infections, even though the mechanism of protection remains obscure. For *A. fumigatus*, we have recently characterized the major allergen Asp f3 as a two-cysteine type peroxiredoxin with high relevance for the protection against ROS. The deletion of *aspf3* resulted in high sensitivity towards certain ROS and avirulence in a mouse model of pulmonary aspergillosis. Since the protective mechanism of Asp f3 during host invasive growth and in the presence of ROS is still unknown, we are aiming to elucidate its cellular function, as well as the primary biochemical targets of ROS. To monitor the direct cellular effects of ROS, we established an *in vivo* assay, which allows specific exposure to external pulses of superoxide (O<sub>2</sub><sup>-</sup>), the primary product of the NADPH oxidase in innate immune cells. Although such O<sub>2</sub><sup>-</sup> treatments were not lethal, these pulses were fully fungistatic for strains lacking Asp f3. To analyse whether high levels of protein oxidation could have caused this growth defect we used a gel-based proteomic approach to identify highly oxidized proteins. Main targets with a different protein redox states in the wildtype and  $\Delta$ aspf3 strain, included not only central metabolic enzymes, but also proteins for which an extracellular function can be proposed. Using *in vitro* assays, we are currently validating whether these candidates could represent central targets of host-derived ROS.

## FBP221

### Fruiting-body formation in *Sordaria macrospora* involves nutrient recycling and remodeling by autophagy

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The homothallic filamentous ascomycete *Sordaria macrospora* is an ideal model organism to study multicellular fruiting-body development. Supply and homeostasis of nutrients are important issues for sexual development. Autophagy is a degradation process in which eukaryotic cells digest their own cell constituents. We have characterized conserved components of the autophagic machinery and could show that autophagy is an essential process to sustain high energy levels for multicellular development. In contrast to non-selective bulk autophagy, selective autophagy is characterized by cargo receptors, which bind specific cargos such as superfluous or damaged organelles, and target them for autophagic degradation. Using the core autophagy protein ATG8 as bait, GFP-Trap analysis followed LC/MS identified a putative homolog of the human autophagy cargo receptor neighbour of BRCA1 (NBR1) in *S. macrospora*. Fluorescence microscopy revealed that SmNBR1 co-localizes with SmATG8 at autophagosome-like structures and in the lumen of vacuoles. Delivery of SmNBR1 to the vacuoles requires SmATG8. Both proteins interact in an LC3 interacting region (LIR)-dependent manner. Deletion of *Smnbr1* leads to impaired vegetative growth under starvation conditions, and reduced sexual spore production under non-starvation conditions. The human *nbr1* homolog partially rescues the phenotypic defects of the fungal *Smnbr1* deletion mutant. The *Smnbr1* mutant can neither use fatty acids as a sole carbon source, nor form fruiting bodies under oxidative stress conditions. Fluorescence microscopy revealed that degradation of a peroxisomal reporter protein is impaired in the *Smnbr1* deletion mutant. Thus, SmNBR1 is a cargo receptor for peroxisomes in filamentous ascomycetes.

## FBP222

### Taxonomic identification of strains from the mycophenolic acid producer *Penicillium brevicompactum* by using mating-type loci

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In heterothallic ascomycetes mating and sexual reproduction are governed by two non-allelic idiomorphs, namely mating-type loci (MAT1-1 or MAT1-2). These loci carry genes encoding transcription factors, which have either an alpha- or a high mobility group- DNA-binding domain. We discovered the MAT loci in the filamentous fungus *Penicillium brevicompactum*, which is used in industry for production of immunosuppressant mycophenolic acid.

After cloning of MAT loci by using PCR primers for conserved sequences flanking the MAT loci, we discovered the genomic organization of the MAT1-2-1 and MAT1-1-1 open reading frames from at least 13 strains of *Penicillium brevicompactum*. The open reading frames were verified by cDNA cloning and sequencing. Comparing MAT amino acid sequences with those from other *Penicillium* species revealed a high homology in the DNA binding domains. However other regions of the proteins were less similar.

Beside 2 molecular markers, Internal transcribed spacer (ITS),  $\beta$ -tubulin, MAT loci were also used for taxonomic characterization of 36 wild type strains provided from different culture collections. Remarkably, from 36 strains, previously described as *P. brevicompactum* 16 were identified as another related species. Further, we identified an almost equal number of MAT 1-1 and MAT1-2 strains, suggesting that sexual reproduction occurs between *P. brevicompactum* strains in nature. Our data suggests that MAT loci can be used as a novel molecular marker to identify strains from *P. brevicompactum* and point to the potential of this gene for the taxonomic identification of other *Penicillium* species. It will be interesting to use MAT genes for the taxonomic identification of other *Penicillium* species.

## FBP223

### Different phagocytic efficiencies of yeast species by a fungivorous amoeba shed light on ancient patterns for the recognition of fungi

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Phagocytic cells are an important aspect of the human innate immune system. They can recognize a myriad of different fungal pathogens. However, these cells display differences in the phagocytic efficiencies of various yeast species at the level of uptake and intracellular processing.

*Protostelium aurantium* is an exclusively mycophagous amoeba which we use as a new model system to study evolutionarily conserved interactions between pathogenic yeasts and phagocytes.

A wide range of fungi are recognized, phagocytosed, and killed in the range of a few minutes with extreme efficiency. Feeding experiments showed that this amoeba has a broad prey range of both filamentous fungi and yeasts including most *Candida* spp., though some species were unable to serve as a food source, such as *Saccharomyces cerevisiae* and *C. glabrata*. Recognition and phagocytosis experiments using fluorescence staining and confocal microscopy revealed that recognition of fungi is independent of intracellular processing. Rapid phagocytic uptake was observed with the preferred food source *C. parapsilosis* but also with two inedible yeasts. In contrast, *C. albicans* cells were rarely ingested. Interestingly, comparably low phagocytosis rate for *C. albicans* have also been observed with human neutrophils and were shown to result from reduced exposure of  $\beta$ -glucan on the surface. This makes  $\beta$ -glucan a promising candidate for the recognition receptor of *P. aurantium* for its prey suggesting that environmental amoeba and innate immune cells recognize fungi by identical molecular patterns.

## FBP224

### Heavy Metal Induced Expression of PcaA Provides Cadmium Tolerance to *Aspergillus fumigatus* and Supports its Virulence

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Most of the metal transporters in *Aspergillus fumigatus* are yet uncharacterized. Their role in fungal metabolism and virulence remains unclear. This paper describes the novel PIB-type cation ATPase PcaA, which links metal homeostasis and heavy metal tolerance in the opportunistic human pathogen *A. fumigatus*. The protein possesses conserved ATPase motifs and shares 51% amino acid sequence identity with the *Saccharomyces cerevisiae* cadmium exporter Pca1p. A *pcaA* deletion, an overexpression and a *gfp-pcaA* complementation strain of *A. fumigatus* were constructed and their heavy metal susceptibilities were studied. The *pcaA* knock out strain showed drastically decreased cadmium tolerance, however, its growth was not affected by the exposure to copper, iron or zinc. Although the lack of PcaA had no effect on copper adaption, we demonstrated that not only cadmium but also copper is able to induce the transcription of *pcaA* in *A. fumigatus* wild type Af293. Similarly, cadmium and copper could induce the copper exporting ATPase *crpA*. Moreover, its gene expression was dependent on the presence of PcaA. These data imply a general response to heavy metals in *A. fumigatus* through the induction of detoxification systems, on the transcriptomic level. Confocal microscopy of the *gfp-pcaA* complementation strain, expressing functional GFP-PcaA, supports the predicted membrane localization of PcaA. The GFP-PcaA fusion protein is located in the plasma membrane as well as it is associated to the septae of *A. fumigatus* in the presence of cadmium. Moreover, virulence assays attest the necessity of PcaA for the virulence of *A. fumigatus* in the *Galleria mellonella* wax moth larvae model.

#### FBP225

##### The trimeric CandA complex of *A. nidulans* comprises the 20 kDa CandA-C1 protein for CullinA E3 ligase activity and fungal growth

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CandA interacts with cullin E3 RING ligases (CRL) thereby acts as an adaptor-receptor exchange factor for CRL assembly control in the ubiquitin proteasome pathway. Evolution changed the subunit composition of the Cand1/A protein, whose sequence and function are conserved across eukaryotes. Higher eukaryotes as human or the plant pathogen *Verticillium dahliae* have a one-subunit Cand1 protein. The human pathogen *Aspergillus fumigatus* CanA is split into an N- and a C-terminal protein. The Af\_CanA C-terminal protein has a fungal specific insertion of about 190 amino acids at its N-terminal end. We identified that this insertion is mostly conserved in *Aspergillus* species and some of them encode in addition a third CandA protein. This was first investigated in *A. nidulans* where we named the 20 kDa protein CandA-C1. The genetic locus is 269 bp upstream of *candA-C* which is five open reading frames upstream of *candA-N*. We show that CandA-C1, CandA-C and CandA-N are separate genes resulting in a trimeric CandA complex. We demonstrate that *A. nidulans* CandA proteins are required to re-activate CulA after deneddylation by the COP9 signalosome. Elevated protein levels of CandA-C1 promote CulA neddylation. Furthermore, CandA-C1 is required for vegetative growth and ascospore production. However, it is not involved in the regulation of the orsellinic acid secondary metabolite production, as CandA-N and CandA-C. We showed that also the *A. fumigatus* CanA orthologs are required for growth and properly timed conidiospore development. The necessity of *A. fumigatus* CandA-C1 ortholog for growth and the fact that this part is

absent in human, suggests this sequence to be a promising drug target to fight aspergillosis.

#### FBP226

##### Biological control of mango dieback disease caused by *Lasiodiplodia theobromae* by actinomycetes in the United Arab Emirates

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Dieback caused by the fungus *Lasiodiplodia theobromae* is a serious disease on mango plantations in the United Arab Emirates. A total of 53 actinomycete strains were isolated from mango rhizosphere, of which 35 were classified as streptomycetes and 18 as non-streptomycetes. Among these isolates, 19 showed antagonistic activities against *L. theobromae* associated with either the production of antifungal metabolites, extracellular cell wall degrading enzymes (CWDEs), or both. In a "novel" mango fruit bioassay, all isolates were tested *in vivo* for their abilities to reduce lesion formation on fruits inoculated with the pathogen. Two *Streptomyces* spp. and one *Micromonospora* sp., showed the strongest inhibitory effect against this fungus *in vitro* and were selected to test on mango seedlings. Our results revealed that the antifungal action of *S. samsunensis* was based on antibiosis, as well as the production of CWDEs; whilst *S. cavourensis* and *M. tulbaghia* were considered as antibiotic- and CWDE-producing isolates, respectively. In greenhouse, pre-inoculation with the isolates showed the highest efficacy against dieback disease on mango seedlings subsequently inoculated with the pathogen. This was evident by the dramatic reduction in the estimated disease severity indices of the mango dieback of individual applications of the biocontrol agents (BCA) compared with the pathogen alone. Prior to *L. theobromae* infection, mango seedlings treated with *S. samsunensis* significantly reduced the number of defoliated leaves and spore counts of *L. theobromae* by 2- and 4-fold, respectively, in comparison with the other two BCA applications. This is the first report of BCA against *L. theobromae* by microbial antagonists and the first report of actinomycetes to control mango dieback.

#### FBP227

##### Chemical control of mango dieback disease caused by the fungal pathogen *Lasiodiplodia theobromae* in the United Arab Emirates

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Mango is affected by different decline disorders causing significant losses to mango growers. In the United Arab Emirates (UAE), the pathogen was isolated from all tissues sampled from diseased trees affected by *Lasiodiplodia theobromae*. Symptoms at early stages of the disease included general wilting appearance of mango trees, and dieback of twigs. In advanced stages, the disease symptoms were also characterized by the curling and drying of leaves, leading to complete defoliation of the tree and discoloration of vascular regions of the stems and branches. To substantially reduce the devastating impact of dieback disease on mango, the fungus was first identified based on its morphological and cultural characteristics. Target regions of 5.8S rRNA (*ITS*) and elongation factor 1- $\alpha$  (*EF1- $\alpha$* ) genes of the pathogen were amplified and sequenced. We also found that the systemic chemical fungicides, Score®, Cidely® Top, and Penthiopyrad®, significantly inhibited the mycelial growth of *L. theobromae* both *in vitro* and in the

greenhouse. Cidely® Top proved to be a highly effective fungicide against *L. theobromae* dieback disease also under field conditions. Altogether, the morphology of the fruiting structures, molecular identification and pathogenicity tests confirm that the causal agent of the mango dieback disease in the UAE is *L. theobromae*. In this study, *L. theobromae* was identified, for the first time, as the causal agent of dieback disease on mango in the UAE.

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## FBP228

### Degradation of fungal dihydroxynaphthalene type melanin: a Proteomics approach

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Melanins have a wide range of roles in fungal pathogenicity. For example, the layer of dihydroxynaphthalene (DHN) melanin located on the outside of spores of the pathogenic mould *Aspergillus fumigatus* has been reported to be involved in masking immunogenic structures on the spore surface, to reduce phagolysosomal acidification and to inhibit apoptosis of macrophages after phagocytosis of the spores. In addition, it contributes to the quenching of reactive oxygen species produced by host immune cells.

The biosynthesis of DHN melanin has been studied in detail and the intermediate products and nearly all enzymes involved have been elucidated. In contrast, nothing is known about the degradation of DHN melanin, which is important for understanding the germination of conidia e.g. in phagolysosomes or to study the immunological impact of breakdown products. Based on the assembly pathway of DHN melanin and the degradation of aromatic compounds with a structure similar to DHN, it can be assumed that enzymes such as laccases and oxygenases are required for the degradation of the melanin. In this study, a proteomics approach was employed in order to identify enzymes with a possible role in DHN melanin degradation. For this purpose, the proteome of germinating conidia and hyphae of *A. fumigatus* was analysed using liquid chromatography-mass spectrometry (LC-MS/MS). Further experiments are performed aiming at the elucidation of intermediate products and ultimately the degradation pathway.

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## FBP229

### The potential of anaerobic fungi for utilization of lignocellulosic biomass

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**Introduction:** Anaerobic fungi (AF) are effective fibre degraders in the digestive tract of herbivores. They decompose lignocellulosic biomass (LCB) mechanically and enzymatically. Due to their fibre degrading skills AF could improve bioenergy production from LCB.

**Objectives:** Due to its recalcitrant structure, LCB is a challenging substrate for bioenergy production. AF were used in pre-treatment experiments to render LCB accessible for biogas production. Practice oriented methods for ensiling maize straw with AF are being developed.

**Material & Methods:** Two AF strains were assessed for their ability to degrade LCB in two phase fermentation. In the first phase, hay was pre-treated with active and heat inactivated AF cultures. In the second phase, a batch assay with the pre-treated material was performed to assess the effect on biogas production.

Novel media based on natural substances were developed for the isolation of AF suitable for the use as silage additives.

**Results:** Hydrolytic pre-treatment with AF cultures led to accelerated fibre degradation, increased volatile fatty acid production and thus to higher initial biogas production. The AF strains were viable and active during the first phase but were deactivated during the batch fermentation phase.

Isolation of AF was feasible in the cattle manure and the grass silage based culture medium but continuous AF growth was only observed in the latter. Thus, for the application of AF as ensiling additives, cultivation of unconditioned AF was possible in simple media.

**Conclusion:** AF could be used in hydrolytic pre-treatment to improve biogas production from LCB. The future use of AF as ensiling additive will be based on the developed culture media. Both approaches may improve bioenergy production from agricultural residues.

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## FBP230

### The *Verticillium dahliae* transcription factors Som1 and Vta3 control microsclerotia formation and sequential steps of plant root penetration and colonization to induce disease

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*Verticillium dahliae* and *Verticillium longisporum* are plant pathogenic fungi that cause severe disease symptoms in different plant hosts. Whereas *V. dahliae* is haploid, *V. longisporum* is amphidiploid. We used a non-adherent yeast strain and expressed a *V. longisporum* cDNA library to find factors which restored adhesion and therefore might be important for the infection process (Tran et al., 2014). We found that the nuclear transcription factors Som1 and Vta3 of *V. dahliae* can rescue adhesion in a *FLO8*-deficient *Saccharomyces cerevisiae* strain. Som1 and Vta3 induce the expression of the yeast *FLO1* and *FLO11* genes encoding adhesins. We further examined the role of Som1 and Vta3 in *V. dahliae*, deleted the *SOM1* and *VTA3* genes and studied their functions in fungus-induced plant pathogenesis using genetic, cell biology, proteomic and plant pathogenicity experiments. Our experiments showed that Som1 and Vta3 are sequentially required for root penetration and colonization of the plant host. Som1 supports fungal adhesion and root penetration and is required earlier than

Vta3 in the colonisation of plant root surfaces and tomato plant infection. Som1 controls septa positioning and the size of vacuoles, and subsequently hyphal development including normal hyphal branching. Som1 and Vta3 also control conidiation and microsclerotia formation by the regulation of the expression of developmental genes. The molecular function of Som1 is conserved between the plant pathogen *V. dahliae* and the opportunistic human pathogen *Aspergillus fumigatus*. Both, Som1 and Vta3, regulate a genetic network for conidiation, microsclerotia formation and pathogenicity of *V. dahliae*.

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### FBP231

#### Time- and development dependent localization of the STRIPAK complex

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**Introduction:** The striatin-interacting phosphatases and kinases (STRIPAK) complex is conserved from mammalian to eukaryotic microbial organisms. In the filamentous fungus *Sordaria macrospora*, the STRIPAK complex is required for the development of three-dimensional fruiting bodies as well as for hyphal fusion events. Previous data from our research group identified an interaction between STRIPAK and the pheromone response (PR) pathway, a highly conserved MAP kinase cascade. The localization of STRIPAK has been initially investigated but is still lacking with respect to time- and developmental changes and impact of other pathways on its localization.

**Objectives:** We aim to unravel the changes in localization of STRIPAK subunits during the development of *S. macrospora*. Furthermore, we want to investigate how the STRIPAK complex and the PR pathway affect each other's localization.

**Materials & Methods:** We used fluorescence microscopy to investigate the development-dependent distribution of the STRIPAK and PR components.

**Results:** We have developed strains expressing GFP with the native promotor and terminator of STRIPAK subunits and present the visualization of STRIPAK gene expression during development. Further, we show that PR deletion mutants are similar to deletion strains of STRIPAK and demonstrate localization of GFP-tagged PR pathway components in *S. macrospora*. Using gene deletion strains, we show the correlation of the STRIPAK complex and the PR pathway with regard to localization.

**Conclusion:** Our investigations will provide insight into the time- and developmental distribution of STRIPAK subunits and its interaction with other pathways. This data will indicate the function and specificity of the STRIPAK complex during eukaryotic multicellular development.

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### FBP232

#### The UspA deubiquitinating enzyme supports multicellular development by influencing abundance of the velvet protein VeA in *Aspergillus nidulans*

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Cullin Ring Ligases (CRLs) catalyze the attachment of ubiquitin molecules to proteins. Ubiquitination can change stability, function and localization of proteins. The COP9 signalosome regulates CRLs by removing the posttranslational modification Nedd8, which renders the ubiquitination cascade inactive. The gene transcript of *ubiquitin-specific protease A (uspA)* is upregulated in  $\Delta csnE$  strain that lacks the catalytically active subunit of COP9 signalosome during *A. nidulans* multicellular development. UspA interacts in yeast-two hybrid experiments with six of eight COP9 signalosome subunits. It reduces the total amount of ubiquitinated proteins during the whole fungal development. The *uspA* gene deletion causes reduction of conidiospores, defects in early sexual development and upregulation of the *derivative of benzaldehyde (dba)* secondary metabolite gene cluster. VeA is a major regulator of fungal development and secondary metabolism. It migrates together with VelB into the nucleus to fulfill its function. VeA-GFP protein abundance is increased by UspA during vegetative growth and at the initiation of multicellular development, whereas it is decreased during late multicellular development. The interaction of VeA with VelB as well as the localization of both to nuclei is not influenced by the deubiquitinase. UspA-GFP itself is localized close to nuclei and pulls proteins related to nuclear transport and transcriptional processing in GFP pull down experiments. The novel fungal deubiquitinating enzyme UspA ensures accurate fungal development and secondary metabolism by influencing protein abundance of the major fungal regulator VeA.

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### FBP233

#### Real-time monitoring of fungal metabolic activity on solid substrates using biocalorimetry

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Solid-state fermentation (SSF) using fungi can convert lignocellulosic biomass into useful chemicals, building blocks, and bioenergy (biogas, liquid biofuels) within the frame of biorefinery applications, or soil fertilizers. Moreover, lignocellulosic residues can be applied as co-substrates in fungi-assisted soil bioremediation schemes; thus using SSF for the attenuation of organic environmental contaminants. A major challenge of SSF processes is related to the commonly inhomogeneous microbial growth on solid substrates, resulting in considerable difficulties with respect to process control and optimisation. Such drawback necessitates the development of adequate control strategies, ideally based on only one (or a few) parameter(s) enabling sufficient real-time characterisation of the overall process. In this regard, biocalorimetry is a potential tool to characterise and monitor SSF processes because the measured parameter (heat production rate) is proportional to the growth rate of the fungal catalyst.

The litter-decay basidiomycete *Stropharia rugosoannulata* is a well-known degrader of many environmental pollutants, and grows on wheat straw. We used wheat straw cultures of *S. rugosoannulata* in order to substantiate the general applicability of biocalorimetry for real-time SSF monitoring. A cement calorimeter was applied to follow fungal metabolic activities.

The calorimetric data indicated that *S. rugosoannulata* started growing after 4 days of incubation. Extracellular laccase and manganese peroxidase activities were concomitantly recorded, which suggests ligninolysis. Briefly,

biocalorimetry seems promising for the real-time monitoring of fungal activity.

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#### FBP234

##### **Development of optimized *Aspergillus niger* strains for highly efficient D-galacturonic acid generation from pectin-rich, agricultural residues.**

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In conjunction with the German national research strategy "BioEconomy 2030", the BMBF fosters the development of novel applications for the use of renewable resources in the chemical industry. As such, pectin is one of the main components in residual agricultural products like sugar beet pulp. Due to the variety of achievable fermentation products from D-galacturonic acid (D-GalA) as the main backbone component of pectin, it is considered a highly promising second generation feedstock for biotechnological fermentations. Naturally, saprotrophic fungi play an important role in biomass degradation. Amongst these, *Aspergillus niger* is known for its strong pectinolytic capabilities making it a perfect candidate for industrial scale pectin de-polymerization. As a consequence of the complex chemical structure, de-polymerization of pectin to D-GalA is facilitated through a large set of secreted enzymes, collectively referred to as pectinases and tightly regulated on the transcriptional level. The panregulon-like regulation of a large subset of pectinase genes by two central transcription factors (TF), namely GaaR and RhaR, has recently been described in *A. niger* (Alazi et al., 2016, Gruben et al., 2014). TF engineering of GaaR and RhaR aiming at a global increase of pectinase expression is therefore applied to realize highly efficient pectin de-polymerization. Notably, GaaR-/RhaR-activities were shown to be modulated by substrate-related inducers (Alazi et al., 2017, Gruben et al., 2014), carbon catabolite repression (CCR) and nuclear exclusion in the case of GaaR (Niu et al., 2017). For that matter, constitutive activity, improved nuclear localization and reduced sensitivity of GaaR/RhaR towards CCR will improve pectinase production in *A. niger* for industrial D-GalA-supply.

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#### FBP235

##### **The Ca<sup>2+</sup>-binding penta-EF-hand protein PEF-1 is part of a fungal resistance mechanism against cell fusion-induced lysis and membrane-destabilizing antifungals**

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To establish a mycelial colony, germinating vegetative spores of *Neurospora crassa* fuse with each other and form a supracellular network. Fusion pore formation involves highly controlled cell wall breakdown and plasma membrane merger. These steps bear the risk of cell lysis and death by membrane rupture.

We identified the Ca<sup>2+</sup>-binding penta-EF-hand protein PEF-1 as part of a proposed membrane repair mechanism. Subcellular localization and live-cell imaging revealed that PEF-1 is recruited to the fusion point of lysing germling pairs. Additionally, PEF-1 accumulates at the plasma membrane after treatment with antifungal and membrane-destabilizing drugs, such as nystatin or the plant defense compound tomatine. The treatment with tomatine also results in PEF-1

recruitment to septa and occlusion of the septal pore. Consistent with this finding, the growth of a *pef-1* knock-out mutant on medium containing tomatine is highly impaired, compared to the wild type strain. We hypothesize that, membrane damage results in the influx of calcium, which activates PEF-1, which in turn mediates plasma membrane repair. PEF-1 functions appear to be conserved in the fungal kingdom. For example, the PEF-1 homologue of the grey mold *Botrytis cinerea* also shows membrane recruitment after tomatine treatment. We are currently testing its contribution to tomatine resistance and fungal virulence using *B. cinerea* as a model. Moreover, our data indicate that Pef-1p, promotes survival of the human fungal pathogen *Candida albicans* inside of macrophages. Additionally, we will investigate the role of Pef-1p -as a potential pathogenicity factor- using *C. elegans* as an infection model for *C. albicans*.

Further studies aim to characterize the molecular bases of this repair mechanism comprehensively.

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#### FBP236

##### **Systematic analysis of UPR crosstalk reveals novel connections to known signaling pathways**

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Coordinate control of fungal development requires perception, conversion and balancing of a multitude of external and internal conditions. Although, conserved sensors, transmitters and regulators have been identified and extensively studied, knowledge on crosstalk between signaling pathways is comparably scarce and demands further investigation. In the corn smut fungus *Ustilago maydis*, the unfolded protein response (UPR), a highly conserved signaling pathway to ensure endoplasmic reticulum (ER) homeostasis, is an important instance of a developmental control. UPR-activation occurs in response to a specific plant environment and serves as trigger for a developmental progression. Interestingly, premature UPR activation prevents previous developmental steps such as conjugation tube-formation, and formation of infectious filaments. Since the pathways controlling these key steps of *U. maydis* development have been extensively characterized, we systematically analyzed the crosstalk between the UPR and MAPK, PKA, as well as the transcription factor network mediated by the bE/bW homeodomain protein complex. Our data suggest a multilayered interaction network that mediates communication of signaling pathways through modified phospho-signaling, transcriptional control and protein-protein interactions.

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#### FBP237

##### **Exploring iron acquisition, transport and utilisation in *Lichtheimia corymbifera***

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*Lichtheimia* species are the second most common cause of mucormycosis, an opportunistic life-threatening infection in immunocompromised and diabetic patients, in Europe. Infections with *Lichtheimia* as with other mucoralean fungi have a rapid progression rate; are usually difficult to

diagnose and have limited therapeutic options which accounts for the high mortality rate. One of the major risk factors for the development of mucormycosis is elevated serum iron levels. Although iron is an essential trace metal for all living cells, its availability is restricted in the environment. As such, fungi have evolved different mechanisms to acquire iron; it includes the reductive pathway, siderophore uptake systems and the utilisation of iron containing proteins. During infection, the availability of free iron is kept extremely low; most of the iron is bound to host proteins such as ferritin, transferrin and haemoglobin. This iron-withholding response is the canonical example of nutritional immunity and it is one of the main strategies used to restrict the growth of invading pathogens. Fungal pathogens use these different high affinity iron acquisition systems to overcome iron starvation inside the host. To date, our knowledge remains limited regarding iron metabolism in *L. corymbifera* during infection. Thus, the aim of our current research is to identify which iron containing proteins *L. corymbifera* sequesters iron from and which host iron sources are important during infection. We also intend to gain insight into how these key virulence factors for iron acquisition are regulated. These will be accomplished by applying both molecular and physiological studies. Answering these key questions will aid our understanding of how these adaptive traits contribute to virulence.

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#### FBP238

##### **The chitin synthase regulator CSR-3 promotes cell fusion fidelity and contributes to stress-induced cell wall remodeling in *Neurospora crassa***

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The synthesis of chitin, an essential component of the fungal cell wall, is a highly regulated process, that contributes to cell wall remodeling during cellular development and in response to stress. The latter is also controlled by the cell wall integrity (CWI) pathway. In the ascomycete fungus *Neurospora crassa*, the MAK-1 MAP kinase cascade and its potential scaffold protein SO are not only part of the CWI signaling but are also indispensable for cell-cell communication preceding fusion. While two germinated spores fuse, the cell walls of the interacting partners are partially degraded before the opposing plasma membranes merge and a fusion pore is formed. This critical event bears the risk of membrane rupture and subsequent lysis.

A Y2H-screen confirmed the physical interaction of SO with the two upstream kinases of the MAK-1 module and revealed a new interaction partner of SO, the chitin synthase regulator 3 (CSR-3). During fusion, SO, MAK-1, its upstream kinase MEK-1 and CSR-3 co-localize, after the two interacting cells established contact, around the forming fusion pore. Germlings lacking CSR-3 tend to lyse during fusion, suggesting that fusion pore formation requires a fine-tuned equilibrium of chitin synthesis and degradation. Together with the potential target chitin synthase 2, CSR-3 also seems to participate in septation in both germlings and hyphae. CSR-3 possesses a c-terminal prenylation motif crucial for its function but not for the specific localization pattern during septation. Additionally, exposure to cell wall stress results in the recruitment of SO, MAK-1, MEK-1 and CSR-3 to the cell surface.

Based on these observations we hypothesize that cell-cell fusion and stress induced cell wall employ a common molecular machinery.

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#### FBP239

##### **Sem1, 26S proteasome degradation and cellular redox state**

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Sem1 is *bona fide* lid subunit of the proteasome. The role of this intrinsically disordered protein was investigated in the multicellular model organism *Aspergillus nidulans* as the human corresponding gene is essential. We found that Sem1 from *A. nidulans* is required for oxidative stress response, mitochondria integrity and proteasome assembly. Sem1 is not required for vegetative fungal growth but it is essential for cellular differentiation and coordination of secondary metabolites. Oxidative stress response in the wild type included increased transcriptional levels of detoxifying enzymes and proteasomal subunits *semA* and *rpn11*, whereas the mutant strain exhibited damaged and dysfunctional mitochondria. EM revealed increased number of 20S proteasomes in  $\Delta$ *semA* mutant strain with multiplied catalytic activity compared to the complementation strain. This enhanced degradation rate of 20S proteasome presumably serve the purpose of dealing with accumulation of damaged proteins due to oxidative stress.

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#### FBP240

##### **How to boost marine fungal research: a first step towards a multidisciplinary approach by combining molecular fungal ecology and natural products chemistry**

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Marine fungi have attracted attention in recent years due to increased appreciation of their functional role in ecosystems and as important sources of new natural products. The concomitant development of various "omic" technologies has boosted fungal research in the fields of biodiversity, physiological ecology and natural product biosynthesis. Each of these research areas has its own research agenda, scientific language and quality standards, which have so far hindered an interdisciplinary exchange. Inter- and transdisciplinary interactions are, however, vital for: (i) a detailed understanding of the ecological role of marine fungi, (ii) unlocking their hidden potential for natural product discovery, and (iii) designing access routes for biotechnological production. In our talk, we describe the two different "worlds" of marine fungal natural product chemists and marine fungal molecular ecologists. The individual scientific approaches and tools employed are summarised and explained. We propose a strategy to find a multidisciplinary approach towards a comprehensive view on marine fungi and their chemical potential.

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**Poster Session 1**  
**Biotechnology (BTP)**  
**16 April 2018 • 14:30–17:30**

**BTP037**

**Solving the diterminal alkane oxidation puzzle**

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Direct and selective terminal oxidation of medium-chain n-alkanes is a major challenge in chemistry. Efforts to achieve this have so far resulted in low specificity and overoxidized products. Biocatalytic oxidation of medium-chain n-alkanes – with for example the alkane monooxygenase AlkB from *P. putida* GPO1- on the other hand is highly selective. However, it also results in overoxidation. Moreover, diterminal oxidation of medium-chain n-alkanes is inefficient. Hence,  $\alpha,\omega$ -bifunctional monomers are mostly produced from olefins using energy intensive, multi-step processes.

By combining biocatalytic oxidation with esterification we drastically increased diterminal oxidation upto 92 mol% and reduced overoxidation to 3% for n-hexane. This methodology allowed us to convert medium-chain n-alkanes into  $\alpha,\omega$ -diacetoxyalkanes and esterified  $\alpha,\omega$ -dicarboxylic acids. We achieved this in a one-pot reaction with resting-cell suspensions of genetically engineered *Escherichia coli*.

The combination of terminal oxidation and esterification constitutes a versatile toolbox to produce  $\alpha,\omega$ -bifunctional monomers from n-alkanes.

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**BTP038**

***Monascus ruber* as cell factory for lactic acid production at low pH**

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A *Monascus ruber* strain was isolated that was able to grow on mineral medium at high sugar concentrations and 175 g/l lactic acid at pH 2.8. Its genome and transcriptomes were sequenced and annotated. Genes encoding lactate dehydrogenase (LDH) were introduced to accomplish lactic acid production and two genes encoding pyruvate decarboxylase (PDC) were knocked out to subdue ethanol formation. The strain preferred lactic acid to glucose as carbon source, which hampered glucose consumption and therefore also lactic acid production. Lactic acid consumption was stopped by knocking out 4 cytochrome-dependent LDH (CLDH) genes, and evolutionary engineering was used to increase the glucose consumption rate. Application of this strain in a fed-batch fermentation resulted in a maximum lactic acid titer of 190 g/l at pH 3.8 and 129 g/l at pH 2.8, respectively 1.7 and 2.2 times higher than reported in literature before. Yield and productivity were on par with the best strains described in literature for lactic acid production at low pH.

**BTP039**

**Structural characterization of the thermostable *Bradyrhizobium japonicum* D-sorbitol dehydrogenase**

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*Bradyrhizobium japonicum* sorbitol dehydrogenase is NADH-dependent and is active at elevated temperatures. The best substrate is d-glucitol (a synonym for d-sorbitol), although l-glucitol is also accepted, giving it particular potential in industrial applications.

Crystallization led to a hexagonal crystal form, with crystals diffracting to 2.9 Å resolution. In attempts to phase the data, a molecular-replacement solution based upon PDB entry 4nbn (33% identical in sequence to the target) was found. The solution contained one molecule in the asymmetric unit, but a tetramer similar to that found in other short-chain dehydrogenases, including the search model, could be reconstructed by applying crystallographic symmetry operations. The active site contains electron density consistent with d-glucitol and phosphate, but there was not clear evidence for the binding of NADH. In a search for the features that determine the thermostability of the enzyme, the T<sub>m</sub> for the orthologue from *Rhodobacter sphaeroides*, for which the structure was already known, was also determined, and this enzyme proved to be considerably less thermostable. A continuous beta-sheet is formed between two monomers in the tetramer of the *B. japonicum* enzyme, a feature not generally shared by short-chain dehydrogenases, and which may contribute to thermostability, as may an increased Pro/Gly ratio.

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**BTP040**

**Valorizing N-acetylmuramic acid as feedstock for production of value-added products by engineered *Corynebacterium glutamicum***

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*Corynebacterium glutamicum* is the workhorse of million-ton-scale amino acid production that has been engineered for access to alternative carbon sources [1] such as N-acetyl-d-glucosamine (GlcNAc), but not yet for the amino sugar acid N-acetylmuramic acid (MurNAc). MurNAc is one of the major components of the bacterial cell wall and its access may be a potential raw material for biorefineries since bacterial biomass is a waste product of fermentation. To enable growth on MurNAc as sole carbon source, the MurNAc-specific PTS components MurP and Crr (EIIA<sub>Glc</sub>) and the etherase MurQ were amplified from the DNA of *E. coli* K-12 [2] and transformed in *C. glutamicum*  $\Delta nanR$ . While MurP and MurQ were essential to allow growth of *C. glutamicum*  $\Delta nanR$  with MurNAc, heterologous Crr was not. Heterologous Crr was beneficial for the growth increasing  $\mu_{max}$  from 0.15 h<sup>-1</sup> to 0.20 h<sup>-1</sup>. Codon optimisation of *murP* for *C. glutamicum* was counterproductive, possibly because its rarely used codons were necessary features of translation and protein folding. When in addition to *murP-murQ-crr* the GlcNAc-specific PTS gene *nagE* from *C. glycinophilum* was expressed, the resulting strain utilized first GlcNAc and subsequently MurNAc. Production of L-lysine, as well as its derivatives cadaverine and l-pipecolic acid and lycopene from MurNAc as sole carbon source and from MurNAc-GlcNAc blends, was successfully established in *C.*

*glutamicum* DM1729 $\Delta$ *nanR* and  $\Delta$ *crtYEb\Delta**nanR*. These proof-of-concept approaches led to product titers of 7mM L-lysine, 39  $\mu\text{g}\cdot\text{g}$  (CDW) $^{-1}$  lycopene, 4mM cadaverine and 4 mM L-pipecolic acid, respectively. This work proves the accessibility to a MurNAc new C source by recycling bacterial biomass in a biorefinery.

#### References:

[1] doi: 10.1016/j.jbiotec.2016.07.022

[2] doi: 10.1128/JB.186.8.2385-2392.2004

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#### BTP041

##### Online Measurement of pH, Oxygen, Biomass and CO<sub>2</sub> in Shake Flasks

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Although novel parallel cultivation systems were developed in the last couple of years, shake flasks are still the workhorse of microbial cultivation. Typically, these vessels are still used as black boxes because no online measurement is integrated. The non-invasive measurement of oxygen and pH using chemical-optical sensors has already been commercially available for several years and online measurement of biomass has recently been introduced. Here, we present the new CO<sub>2</sub> sensor that was developed recently and integrated into a multi-parameter platform.

Applications are various: Although the CO<sub>2</sub> sensor is only a prototype it is possible to follow a diauxie of *E. coli* cultivations online, while small changes in the growth curve detected by the biomass sensor indicate the exact time of limitations which was shown for different organisms. Non-invasive oxygen measurements deliver the critical process parameter kLa – which is even the basis of online OUR determination.

However, our focus was not just to add more parameters but also to let them complement each other. Measuring several parameters - with all of them showing the same characteristics - enhances the measurement security. It was shown that combined oxygen and biomass measurement offers a solid conclusion about the metabolic status of the culture. In summary, multisensory monitoring enables to adjust the conditions in shake flask cultivations to be far more comparable to stirred bioreactors. Therefore, scale-up with yield optimization can be performed more reproducibly.

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#### BTP042

##### Identification of monoterpene resistance mechanisms in *Pseudomonas putida*

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Product toxicity is a common challenge for biotechnological production processes. Therefore, it is essential not only to increase the product formation rate by optimizing metabolic pathways, but also to enhance the strain's tolerance towards the educts and products and thereby avoid limitations due to inhibitory effects. A high toxicity is known for many monoterpenoids, which are widely used in

medicines/pharmaceuticals, flavor and fragrance and agriculture.

The bacterium *Pseudomonas putida* shows an inherent extraordinarily high tolerance towards solvents including monoterpenoids. To increase the already high native monoterpene resistance of *P. putida* and transfer the underlying resistance mechanisms to suitable host strains for biotechnological production processes, the molecular factors of monoterpene tolerance have to be determined. Therefore, an approach, comprising the creation of a mutant library, the selection for monoterpene-hyperresistant mutants and further characterization via genome sequencing, deletion and complementation experiments and growth tests, was conducted.

The results show that increased or decreased tolerance for monoterpenoids is mainly related to altered expression levels of efflux pumps. In addition, first evidences for a specificity of the tolerance mechanisms to certain molecular structures were deducible.

The understanding of the underlying factors can help to create suitable monoterpene production strains with an improved tolerance towards the bioprocess educts and products, aiding the efficient monoterpene production with this organism. Our data also allows a deeper insight into how bacteria can oppose monoterpene containing antimicrobials, like tea tree oil, and demonstrates new strategies to improve their effectiveness.

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#### BTP043

##### *Vibrio natriegens* as novel production host for industrial biotechnology

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High productivity is an important pillar of economic success for the microbial production of commodities. The productivity of industrial fermentation processes is restricted by the biomass specific substrate consumption rate ( $q_s$ ) of the microbial production system. Since  $q_s$  depends on the growth rate ( $\mu$ ), we highlight the potential of the fastest growing non-pathogenic bacterium, *Vibrio natriegens*, as novel powerhouse for future biotechnological processes. Fermentations in minimal medium with glucose showed that *V. natriegens* exhibits an exceptionally high  $q_s$  under aerobic and anaerobic conditions. Fermentations with resting cells of genetically engineered *V. natriegens* under anaerobic conditions yielded an overall volumetric productivity of  $0.56 \pm 0.10$  g alanine L $^{-1}$  min $^{-1}$  (i.e. 34 g L $^{-1}$  h $^{-1}$ ). These inherent properties render *V. natriegens* a promising new microbial platform for future industrial fermentation processes operating with high productivity.

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#### BTP044

##### Two UDP-glucose dehydrogenases originating from Actinobacteria

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UDP-glucuronate could be synthesized by UDP-glucose dehydrogenase (UGDH; EC 1.1.1.22). This reaction and so the UGDH is of importance for the biosynthesis of polysaccharides and ascorbic acid. However, the number of enzymes studied is limited and also the biocatalytic potential was not investigated so far.

In this study, we describe the cloning, expression and biochemical characterization of two UDP-glucose dehydrogenases obtained from *Rhodococcus opacus* 1CP and *Thermocrispum agreste* DSM 44070, both belonging to the class of Actinobacteria.

Genes were codon-optimized and as synthetic constructs obtained. After cloning into the pET16bp vector heterologous expression of the recombinant RoUGDH and TaUGDH in *E. coli* BL21(DE3) pLysS was established and optimized. For enzyme production a scale up was performed into a 10l bioreactor. Afterwards, the respective proteins were purified by utilizing nickel affinity chromatography. Typical NADH-based spectrophotometric as well as HPLC analysis were carried out to assay enzymes and productivity.

Both proteins could be produced successfully and were assayed by following UDP-glucuronate and NADH formation. The optimum pH was identified for RoUGDH at 8.5 and for TaUGDH at 8.7. Reaction temperatures of 45°C or 57°C are best for RoUGDH or TaUGDH, respectively. At these optimum conditions a maximum activity of 23 and 35 U mg<sup>-1</sup> could be determined. Both enzymes are very specific for UDP-glucose. No other nucleotide sugars were accepted. Only NAD<sup>+</sup> could be used as cofactor, respectively.

It can be concluded that both UGDHs of Actinobacteria are highly active and can be used to convert only UDP-glucose. The pH- and temperature-stability make these enzymes interesting candidates to develop a biocatalytic system to produce UDP-glucuronate.

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#### BTP045

##### Engineered peroxisomes as a new platform for the production of monoterpenoids in yeast

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Terpenoids are a chemically diverse group of cyclic and acyclic functionalised terpenes and commercially valuable with a variety of uses as drugs, nutraceuticals, cosmetics, and agriculturals. Monoterpenoids, the smallest group derived from only two isoprene units, are mainly produced in plants and used as flavours and fragrances, insect repellents, as well as precursors for fine chemicals and pharmaceutical molecules. They are major constituents of plant essential oils and can be sourced from e.g. lemon peel, lavender, or rose oil. However, the natural production of commercially relevant monoterpenoids is limited, since their extraction from plants is costly and inefficient, and the alternative chemical synthesis involves long and complex, multi-step protocols.

Monoterpenes produced from microorganisms using heterologous biosynthesis pathways would be ideal for reasons of sustainability, compound diversity, as well as regio- and stereo-selectivity of the products. We developed a new platform for the production of monoterpenoids in *Saccharomyces cerevisiae* by engineering peroxisomes as production site. This bypasses many of the production constraints associated with chemical synthesis and isolation from natural resources and the platform might be extended to the production of higher terpenoids and to different yeasts.

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#### BTP046

##### Towards a competitive expression platform: Strategies to optimize protein export via unconventional secretion in *Ustilago maydis*

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Proteinaceous industrial products often have highly specific demands on their expression host and production conditions. Therefore, a broad variety of expression platforms is required to overcome existing bottlenecks. One promising candidate host to fill challenging niches is *Ustilago maydis*. This fungus is capable to secrete proteins via an unconventional secretion pathway which circumvents *N*-glycosylation. Fusions of heterologous proteins to endogenous unconventionally secreted proteins allow hitchhiking of this mechanism: Heterologous proteins are co-exported into the culture supernatant avoiding potential devastating effects linked to an unsuitable *N*-glycosylation pattern. Towards yield improvement, we currently focus on developing a high-throughput screen for an enhanced unconventional secretion capacity. To this end, a screening strain harboring different reporter for unconventional secretion is UV-mutagenized and cells with enhanced extracellularly reporter activity are selected and further characterized.

Additionally, to evaluate the system and to demonstrate improvements during development, different heterologous proteins are characterized with respect to their production and secretion efficiency. While previous studies focused on pharmaceutically relevant nanobodies, recent attempts focus on antimicrobial peptides like legume's symbiotic peptide Nodule-specific Cysteine-Rich peptide (NCR) 247 which is involved in bacteroid differentiation. Heterologous expression of such proteins is challenging due to direct effects on the expression host. Furthermore, conventional secretion might lead to inactivity due to extensive disulfide bond formation. Exploitation of *U. maydis* for production of NCR247 might be an elegant way to circumvent these bottlenecks.

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#### BTP047

##### Production of levan-based prebiotics in *Gluconobacter* sp.

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Levan is a  $\beta$ -2,6-glycosidic linked fructan polymer that can be used for a broad range of industrial applications. Due to its prebiotic character levan is attracting attention for large-scale production and commercial distribution. Industrial production of levan is limited by its enormous molecular weight of up to 100 tons per mol, which prevents economical and efficient purification of the polymer. Here we present a strategy for the production of levan-based prebiotic

fructooligosaccharides (FOS) that can be formed by a potent levan producer from the genus *Gluconobacter* and the hydrolytic activity of specific endolevanases. We identified a *Gluconobacter* strain (*Gluconobacter* strain MaHo) that achieved a Levan yield of ~ 90 g/L, which is comparable to the most productive levan producers *Zymomonas mobilis* CCT 4494 and *Bacillus licheniformis* NS032<sup>1</sup>. *Gluconobacter* strain MaHo possesses a very high osmotic tolerance, so that it is possible to grow the organism at high sucrose concentrations for levan production. To convert levan into short-chain FOS that can be easily isolated from industrial fermentation by filtration, we analyzed the product spectra of three different endolevanases by HPLC. The enzymes of interest were overexpressed in *E. coli* and purified by strep-tag affinity chromatography. Two promising enzymes from *Azotobacter* sp. (528 U/mg) and *Bacillus* sp. (1.5 U/mg) were identified which almost exclusively formed FOS with a degree of polymerization > 2. Our results demonstrate that by heterologous expression and subsequent secretion of the examined endolevanases the mentioned *Gluconobacter* strain could be used as an efficient host for a scalable production of levan-based prebiotic fibers.

#### References:

[1] Öner *et al.* (2016). *Biotechnology advances*, 34 (5), 827-844.

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#### BTP048

##### Pyrophosphorylases of Actinobacteria for the enzymatic production of UDP-glucose

A. Kumpf<sup>1,2</sup>, A. Partzsch<sup>1</sup>, A. Pollender<sup>1</sup>, D. Tischler<sup>3</sup>, D. Kowalczykiewicz<sup>4</sup>, M. Musiol<sup>4</sup>, K. Szymanska<sup>4</sup>, I. Bento<sup>2</sup>

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UDP-glucose is in nature an important precursor for many compounds within sugar and nucleotide metabolism. A key enzyme for UDP-glucose synthesis is UDP-glucose pyrophosphorylase (GalU) that converts UTP and glucose-1-phosphate into UDP-glucose and pyrophosphate.

A highly active and stable GalU enzyme is needed to efficiently produce UDP-glucose, which can then be employed to produce other compounds such as trehalose or UDP-glucuronate.

By following a genome mining approach the *galU* genes of *Thermocrispum agreste* DSM 44070 (*TagalU*), a thermophilic Actinobacterium, and of *Rhodococcus opacus* 1 CP (*RogalUa* and *RogalUb*), a soil bacterium, were identified. These have been codon optimized, cloned and expressed in the recombinant host *E. coli* BL21 (De3) pLysS. A biochemical characterization and initial biotransformation were conducted.

First interesting characterization data of *TaGalU* (2877 U mg<sup>-1</sup>) and *RoGalUb* (5.8 U mg<sup>-1</sup>) could underline a potential use as biocatalysts, especially of the *Thermocrispum* variant, as *TaGalU* could be shown to have a 500 times higher activity than *RoGalUb*. The *TaGalU* seems to be rather stable (50 % activity after 2 weeks at 4 °C) and active at higher temperatures (60 to 70 °C). First immobilization experiments of *TaGalU* have also been very successful, as the immobilization could improve stability by remaining activity at

the same level. No significant loss of activity could be observed after storage at 4 °C for several weeks.

In conclusion, it can be stated that the genome mining approach was successful, as the highest GalU-activity was found compared to literature. This enzyme will be now studied from biochemical and structural point of view in order to improve stability and turnover. Best variants will be employed as biocatalysts.

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#### BTP049

##### *In vitro* characterization of the thioester reductase FcIG in the fabclavine biosynthesis

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**Question:** Fabclavines are bioactive secondary metabolites which can be found in *Xenorhabdus szentirmai* or *X. budapestensis* both living in symbiosis with entomopathogenic nematodes[1]. Structural they consist of a NRPS-PKS-hybrid connected with an unusual polyamine[1]. Their biosynthesis is supposed to be a parallel assembly line at which the polyamine-part is biosynthesized, reductively released by the proposed thioester reductase FcIG and then connected to the enzyme-bound NRPS-PKS-part[1]. To better understand the essential release step we wanted to analyse the proposed thioester reductase-function of FcIG by *in vitro* spectrometric and mass spectrometric assays to determine possible substrates, products and cofactors.

**Methods:** To determine substrates and cofactors we measured the consumption of NAD(P)H spectrometrically by incubation it with purified FcIG and a set of different Coenzyme A derivatives as mimics of the natural substrate. Possible products were derivatized and detected by GC-MS.

**Results:** We could establish a combined method of spectrometric assay followed by GC-MS to characterize reductases which allows to determine substrates, products and cofactors. In our case we could show that FcIG prefers NADPH over NADH and could detect long chain aldehydes as products following its incubation with acyl-CoA derivatives like myristoyl-CoA or lauroyl-CoA. We could also define a acyl chain length range of the CoA derivatives which is preferred by FcIG.

**Conclusions:** We confirmed FcIG as a thioester reductase utilizing NADPH to convert acyl-CoA derivatives to aldehydes *in vitro* which supports its key function for the reductive release of the unusual polyamine part in the fabclavine biosynthesis.

#### References:

[1] S. W. Fuchs *et al.*, *ChemBioChem* **2014**, 15, 512 – 516.

## BTP050

### RNAseq analysis of acetic acid bacterium

#### *Gluconobacter oxydans* 621H

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**Introduction:** *Gluconobacter oxydans* incompletely oxidizes a great variety of carbohydrates in the periplasm regio- and stereoselectively. This capability is used industrially, e.g. in vitamin C production. The genome-wide transcriptional profile of this cell factory is rather unexplored.

**Objectives:** RNA sequencing of primary and whole transcriptomes to identify transcription start sites (TSSs) and operon structures.

**Methods:** Cells for RNA isolation were cultivated in complex medium under standard and several stress conditions. Whole transcriptome libraries were generated using a strand-specific protocol (Illumina). To determine TSSs, libraries enriched for primary 5'-transcript ends by digestion of processed transcripts were prepared. MiSeq was used for sequencing of the libraries.

**Results:** Overall, 2449 TSSs were detected, classified according to their genomic context and used for further analysis. Both the -10 and -35 region of the consensus promoter motif were weakly conserved. Analysis of 5'-UTRs showed that less than 5% of the transcripts are leaderless. Detection of TSSs also allowed identification of intragenic transcripts for 12% and antisense transcripts for 11% of all ORFs. The most frequent initiation nucleotides of sense transcripts are purines (65%), whereas this frequency is lower for intragenic (51%) and antisense transcripts (47%). In total, 1144 monocistronic transcripts and 571 operons comprising 1634 genes were identified by whole transcriptome sequencing.

**Conclusion:** RNA-Seq analysis provided detailed insights into the transcriptional landscape of *G. oxydans*<sup>1</sup>. The data obtained will benefit both basic and applied research with this cell factory.

#### References:

[1] Kranz et al. (2018) BMC Genomics 19:24.

## BTP051

### Compatible Duet expression vectors for co-expression of recombinant proteins in *Corynebacterium glutamicum*

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**Introduction:** *Corynebacterium glutamicum* is a globally employed industrial workhorse owing to its advantageous traits such as fast growth, substrate co-utilization, low extracellular protease activity and presence of two protein secretion pathways. Despite these advantages it failed to replace *E. coli* and *Bacillus subtilis* as leading bacterial protein expression systems at least partially, due to its lower

transformation efficiency and the availability of less expression vectors.

**Objectives:** The present study aims to develop compatible Duet expression vectors for use in *C. glutamicum* to allow the co-expression of several target genes for metabolic engineering studies and for studying protein complexes.

**Material and methods:** Plasmid compatibility was established using PCR assays and agar plate selection (plasmids co-transformation). Functionality and co-existence of plasmids were proven by the determination of plasmid-based enzyme activities.

**Results:** Two shuttle Duet expression vectors for *E. coli/C. glutamicum* were constructed utilizing p15A/pCG1 (pRG\_Duet1) and colE1/pBL1 (pRG\_Duet2) replicons. For the stable maintenance of both plasmids different antibiotic resistance genes were introduced along with compatible origins of replication.

**Conclusion:** We constructed novel, compatible Duet expression vectors for use in *C. glutamicum*. Expression of genes cloned in these vectors is inducible by either IPTG or by anhydrotetracyclin and thus can be utilized for independent expression of two genes at the same time.

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## BTP052

### Engineering *Pseudomonas putida* KT2440 for isobutanol production

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**Introduction:** Isobutanol is an attractive choice as a sustainable biofuel. It has a high energy density, low vapor pressure and an octane number comparable to gasoline. Biological isobutanol production has been demonstrated in *E. coli* and various other important industrially used microorganisms.

**Objectives:** *P. putida* has a high intrinsic tolerance against many organic compounds such as isobutanol. Therefore, we aim to establish the production of this target molecule in this versatile soil bacterium.

**Methods and results:** As a basis for isobutanol production and to prevent product degradation, *P. putida* strain GN346 was evaluated, which contains several knockouts (*upp*, *pedE*, *pedI*, *pedH* and *aldB-I*) (1). These genes are involved in isobutanol degradation and strain GN346 was unable to grow on isobutanol. Deletion of the gene *bkdAA* (subunit A branched-chain keto acid dehydrogenase complex) prevented degradation of the precursor 2-ketoisovalerate while the inactivation of the transhydrogenase *Stha* should improve NADPH availability. In the resulting *P. putida* strain plasmid pEMP04 has been introduced which contains the genes for isobutanol formation: *kivD* (keto acid decarboxylase from *L. lactis*), *yqhD* (alcohol dehydrogenase from *E. coli*) as well as *alsS* (acetolactate synthase from *B. subtilis*) and the native l-valine biosynthesis genes *ilvCD*. Shaking flask experiments in minimal medium with 30 mM glucose as carbon source showed the production of  $11 \pm 1$  mg isobutanol L<sup>-1</sup> after 10 hours of cultivation. A dual-phase Fed-Batch fermentation resulted in the production of up to

10.7 g 2-ketoisovalerate L<sup>-1</sup> and 1.56 g isobutanol L<sup>-1</sup> after 31 hours of cultivation.

#### References:

[1] Simon et al. (2015). *J Proteomics*, 122, 11–25.

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#### BTP053

##### Change of co-substrate specificity from FADH<sub>2</sub> to NADH in the flavin monooxygenase PqsL

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#### Introduction:

The class A flavin dependent monooxygenase (FMO) PqsL catalyzes the highly unusual *N*-hydroxylation of 2-aminobenzoylacetate, a central intermediate in alkyl quinolone biosynthesis, to form 2-hydroxylaminobenzoylacetate, and therefore acts as the key enzyme in AQ-*N*-oxide biosynthesis. In contrast to all known class A FMOs, PqsL does not utilize NAD(P)H as co-substrate but accepts electrons from free reduced flavin *in vitro*.

#### Objectives:

We studied the co-substrate utilization of PqsL and its impact on AQNO production in *P. aeruginosa*. Using a structure-based protein-engineering approach, we identified and replaced amino acid residues crucial for co-substrate binding, aiming at installing NADH-dependent catalytic activity.

**Methods:** Protein variants generated by site-directed mutagenesis of *pqsL* were screened for HQNO formation using a *Pseudomonas putida* model. Flavin redox potential, co-substrate utilization, *in vitro* enzyme activity and substrate affinity were analyzed using spectroscopic and chromatographic methods.

**Results:** The redox potential of the flavin cofactor of PqsL is remarkably positive compared to other class A FMOs, supporting electron transfer from FADH<sub>2</sub> to PqsL. A single amino acid turned out to be crucial for NADH interaction, enabling flavin reduction and *in vitro* activity of PqsL with NADH as sole electron donor. The reductive half-reaction could be accelerated by a second amino acid exchange. However, the protein variants appeared to be less active *in vivo*.

**Conclusion:** Manipulation of co-substrate specificity gives insights into the reaction mechanism of the unique class A FMO PqsL. It allows a deeper understanding of how co-substrates are utilized in FMOs and might be applicable to medically and industrially relevant enzymes.

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#### BTP054

##### Metabolic engineering to guide evolution - creating a novel mode for L-valine production with *Corynebacterium glutamicum*

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**Introduction:** Evolutionary approaches are often mutagen-based, thus yielding numerous mutations, which need

elaborate screenings to identify relevant targets. *Metabolic Engineering to guide evolution* (MGE) is an evolutionary approach to identify and evolve new targets to improving microbial producer strains.

**Objectives:** Developing an evolutionary approach that yields a manageable amount of mutations for further target identification.

**Materials&Methods:** MGE consists of 3 phases: (i) Metabolic Engineering to put evolutionary pressure upon the strain of choice, (ii) a growth phase that eventually leads to an evolutionary event, (iii) whole genome sequencing (WGS), to identify targets.

**Results:** Applying MGE, the genes *ppc* and *pyc* (encoding the anaplerotic carboxylases for phosphoenolpyruvate and pyruvate) were deleted. The resulting strain *C. glutamicum*  $\Delta ppc \Delta pyc$  showed negligible growth. It was sequentially transferred for 14 days including screening for faster growing mutants. After evolutionary events, WGS was used to identify the mutational intersection. In contrast to the initial strain, 3 independently evolved mutants showed growth rates of up to 0.32 h<sup>-1</sup> (80% of WT). The intersection of the mutations revealed isocitrate dehydrogenase (ICD) as consistent target. Upon re-engineering, the mutations led to lower ICD activities, which activated the glyoxylate shunt. Suitability for production was demonstrated by introducing an overexpression plasmid of the L-valine biosynthesis genes. These strains accumulated up to 8.1 ± 1.0 g L-valine L<sup>-1</sup> (0.22 ± 0.01 g L-valine per g glucose).

**Conclusion:** MGE is a suitable evolution approach and its application identified ICD mutations as potent alternative to pyruvate dehydrogenase complex attenuated/deficient L-valine producers.

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#### BTP055

##### RGB-S Reporter: a Novel Multi-Stress Whole-Cell Biosensor

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Bacterial whole-cell biosensing is a sturdy tool for assessment of environmental as well as biotic factors, exploiting the impressive capability of bacteria to sense and respond to various influential agents. The transcriptional fusion-based reporter is one of the common biosensing strategies where a stress responsive transcriptional factor is fused to a reporter element allowing for a sensitive monitoring of the corresponding stress response activity. Based on this strategy and using synthetic biology designing tools, we show here the development and test of a novel three-color whole-cell biosensor, called RGB-S Reporter, which enables the measurement of three stress categories: physiological stress, genotoxicity and cytotoxicity in three fluorescence colors: red, green and blue respectively. The new biosensing system, hosted in *E. coli*, is able to measure the three mentioned stress categories simultaneously and independently, giving the possibility for a reliable qualitative and quantitative data acquisition. In addition to its reliable measurements, RGB-S Reporter showed compatibility to high throughput stress quantification in bulk solutions as well as single-cell imaging. This new multi-stress sensing system has a wide range of possible applications in biotechnology and basic science research, as amongst them environmental toxicity assessment,

bioprocess and fermentation optimization as well as bacterial stress response studies.

#### BTP056

##### Ferulic acid synthesis in engineered *E. coli* is limited by methyl-group supply

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The phenylpropanoic acids coumaric acid, ferulic acid and sinapic acid are three precursors of lignin as well as a set of secondary metabolites of which some are predicted to have beneficial biological activity on human health. To identify potential biotechnological routes for their synthesis, we investigated the production of ferulic acid in *E. coli*. We designed an inducible polycistronic expression construct comprising four enzymes catalysing the immediate three step conversions from tyrosine to ferulic acid by desamination (TAL), aromatic hydroxylation (HpaBC) and methylation (OMT). Recombinant cells cultivated in minimal medium were pulse fed with tyrosine and the concentration of intermediates and product were followed by quantitative HPLC measurements. In initial shake flask cultivations a set of homologous enzymes were assessed individually and the best candidates implemented in the synthetic cascade. Cells expressing all enzymes of the cascade transformed 2 mM tyrosine in 0.5 mM ferulic acid in a shake flask in 24 h, by concomitant accumulation of 0.3 mM caffeic acid. Addition of methionine and serine, precursors of S-adenosyl-methionine, improved the final ferulic acid concentration to 1 mM by improved methylation of caffeic acid. Theoretical flux analysis based on kinetic data of the first two enzymes of the cascade revealed a substantial downshift in the flux by a strong product inhibition of the first enzyme ( $K_i = 8 \mu\text{M}$ ) together with a high  $K_M$  of the second enzyme ( $K_M = 1 \text{ mM}$ ). Product inhibition is partially relieved in a  $\Delta\text{tyrR}$ -background. In a controlled reactor environment with constant feeding of 1.5 mM/h tyrosine, the  $\Delta\text{tyrR}$ -strain yielded 5.5 mM ferulic acid and 1 mM caffeic acid after 24 h.

#### BTP057

##### MenD, a thiamine-dependent enzyme having 1,2- and 1,4-addition activity with Michael acceptors

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**Introduction:** Thiamine diphosphate (ThDP)-dependent enzymes are well-established catalysts in the field of asymmetric synthesis [1]. The ThDP-dependent enzyme MenD (SEPHCHC synthase) from *E. coli* uses its physiological donor, 2-oxoglutarate, which is decarboxylated and performs a unique Stetter-like 1,4 addition to an  $\alpha,\beta$ -unsaturated Michael acceptor (isochorismate, 2,3-*trans*-CHD) [2,3]. As well, MenD is able to perform 1,2-addition reactions with a variety of aliphatic or benzylic aldehydes [3,4] to form stereospecific S-hydroxyketones. This makes MenD a promising novel biocatalyst for C-C bonding reactions.

**Objective:** Here we report that MenD is able to utilize the alternative donor, 4-hydroxy-2-oxoglutarate (HOG) as a novel donor compound for 1,4- as well as 1,2-addition reactions and leads to more functionalized products.

**Material and Methods:** HOG was synthesized either by a chemical reaction (product: R,S-HOG) or by aldolase reactions from glyoxylate and pyruvate with Eda [5] or DgoA [6].

**Results:** We present data on the donor and acceptor substrate spectrum of MenD with HOG [7].

#### References:

[1] a) Müller, M. et al. (2009) *FEBS Journal* **276**, 2894. b) Müller, M. et al. (2013) *Curr. Opin. Chem. Biol.* **17**, 261.

[2] Jiang, M. et al. (2007) *Biochemistry* **46**, 10979.

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#### BTP058

##### Stereochemistry of the ene-reductase OYERo2a and its cysteine variants

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The ene-reductase OYERo2a from *Rhodococcus opacus* 1CP belongs to class III of the old yellow enzyme (OYE) family. These flavoenzymes reduce various  $\alpha,\beta$ -unsaturated substrates at the expense of a nicotinamide cofactor, producing chiral molecules. This makes them potentially interesting for biocatalysis. An N-terminal cysteine residue, which is highly conserved in class III OYEs, is involved in the binding of the flavin cofactor and the substrate.

OYERo2a as well as its engineered variants C25A, C25S and C25G were characterized with respect to conversion and enantioselectivity.

Conventional cloning, expression and protein purification methods were employed. Enzyme assays were followed by product extraction and subsequent determination via HPLC or GC.

Maleimides inhibit the wild type due to a Michael addition with the cysteine, forming a thioether. However, the cysteine lacking variants are not inhibited, showing high specific activities on N-methylmaleimide with NADPH as the corresponding cofactor. Conversion of most substrates were worse with the variants than with the wild type. This concerns especially the C25G and C25S variant. Nevertheless, conversion of ketoisophorone could be slightly increased to 34 % using the C25A variant but the enantiomeric excess decreased to 65 %. Mutagenesis of the cysteine residue had

only a small impact on the enantiomeric excess. Instead of NADPH the more cost efficient BNAH can be used for the reduction. The conversion of 2-methyl-*N*-phenylmaleimide was even higher when BNAH was used.

In conclusion it can be stated that the cysteine variants show an improved biocatalytic performance on maleimides, especially with the synthetic cofactor BNAH. But, the wild type is in favor for other substrates while employing NADPH as reductant.

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#### BTP059

##### Stable Immobilization of Lipase Variants by Site-Directed Coupling Methods

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The versatility of lipases (E.C. 3.1.1.3) to utilize a wide range of substrates in hydrolysis as well as synthesis reactions offers various applications in the fields of food, pharmaceutical and fine chemicals industry. With respect to an efficient use of the enzyme immobilization could be a useful tool to enable a recycling strategy of the biocatalyst.

In this study, the site-directed immobilization of lipase T1 from *Geobacillus zalihae* (LipT1) was investigated by utilizing different coupling strategies. In separate approaches, several variants of the lipase were designed containing either amino acid substitutions of lysyl and cysteinyl residues or fusions with histidine and glutamine peptide tags.

The usage of glutaminyl residues as reactive groups (Q-tag) was combined with the application of microbial transglutaminase (MTG; E.C. 2.3.2.13). MTG is able to form isopeptide bonds between the  $\gamma$ -carboxamide group of glutaminyl residues and various amines. Therefore, the enzyme can be used to catalyze the covalent coupling of proteins to solid supports containing reactive amino groups. Following this approach, the Q-tagged lipase variants in this study were successfully immobilized on activated amino supports with bound activities of up to 2.3 U<sub>g<sup>support</sup>-1</sub> (model substrate *p*-nitrophenyl palmitate; *p*NPP). After 12 cycles of repeated use in hydrolysis of *p*NPP the immobilized lipase showed a residual activity of up to 76 % proving a stable enzyme immobilization.

In conclusion, the use of MTG in combination with the designed Q-tag offers a new way to reach the site-specific and stable immobilization of lipases that could be used in further investigations on the conversion of biotechnological relevant substrates.

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#### BTP060

##### Electrode-assisted fermentation of acetoin in *Escherichia coli* and *Shewanella oneidensis*

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**Question:** Electrode-assisted fermentation is a new strategy in anaerobic biotechnology. It can be used for reactions in which the average oxidation state of the end product is higher than the substrate. Conventionally, these reactions are catalyzed under oxic process conditions which is connected to the disadvantage of higher biomass formation and high energy input in the system. Here we show two

applications of an electro-fermentation for the anoxic production of acetoin.

**Methods:** The presented approaches in *Shewanella oneidensis* and *Escherichia coli* utilize engineered metabolisms steering the carbon flux towards pyruvate in order to achieve high product yields. The heterologous expression of *alsS* and *alsD* from *Bacillus subtilis* enables acetoin formation branching from pyruvate. Utilizing these strains, acetoin production was investigated under anoxic conditions using soluble electron acceptors. In order to meet biotechnological demands, the next step was to implement this process in bioelectrochemical fermenters with an anode as a non-depletable electron acceptor.

**Results:** Both strains were able to produce acetoin with high yields up to 90% in batches with nitrate or fumarate. Further, we were able to show acetoin production in anode-assisted fermentations. Those bioelectrochemical processes were able to sustain a yield of roughly 80% with both strains, comparable to the previous experiments with soluble electron acceptors.

**Conclusions:** Both bioelectrochemical approaches, highlight the anoxic fermentation of acetoin as a potential future biotechnological strategy. However, productivities are rather low in both setups but can be improved via different approaches to be competitive with oxic process routines.

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#### BTP061

##### Indigoid dyes produced by FAD-dependent epoxidases of soil bacteria

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An overview on oxidative indole conversions and respective products is presented. A focus is on the formation of pure indigo and some derivatives. FAD-dependent epoxidases were used. They allow the selective oxygenation of indole leading to pure products without the formation of by-products (e.g. indirubin).

Genes of FAD-dependent epoxidases of four soil bacteria were cloned and expressed by a pET system in *E. coli* BL21 (DE3). Proteins obtained comprised an *N*-terminal His10-tag which allowed a one-step purification. Proteins were used to convert styrene and indole like substrates. Spectrophotometric assays were used to follow indigo production and HPLC as well as MS and NMR were employed to determine reaction intermediates / products to uncover the mechanism.

All enzymes were successfully produced and allowed the stereoselective epoxidation of the model substrate styrene to (*S*)-styrene oxide. BNAH was used as source of electrons to provide reduced FAD for oxygen activation for the epoxidation reactions. Optimal BNAH concentrations were determined to be about 8 to 12 mM in dependence of the enzyme. This setup was subsequently used to convert (halogenated) indole(s) to corresponding indigoid dyes. Assays were performed in 96-well-plates to determine rates and scaled up (10 ml) to obtain products for characterization. Dyes obtained were dried under low pressure and dissolved in DMSO for NMR analysis. Intermediates were sampled by an epoxide specific derivatization procedure and analyzed by

HPLC-UV and MS. Thus, an epoxidation as initial step of the indigo formation was proven.

It can be concluded that the tested bacterial epoxidases allow the initial and fast epoxidation of indole, and in presence of oxygen a rapid and specific indigoid dye production takes place.

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#### BTP062

##### Preparative synthesis of Dolichol phosphate mannose by immobilized Yeast Dpm1 expressed in *E. coli*

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Glycosylation of proteins is conserved within the domains of eukaryotes, prokaryotes and archaea. In eukaryotes mannose is provided to glycosyltransferases by GDP-mannose (GDPM) on the cytoplasmic face of the endoplasmic reticulum and by dolichylphosphate-mannose (DoIPM) on the luminal side [1]. DoIPM is formed by the strict divalent metal ion dependent dolichylphosphate-mannose synthase 1 (Dpm1, EC 2.4.1.83) transferring the mannose from GDPM to membrane integrated polyisoprene dolichylphosphate (DoIP) in *S. cerevisiae*. In the past the enzymatic activity of Dpm1 was measured by scintillation counting applying radioactive substrates [2]. In this work a label-free assay is described to analyze Dpm1 kinetics with the natural substrate DoIP. Additionally phytanylphosphate – a C20 fully saturated lipid analogue – was used as substrate and the substitution of naturally used divalent cofactor Mg<sup>2+</sup> was compared with Ni<sup>2+</sup>, Co<sup>2+</sup> and Ca<sup>2+</sup>. The thermodynamic constant KD characterizing the biochemical binding of GDPM to DPM1 was shown to be 103 ± 18 µM by ITC. Finally the employment of a recombinant hexa-histidine tagged small ubiquitin modifier (SUMO) at the N-terminus of Dpm1 could be used to easily purify the fusion protein and account for immobilized enzyme activity. Immobilized SUMO-Dpm1 was used to produce DoIPM continuously in milligram quantities. Purified DoIPM was investigated by ESI-MS analysis showing the expected m/z ratio. These results help in understanding the mechanism of lipid-monophosphate using glycosyltransferase and provide for the first time the continuous lipid-linked-monosaccharide production.

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#### BTP063

##### Physiology of the thermophilic acetogenic bacterium *Thermoanaerobacter kivui* during growth on mannitol

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**Introduction:** The thermophilic acetogenic bacterium *Thermoanaerobacter kivui* uses the Wood-Ljungdahl Pathway for CO<sub>2</sub> reduction. As an acetogen, *T. kivui* converts one mole of hexose into three moles of acetate as major product. Recently, we discovered that *T. kivui* grows on the more reduced sugar alcohol mannitol.

**Objectives:** We aimed to investigate the physiology and biochemistry of mannitol metabolism in *T. kivui*.

**Materials & methods:** Mannitol utilization and product formation were studied in cell suspension experiments. Genetic (1) and biochemical experiments were performed to identify the key enzyme(s) in mannitol metabolism.

**Results:** *T. kivui* converted mannitol to mainly acetate. We identified a possible PTS for mannitol uptake and a mannitol-1-phosphate dehydrogenase (MtlD) in the genome (2). Using the recently developed genetic system (1), we deleted *mtlD* and found that, unlike the wild type, the deletion mutant did not grow on mannitol anymore. The cell-free extract only of *T. kivui* grown on mannitol (but not on glucose) catalyzed NADH-dependent reduction of fructose-6-phosphate, an activity which has been attributed to other MtlDs before.

**Conclusion:** Acetate is a major product of mannitol utilization in *T. kivui*, with a reduced product yet to be identified. We identified a gene cluster including *mtlD* that is likely responsible for mannitol uptake and conversion.

#### References:

[1] Basen M, Geiger I, Henke L, Müller V: *Appl Environ Microbiol* 2017. doi:10.1128/AEM.02210-17.

[2] Hess V, Poehlein A, Weghoff MC, Daniel R, Müller V. 2014 *BMC Genomics* **15**:1139

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#### BTP065

##### Development of a genetic system for the thermophilic acetogenic bacterium *Thermoanaerobacter kivui*

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**Introduction:** *Thermoanaerobacter kivui* is one of the few acetogenic thermophilic bacteria (T<sub>OPT</sub> 65°C). It grows on sugars, pyruvate and formate, and autotrophically on components of syngas: H<sub>2</sub>+CO<sub>2</sub> or on CO; producing acetate as main product (1). CO<sub>2</sub> is reduced through the Wood-Ljungdahl pathway, while H<sub>2</sub> is oxidized by a hydrogen-dependent carbon dioxide reductase (HDCR) and an electron-bifurcation hydrogenase. A proton gradient is proposed to be built up by Energy-converting hydrogenases (Ech) (2).

**Objectives:** We aimed to develop a genetic system for modifications on the *T. kivui* chromosome.

**Materials and methods:** We tested the ability for DNA uptake using the replicating plasmid pMU131, conferring resistance to kanamycin. Subsequently, we aimed for the deletion of the *pyrE* gene encoding a key enzyme in pyrimidine biosynthesis, via homologous recombination and using 5-FOA for mutant selection.

**Results:** *T. kivui* was naturally competent for DNA uptake with transformation frequency of 1\*10<sup>-6</sup>. We succeeded overproducing its own HDCR, using a pMU131-derived plasmid. Then, we identified an uracil-auxotrophic *pyrE* deletion mutant. Reintroduction of *pyrE* on a plasmid or into the genome restored the ability to grow without uracil. Finally, using *pyrE* as selective marker, *fruK* (encoding 1-phosphofructokinase) was deleted, leaving a  $\Delta fruK$  mutant not able to grow on fructose anymore.

**Conclusion:** We demonstrated proof-of-concept for a genetic system to decipher the bioenergetics in the acetogen *T. kivui*, and to subsequently engineer this biotechnologically relevant syngas-fermenting bacterium to produce fuels or chemicals.

## References:

[1] Weghoff MC, Müller V (2016), Appl Environ Microbiol **82**:2312–2319

[2] Hess V, Poehlein A, Weghoff MC, Daniel R, Müller V (2014), BMC Genomics **15**:1139

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### BTP066

#### Application of ICEBs1, an integrative and conjugative element of *Bacillus subtilis*, for DNA transfer and genome editing of *Bacillus* strains with low competence

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Integrative and conjugative element ICEBs1 of *B. subtilis* is a mobile genetic element providing the rapid gene transfer and playing a profound role in bacterial evolution. ICEBs1 exhibits high mating frequency and its relatively small size facilitates genetic manipulations. Hosts of ICEBs1 are species such as *B. subtilis*, *B. licheniformis*, *B. anthracis* and *Listeria monocytogenes*.

The goal of study is to develop genetic tools based on ICEBs1 element for plasmid transfer and gene deletion in hardly transformable bacillary species. To do this, inducible promoter of *B. subtilis* mannose operon was inserted upstream of ICEBs1 transfer genes on the chromosome and one side of the element was deleted to inhibit transfer of ICEBs1. Resulted *B. subtilis* JA-Bs21 strain was used as a plasmid donor during the mating procedure with *B. subtilis* NCIB3610 as recipient strain. NCIB3610 strain harbors pBS32 plasmid with *comI* gene, an inhibitor of genetic competence. A range of plasmids containing pE194ts thermosensitive replication origin, *cas9* gene under control of xylose regulatory elements and kanamycin resistance gene were constructed. To optimize the mating assay protocol donor and recipient cells were mixed and put on solid minimal nutrient medium with mannose in different concentration overnight for conjugation. The highest efficiency of conjugation was found under 0.1% of mannose concentration. The efficiency became tremendously higher when the donor was induced by mannose before mixing with the recipient and mating performed on a filter overnight. Deletion of the *amyE* gene on the chromosome, *comI* gene on pBS32 plasmid and curing of pBS32 plasmid were successfully performed in the NCIB3610 strain. Attempts are underway to apply this technique in other *Bacillus* species.

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### BTP067

#### Molecular and optogenetic tools for efficient biosynthesis of structurally diverse secondary metabolites

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The inexhaustible variety of secondary metabolites produced by plants and microorganisms has proven to be a valuable source of compounds with pharmaceutical or biotechnological potential. However, an efficient biosynthesis

of a desired metabolite in a heterologous host often underlies certain bottlenecks, e.g. inefficient metabolic fluxes towards the aimed compound, or accumulation of unwanted or toxic intermediates. Such unwanted effects can be abolished by applying modular systems allowing for a strict control over the expression of key enzymes within recombinant biosynthetic cascades.

This project therefore aims to achieve a precise and straightforward control over complex gene cluster-encoded pathways by the application of light-triggered optogenetic switches such as photocaged compounds.

As a proof of concept, one-step cleavable photocaged arabinose was synthesised and applied as a novel light-responsive inducer for heterologous gene expression in *E. coli*. Exposure of caged arabinose to UV-A light resulted in a rapid induction of protein production. The applicability of this phototrigger in a biotechnological context was demonstrated by successful expression of the violacein biosynthetic pathway from *Chromobacterium violaceum* and its gradual induction in *E. coli* by increasing light-intensities.

Next, the photoswitch principle will be transferred to the alternative production hosts *Rhodobacter capsulatus* and *Pseudomonas putida*, in order to optimize the synthesis of novel sesquiterpenes as well as prodiginines by light-triggered fine-tuning of the respective metabolite fluxes. First expression studies with  $\beta$ -caryophyllene synthases were performed in *R. capsulatus*, resulting in a substantial production of the heterologous sesquiterpene with final yields of  $\geq 1$ g/L.

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### BTP068

#### Engineering *Hydrogenophaga pseudoflava* for carboxydrotrophic aerobic production of chemicals and fuels from synthesis gas

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Synthesis gas (syngas), a varying mixture of carbon monoxide, carbon dioxide and hydrogen, is a promising alternative carbon source for bacterial fermentation. It can be generated from previously unusable resources or is a byproduct in heavy industry. Currently, for syngas-based fermentation processes different *Clostridium* species are commonly applied. However, their anaerobic nature results in a rather low ATP supply, thereby preventing the biosynthesis of ATP-intensive products. To address this issue, we searched for fast growing, aerobic autotrophic microorganisms and identified the  $\beta$ -proteobacterium *Hydrogenophaga pseudoflava* DSM1084 as a potential host for aerobic syngas fermentation with the goal to produce high value chemicals and fuels. We performed whole genome sequencing (WGS) on *H. pseudoflava* and developed the basic genetic tools and methods to allow genetic engineering. Cultivation experiments were performed in shaking flasks and in a syngas-operated bioreactor. Using CO-Ox minimal medium we demonstrate rapid growth ( $\mu = 0.43 \pm 0.01$  h<sup>-1</sup>) under heterotrophic conditions (10 g sucrose L<sup>-1</sup>) in shaking flasks. With synthesis gas as the sole carbon and energy source we show growth in shaking flasks and under controlled conditions in a bioreactor. Tools to enable genetic manipulation were established and used to generate producer strains for different isoprenoid-derived chemicals. We show the production of E- $\alpha$ -bisabolene, a C<sub>15</sub>-isoprenoid, under both, heterotrophic and autotrophic

conditions. We report the cultivation of the wildtype in a syngas bioreactor, as well as the production of E- $\alpha$ -bisabolene heterotrophically and autotrophically, showing that *H. pseudoflava* is a well suited organism for the production of higher value chemicals from syngas.

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#### BTP069

##### **In vitro modifications of bacterial cyanophycin and cyanophycin-dipeptides using chemical agents.**

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Variations of the common composition of the polyamide cyanophycin (CGP) have been investigated since the early 2000s. Modifications of the polymer are not only of academic interest, an increased structural variety also expands the number of putative applications for CGP and its dipeptides in fields like food supplementations, medical and cosmetic applications. Usually variations of the composition occurred only *in vivo*, either naturally as a characteristic of the producing organism, or intended by using selectively chosen and modified strains and/or specifically optimized culture conditions. However, in recent years two novel procedures using chemical or enzymatic *in vitro* modification of the polymer were successfully applied. While both methods can successfully introduce modifications, chemical treatments appeared to be more effective and reach higher conversion rates of the targeted amino acid. Recently a broad search for interesting and promising reactions was conducted and resulted in a set of putative reactions that were subsequently tested. The reaction of CGP with methylisocyanate resulted in the conversion of 50% of lysine residues while only 3% of the arginine was modified. However, using digested CGP dipeptides the conversion rates of lysine increased slightly to 72% while the conversion of arginine reached 96%. Using formaldehyde, CGP could also be methylated with a conversion rate of 84% of lysine and 15% of arginine. Acetylation of lysine residues was obtained using acetic anhydride, resulting in a conversion rate of 100% for a single acetylation, where 63% of the residues were acetylated twice. Arginine residues could be acetylated at a rate of 89%. Overall, the tested reactions confirm the viability of chemical CGP modification for future approaches.

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#### BTP070

##### **Lactate production with metabolically engineered strains of *Acetobacterium woodii***

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**Question:** *Acetobacterium woodii* represents a promising candidate for the biotechnological production of high-value platform chemicals from CO<sub>2</sub>. As such, lactate exhibits versatile applications e.g. as a food additive with acidifying and preserving properties, or after polycondensation as a biodegradable and biocompatible polymer with numerous possible implementations. This work was intended to genetically modify *A. woodii* to produce lactate from CO<sub>2</sub> and H<sub>2</sub>. The major goal was shifting the biosynthesis pathway of *A. woodii* towards lactate production consistent with a concomitant circumvention of the native metabolism of lactate consumption.

**Methods:** The genes of the lactate dehydrogenase complex in *A. woodii* were knocked-out using Allele-Coupled Exchange (ACE) with the *pyrE* gene as a counterselection marker. Several plasmids were cloned into a Gram positive/Gram negative shuttle vector harboring a heterologous lactate dehydrogenase gene from *Leuconostoc mesenteroides* alone, or in combination with the native pyruvate: ferredoxin oxidoreductase gene under the control of an inducible promoter. The respective plasmids were transformed into the previously generated knock-out strain of *A. woodii*. Heterotrophic and autotrophic growth experiments were conducted in serum bottles followed by quantitative analysis of the fermentation products.

**Results:** Growth experiments revealed that the engineered *A. woodii* strains were capable of producing up to 70 mM lactate from fructose and up to 10 mM lactate when cells were growing on CO<sub>2</sub> and H<sub>2</sub>.

**Conclusion:** Our results indicate that the native lactate metabolism of *A. woodii* could be circumvented using knock-out mutants and first lactate production from CO<sub>2</sub> and H<sub>2</sub> via expression of a heterologous lactate dehydrogenase gene was achieved.

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#### BTP071

##### **Development of an enzyme cascade process for the production of chiral $\beta$ -amino acids**

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Chiral  $\beta$ -amino acids are valuable building blocks for pharmaceuticals and fine chemicals (Cabrele, Martinek *et al.* 2014). Within this project chiral  $\beta$ -amino acids are to be produced applying a modified hydantoinase process using racemic dihydropyrimidines as educts. The process is to be based on two enzymes. A cyclic amidase will be used for hydrolytic cleavage of the dihydropyrimidine ring followed by the reaction of a linear amidase able to decarbamoylate *N*-carbamoyl  $\beta$ -amino acids. Both enzymes are to be immobilized and applied in a microfluidic system.

While a screening for enzymes catalyzing the decarbamoylation step is ongoing it was already demonstrated in previous work that hydantoinases can hydrolyze racemic 6-substituted dihydropyrimidines to the corresponding *N*-carbamoylated  $\beta$ -amino acids (Engel, Syldatk *et al.* 2012). Expression conditions for several recombinant dihydropyrimidinases were optimized to achieve soluble expression and acceptable amounts of enzyme. Additionally first immobilization experiments were conducted.

Next steps are the determination of kinetic parameters for the model substrate phenyldihydrouracil, the evaluation of optimal reaction conditions and immobilization of a dihydropyrimidinase in a microfluidic system.

#### **References:**

[1] Cabrele, C., Martinek, T. A., Reiser, O. and Berlicki, Ł., *J. Med. Chem.*, **57**(23), (2014), 9718-9739.

[2] Engel, U., Syldatk, C. and Rudat, J., *Appl. Microbiol. Biotechnol.*, **94**(5), (2012), 1221-1231.

## BTP072

### How to program your TA to like it hot

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$\omega$ -Transaminases ( $\omega$ -TAs) are important biocatalysts for the synthesis of chiral amines and amino acids as building blocks of active pharmaceutical ingredients. However, the application of  $\omega$ -TAs is limited by the availability of enzymes with high conversion rates and their process stability.

For the synthesis and optical resolution of  $\beta$ -phenylalanine and other important aromatic  $\beta$ -amino acids we designed thermostable variants of an (S)-selective  $\omega$ -TA from *Variovorax paradoxus* by site-directed mutagenesis on the basis of predictions calculated by using the FoldX software.

The melting point ( $T_m$ ) of our best-performing mutant was increased to 59.3 °C while fully retaining its specific activity relative to the wild-type enzyme.

This is the first report on FoldX-based thermostabilization of a formerly nonthermostable  $\omega$ -TA, thereby laying a foundation for a more efficient production process due to (1) increased substrate solubility (2) higher turnover rates and (3) improved enzyme stability at elevated temperatures.

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## BTP073

### Microbial Leaching of Skutterudite by an Acidophilic Mixed Culture

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As a cobalt arsenide, the mineral Skutterudite combines the drawbacks and advantages of mining. On the positive side it poses an important source of the highly valuable cobalt, which is regarded as a strategic element. Unfortunately, the arsenic is not only a highly toxic pollutant but also a major hindrance in the pyrometallurgical exploitation.

However, we were able to use an acidophilic iron oxidising mixed culture which was not only able to cope with the arsenic but also to liberate up to 80% of both, the nickel and the cobalt. Due to ferric arsenate precipitates, which are hardly dissolvable, only 50% of the arsenic got into solution. The abiotic controls yielded <5% cobalt and <20% nickel.

Consequently, we are able to propose a possible technique not only to process cobalt bearing arsenides, but also to do so in an ecologically friendly way, and thus to provide the for the modern industry pivotal cobalt.

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## BTP074

### Combining ABE and Syngas Fermentations

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In the last decades, the fermentation of Syngas to produce platform chemicals in a Bioeconomy scenario has gained continuously more importance.

Syngas is, in this context, a mixture of carbon monoxide (CO), carbon dioxide (CO<sub>2</sub>) and hydrogen (H<sub>2</sub>).

Certain anaerobic microorganisms, known as acetogens, can use Syngas as carbon and energy sources, producing organic acids and alcohols, mainly acetate and ethanol. Some other products are also possible, like butyric acid, butan-2,3-diol or butanol, but generally in lower amounts.

Another possibility to obtain platform chemicals via bacterial fermentation is the well-known ABE process, where acetone, butanol and ethanol are produced using carbohydrates from different sources. This fermentation process has a drawback, though: there is always CO<sub>2</sub> loss during glycolysis.

By combining both Syngas and ABE Fermentations in a mixed culture, the butanol producing ability of the ABE organisms can be exploited, and the CO<sub>2</sub> produced could be recaptured. Besides, the presence of acetate in the culture media might be beneficial for product formation for the ABE organisms. The characterisation of this mixed culture fermentation is industrially relevant, and this could represent a new platform for the production of green and renewable chemicals.

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## BTP075

### A novel $\alpha$ -amylase with remarkable characteristics for biotechnological applications

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**Introduction & Objectives:** During the last decades, starch-converting enzymes have entered various sustainable biotechnological processes and displaced acid hydrolysis to the greatest possible extent. Especially,  $\alpha$ -amylases are a group of enzymes which depolymerize the complex structure of starch into oligosaccharides and smaller sugars. Therefore, they are used in large-scale processing industries, such as for starch liquefaction, as well as in daily life, like laundry cleaning.  $\alpha$ -Amylases are of great significance in present day biotechnology, constituting a class of industrial enzymes that represents approximately 30 % of the world enzyme market<sup>1</sup>. For the optimization of established applications there is still a great need in finding novel amylases with unique properties.

**Material & Methods:** Aiming at the isolation of novel starch-degrading enzymes a metagenome library from an enrichment culture using algae compost as inoculum was constructed. More than 4,000 clones were screened and one active clone was found. An open reading frame of 1662 bp encoding a putative  $\alpha$ -amylase with the highest sequence identity (85 %) to an GH13  $\alpha$ -amylase from the marine organism *Microbulbifer thermotolerans* was identified.

**Results & Conclusion:** The gene *amy1* was successfully cloned in *Escherichia coli*, resulting in high-level expression of the recombinant protein. Over a two-step FPLC-based purification Amy1 was purified to homogeneity. Amy1 is characterized by a high specific activity of 2237 U/mg and high production of maltose as well as thermostability at temperatures up to 50 °C. These characteristics make Amy1 an ideal candidate for biotechnological applications.

## References:

[1] Bertolini, Andrea, ed. *Starches: Characterization, Properties, and Applications*. CRC Press, 2009.

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### BTP076

#### **Sarocladium strictum - as new producer of a complex thrombolytic enzymes with plasminogen activator activity**

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Medicine increasingly needs plasminogen activators. But most of the drugs have significant disadvantages. Therefore, the search and development of new drugs is an urgent task. Very promising is the complex of thrombolytic enzymes formed by micromycete *Sarocladium strictum*.

Fractionation of the complex thrombolytic drug formed by *Sarocladium strictum*, and the study of the substrate specificity of its constituent proteinases.

*S. strictum* was cultivated under submerged conditions, after which a complex of enzymes was isolated from the culture liquid. The resulting complex was subjected to isoelectrofocusing. The activity of the obtained fractions was determined with chromogenic substrates of blood plasma proteinases.

As a result, four fractions were found which possessed high activity against duplicated proteins of the hemostatic system with respect to the chromogenic peptide substrates studied. Protease of peak I showed a narrow substrate specificity and pronounced urokinase activity, which was 174.8  $\mu\text{M pNA/ml}\cdot\text{min}$ . Protease II and III peptides also showed high urokinase activity, which were 89.3 and 78.8  $\mu\text{M pNA/ml}\cdot\text{min}$ , respectively, but were also able to weakly hydrolyze other substrates of blood plasma proteinases: thrombin, plasmin and tissue plasminogen activator substrate. Proteinase IV peak actively cleaved all the above listed substrates.

Proteinases I, II and III have a narrow substrate specificity with pronounced urokinase activity, and the proteinase IV of the peak shows a wide substrate specificity. Apparently, it is the urokinase activity that causes the activator-to-plasminogen-induced action. The presence in the proteolytic complex of proteinase with broad substrate specificity is the cause of the overall effect.

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### BTP077

#### **Combinatorial antibiotic effects of prodigiosin and surfactants**

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The worldwide increase of antibiotic resistances of life-threatening pathogenic bacteria represents one of the most important therapeutical challenges. Thus, new solutions to combat these pathogens need to be found urgently. Here, nature can be taken as an inspiration to identify classes of antimicrobial compounds that are co-produced by microbes

and thus can potentially be applied for combinatorial drug therapies. For example, the antibacterial red pigment prodigiosin is co-produced with the antibacterial biosurfactant serrawettin W1 by the bacterium *Serratia marcescens*. Thus, we present here the evaluation of prodigiosin antibacterial activity when used in combination with serrawettin W1 and other surfactants including the biosurfactant N-myristoyltyrosine as well as other industrially applied surfactants. *Corynebacterium glutamicum* was chosen as a model for pathogenic mycobacteria in disk diffusion assays. The results demonstrated a clear combinatorial effect of prodigiosin and surfactants. Prodigiosin together with the naturally occurring N-myristoyltyrosine showed an especially pronounced concentration-dependent antimicrobial activity. Subsequently, detailed analysis of prodigiosin and N-myristoyltyrosine revealed minimal inhibitory and bactericidal concentrations of 2.56  $\mu\text{g/ml}$  and 32  $\mu\text{g/ml}$ , respectively. In combination, MIC values of both compounds declined significantly to 0.005  $\mu\text{g/ml}$  prodigiosin together with 16  $\mu\text{g/ml}$  N-myristoyltyrosine, thereby demonstrating a synergistic antibacterial effect. Therefore, natural mixtures such as the combination of an antibiotic compound together with an antimicrobial surfactant may serve as a promising blueprint for further effective drug developments.

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### BTP078

#### **German Network for Bioinformatics Infrastructure**

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In recent years, the ever-growing application of the omics techniques and the exploitation of the resulting data have revolutionized many fields of science and are furthermore opening new areas of basic and applied research with considerable opportunities for life sciences. The bottleneck that prevents realization of the full potential of the different omics technologies is not the data generation itself, but the subsequent data analysis.

The German Network for Bioinformatics Infrastructure (de.NBI) takes care of this challenge in life sciences with its mission to provide, expand and improve a repertoire of specialized bioinformatics tools, appropriate computing and storage capacities and high-quality data resources. These efforts are supplemented by a training program providing courses on the supplied tools.

de.NBI is a distributed bioinformatics infrastructure which started in March 2015 as an academic funding initiative of the German Ministry of Research and Education (BMBF). The consortium currently consists of 39 project partners organized in eight service centers and one central administration and coordination unit. The service centers offer a variety of training courses and bioinformatics services, online databases, software libraries, and tools as webservices and/or for download. Services are aimed at application users in life sciences as well as bioinformaticians and developers. The de.NBI services will be unified with regard to standards, interchangeability and reproducibility. The network has recently been supplemented with a federated cloud at five locations. This hardware is enabling big data exploitation in all areas of life sciences.

## BTP079

### Biotransformation of the pectin degradation product D-galacturonic acid to L-galactonate in *Saccharomyces cerevisiae*

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As one of the most abundant polysaccharides in nature and a waste product of sugar industry, pectin ranks as one of the major potential second-generation feedstocks (non-food plant material) for biotechnological production of different compounds, such as fuels, fine chemicals and additives for cosmetic and nutraceutical industry. Pectin mainly consists of  $\alpha$ -1,4-glycosidically linked D-galacturonic acid units (D-GalA), which is naturally not metabolized by the prominent biotechnological production host *Saccharomyces cerevisiae*. We aim to engineer this yeast for biotransformation of D-GalA to L-galactonate (L-GalOA), which has potential applications as chelator, moisturizer, pH-stabilizer and leaving agent in food and cosmetic industry. This requires the expression of heterologous D-GalA transporters and reductases (Benz et al., 2014), but also extensive interventions into the central carbon metabolism of the host cell. Previous attempts to construct D-GalA utilizing *S. cerevisiae* strains have revealed that the higher oxidation state of D-GalA (compared to sugars) is one of the challenges for its funneling into the endogenous metabolism of yeast (Biz et al., 2016), since surplus reducing equivalents are required. These can be derived from the fermentation of sugars present in the pectin biomass, mainly glucose, galactose and arabinose by blocking the ethanol formation. In this way, a nearly complete valorization of the pectin feedstock, which is currently underused, can be achieved.

## BTP080

### Integrated Succinic acid production using xylose from pretreated Lignocellulose and carbon dioxide from biogas production

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The environmental and climate challenges caused by the excessive fossil fuels consumption increased the demand of renewable materials. Beside the energy industry, the petrochemical industry also need alternatives for oil. Succinic acid, a dicarboxylic acid, was regarded as one of the most important platform chemicals for the future. Today, succinic acid is produced mainly by catalytic hydrogenation of petrochemical derived maleic acid. But the production based on renewable biomass by anaerobic bacteria has become more attractive economically. The pretreatment of lignocellulosic biomass released great amounts of xylose as hydrolysate. Producers of succinic acid like *A. succiniciproducens* could utilize a wide range of different sugars like glucose and xylose. The idea is to use the xylose-rich hydrolysate as substrate for the succinic acid production. As a great benefit, the succinic acid production needed CO<sub>2</sub>. This will improve the CO<sub>2</sub> balance of the production process. A cheap high-concentrated CO<sub>2</sub> source is necessary. Biogas from biogas plants contained between 25 – 45 % CO<sub>2</sub>. Today, the CO<sub>2</sub> portion is released useless in the atmosphere. The idea in the here presented project was to use the CO<sub>2</sub> from the biogas in the succinic acid production. The combination with the xylose-rich hydrolysate from wheat straw pretreatment will increase the sustainability of the whole process. Simultaneously, the biogas will be purified to biomethane which could be used as

natural gas. The production of the succinic acid will be done in a fixed-bed reactor with bacteria on a solid state. Typically, the bacteria also produced other acids like acetic or formic acid. Genetic manipulations should eliminate the by-product formation and increase the succinic acid production.

## BTP081

### A Low-cost Thin Layer Spectroelectrochemical Cell to Study Bacterial Redoxproteins

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Redoxactive metal-containing enzymes and their associated redox partners such as cytochromes c, iron-sulfur cluster proteins and cupredoxins play key roles in microbial energy conservation. Important parameters to characterize such redox cofactors are their mid-point reduction potentials at physiological pH ( $E_0'$ ). Although **Optically Transparent Thin-Layer Electrochemical (OTTLE) Cells** (e.g. Baymann, 1991) are convenient devices to determine mid-point potentials, their construction often appears cumbersome. Here we present the construction and application of a thin-layer spectroelectrochemical cell based on a standard microscopy glass slide that can be prepared using common laboratory equipment. Ultrathin gold electrode connections (< 1  $\mu$ m thickness) were prepared by spray coating with gold paint ("Glanzgold", Heraeus, Hanau, Germany) followed by curing of the metal layer by oven heating. An optically transparent gold mesh (10  $\mu$ m thickness) modified with 4,4'-dithiodipyridine (DTP) served as working electrode. A surface-mounted silver/silver chloride reference electrode patch used in the presence of 100 mM KCl in the sample buffer completed the electrode setup. The sample was filled in between the gold-coated glass slide electrode assembly and a glass coverslip. Small amounts of sample solution (< 10  $\mu$ L) allowed fast equilibration times (< 1 min) per applied potential step. The presented thin-layer spectroelectrochemical cell is easy to manufacture at low costs and convenient to use. Measurements of the mid-point potentials of several bacterial multiheme cytochromes were successfully performed.

## BTP082

### Modulation of the N-metabolism for an optimized secondary metabolite production in *Streptomyces coelicolor*

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There is an urgent need for novel antibiotics to fight life-threatening infections and to counteract the increasing problem of antibiotic resistance. New molecular genetic and biochemical tools have provided insight into the enormous unexploited genetic pool of environmental microbial biodiversity for the synthesis of potential new bioactive compounds. However, new tools for antibiotic discovery and production are needed to more efficiently bring novel antibiotics to market.

This project aims to develop the model actinomycete *Streptomyces coelicolor* into a 'Superhost' for the efficient heterologous production of bioactive compounds. Central to this approach will be an iterative Systems Biology process, combining microbiology, genetics, biochemistry, and fermentation technology with modelling. As project partners we work on optimizing the nitrogen metabolism of the 'Superhost'. We have identified several genes of the nitrogen

regulatory network, which show potential to have an overall positive effect on secondary metabolite production when modified. By that means, we aim to help generate a stepwise improved 'Superhost' for the production of antibiotics in which metabolic bottlenecks and regulatory restrictions are greatly mitigated. The optimized strains will be tested concerning their applicability for an improved production of commercially relevant antibiotics and the expression of novel bioactive gene clusters identified in new actinomycete strains and environmental metagenomes.

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#### BTP083

##### Production of monolignols in engineered *E. coli* is limited by a negative feedback mechanism

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The monolignols coumaryl, sinapyl and coniferyl alcohols are precursors of lignin and a variety of secondary metabolites with beneficial properties for human health. The biotechnological synthesis of these compounds is still insufficient for their economical production. By implementing the shortest possible three step pathway from tyrosine to coumaryl alcohol we aimed to improve productivity in *E. coli*. Therefore, we designed a polycistronic expression construct with ascending order of catalytic proficiency of the encoded gene products tyrosine ammonia lyase, carboxylic acid reductase and cinnamoyl alcohol dehydrogenase. Optimization was performed for coumaryl alcohol biosynthesis but is transferable to coniferyl and sinapyl alcohol due to substrate promiscuity of the heterologous enzymes. We improved the coumaryl alcohol production in stirred tank reactors with resting and growing cells by (1) decreasing the expression temperature of the biosynthetic genes, (2) feeding of glucose and a source of amino acids and (3) utilizing *E. coli* with a deletion in the *tyrR* gene to the maximum titer of 5 mM coumaryl alcohol. However, in all experiments a concomitant decline in productivity within the first 5 h of the reaction was obvious from a maximum of 700  $\mu\text{mol/h/g}_{\text{BDW}}$  to less than 200  $\mu\text{mol/h/g}_{\text{BDW}}$ . The loss of productivity was neither due to (1) the loss of recombinant enzyme activity, nor (2) a soluble inhibitor in the medium or (3) substrate depletion. In contrast to living cells activity of the three step pathway in cell extracts showed a decrease in activity to 57 % after 5 h of reaction resulting in a nevertheless high productivity of 604  $\mu\text{mol/h/g}_{\text{BDW}}$ . As the loss occurs predominantly *in vivo* by the intermediates of the heterologous pathway it indicates a feedback inhibition.

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#### BTP084

##### Cationic biocides as potential antifouling agents for polymeric coatings/plastic systems.

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There are many surfaces need to remain biofouling-free due to technological processes and contamination risks. The corrosion-related microorganisms able to form of biofilms resulted in corrosion induction. A coating with antifouling characteristics can protect the metal surfaces from biofouling, and, hence, decrease corrosion risks. Undesirable biofouling of different plastic surfaces contributes to hospital-

acquired infections and multi-drug resistant bacteria persistence. From this point of view, cationic biocides including long-chain ionic liquids (IL) or ionene polymers, compatible with marine paints and thermoplastic polymers are under focus. In order to assess antifouling effects of cationic biocides, *E. coli* DH 10B was used as a model biofilm-forming strain. Polycarbonate films containing from 3% to 10 wt% of water immiscible IL 1-dodecyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide (C12C11M-TFSI), as well as polyamide 11 containing from 3 to 10 wt% of cationic biocide polyhexamethylene guanidine 2-naphtalenesulfonate (PHMG-NS) were used in a biofilm assay. Both crystal violet staining and a MTT test showed a reduction in biofilm formation compared to a control. Another two ILs, 1-dodecyl-3-methylimidazolium bromide (C12C11M-Br) and 1-hexadecyl-3-methylimidazolium bromide (C16C11M-Br), were used for marine paint formulation. Metal coupons covered by ILs-containing paint were incubated in microcosms containing *Thiobacillus* sp.-*Stenotrophomonas maltophilia* and *Alteromonas nucleodii*-*Shewanella baltica* associations; the level of biofouling were also measured. In both assays ILs-containing polymers showed biofouling reduction. ILs are a promising class of antifouling anchored compounds to be applied in marine-associated and hospital environments.

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#### BTP085

##### Process engineering and microbiological characterization of on demand biogas production through sugar beet silage application

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**Introduction & Objectives:** The generation of electricity from biogas production is the only form of regenerative energy production that allows flexible supply. We aim for on demand biogas production by quickly available carbohydrate in sugar beet silage (SBS). Metabolic activities of microorganisms are determined by measuring specific enzyme activities. Direct correlation of metabolic activity with process parameters may allow assessment of limitations during anaerobic digestion (AD).

**Materials and Methods:** AD was carried out in four 15 L continuous fermenters (CF) supplied with different ratios of maize silage (MS) and SBS (CF1 1:0, CF2 1:6, CF3 1:3, CF4 1:3) AD was divided into two different phases: 1) hourly substrate supply of MS and SBS and 2) hourly supply of MS, and SBS twice per day. Samples of fermenter content for enzyme activity measurements were drawn weekly.

**Results:** Average methane yields (MY) for CF1-3 (223.6 $\pm$ 10.1 NI/kg oTS) were in same range as for CF4 (205.3 $\pm$ 80.5 NI/kg oTS). During phase 1, enzyme activities of acetate kinase (ACK) in CF4 were found to be ten times higher (6.40 $\pm$ 2.43 U/mg) compared to activities in CF1-3 (1.08 $\pm$ 0.08 U/mg). During phase 2, SBS induced methane production immediately. Averaged specific MY for CF1-3 were 260.48 $\pm$ 14.92 NI/kg oTS and ACK activities 0.75 $\pm$ 0.27 U/mg, respectively. In CF4 MY were 179.03 $\pm$ 28.14 NI/kg oTS and ACK activities 3.52 $\pm$ 0.91 U/mg. In phase 2 enzyme activities correlated negatively with MY.

**Conclusion:** On demand methane production can be achieved by addition of SBS. Moreover, metabolic activities of microorganisms were not affected by applying moderate

ratios of MS:SBS such as 1:6 and 1:3. However to large quantities of SBS (MS:SBS ratio of 1:3) impaired the metabolic activity of microorganisms.

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#### BTP086

##### **Different growth properties of *ackA* compared to *pta* and *ackA-pta* mutants of *Escherichia coli* K-12 at aerobic and anaerobic conditions**

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During growth on glucose, acetate is a characteristic by-product of *E. coli* K-12, at aerobic as well as fermentative conditions. While the reasons for the aerobic acetate production, the so called overflow metabolism, are still discussed, under anaerobic conditions, acetate production is important for ATP synthesis. At both conditions, acetate is produced by a pathway consisting of phosphate acetyltransferase (Pta), producing acetyl-phosphate from acetyl-coenzyme A, and acetate kinase (AckA), producing acetate from acetyl-phosphate, coupled to the production of ATP. Mutants in *ackA* and *pta* differ in the potential to produce and accumulate acetyl-phosphate. In the publication at hand, we investigated different mutants in the acetate pathway at aerobic as well as fermentative conditions. We performed growth assays and determined growth rate as well as production of by-products. The studies were extended by analysis of gene expression, phosphorylation of the response regulator ArcA and of protein acetylation. While the growth defect at aerobic conditions was only marginal for the different mutants, at anaerobic conditions all acetate mutants showed severe reduction in growth rate, coupled to changes in the by-product pattern. The most severe growth defect was observed for the *ackA*-mutant. This mutant exhibits strong changes in gene expression and unexpectedly showed a lowered phosphorylation of ArcA at anaerobic conditions. Also, significantly increased protein acetylation was observed for this mutant growing anaerobically. The data hence show, that the production and accumulation of acetyl-phosphate has a strong impact on anaerobic growth of *E. coli* K-12. They hint to an important function of protein acetylation in the regulation of metabolic fluxes.

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#### BTP087

##### **Discovery of novel cystobactamid variants with superior activity in a multidisciplinary screening approach**

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Nature is the most promising source for molecules, which can be used as starting point for a drug development program, especially when it comes to antibiotics[1]. Antibiotic discovery and development is yet challenging since molecules with novel modes of action and broad-spectrum activity rarely occur in screenings and their discovery is very often hampered by low product titers[2].

Herein we report a combination of methodologies, which led to the discovery of new cystobactamids with a significantly improved antibacterial profile through detailed screening of a myxobacterial producer strain[3]. Careful MS/MS analysis of

active fractions from extracts, media and process development, and a sophisticated purification strategy enabled the discovery, purification and characterization of these molecules.

Some of these new derivatives display antibacterial activities (through inhibition of DNA gyrase) in the sub  $\mu\text{g mL}^{-1}$  range against Gram-positive and Gram-negative pathogens, including clinical isolates of *Klebsiella oxytoca*, *Pseudomonas aeruginosa*, and fluoroquinolone-resistant *Enterobacteriaceae*, which were not observed for previously reported cystobactamids. Our findings provide structure-activity relationships and show how pathogen resistance can be overcome by natural scaffold diversity. The most promising derivative 861-2 was subsequently prepared by total synthesis, which will enable further chemical optimization of this privileged scaffold.

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[1] D. J. Newman, G. M. Cragg, *J. Nat. Prod.* 2016, 79, 629-661.

[2] S. Cociancich *et al.*, *Nat. Chem. Biol.* 2015, 11, 195-197.

[3] S. Hüttel *et al.*, *Angew. Chem. Int. Ed.* 2017, 56, 12760-12764

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#### BTP088

##### **Self-immobilizing Biocatalysts for fluidic Reaction Cascades**

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A rich source of innovation in biocatalysis is currently drawn from biomimetic fluidic approaches for the compartmentalization and cascading of multiple enzymatic transformations. One key aspect in the establishment of such artificial fluidic cascades concerns the immobilization of accessible and active biocatalysts. Usual approaches use a biocatalyst which is non-specifically immobilized (e.g. physically adsorbed, chemically cross-linked or entrapped) and, thus, often decreased in its overall catalytic activity. Therefore we engineered biorthogonal self-immobilizing enzymes and cells which enable, due to their high binding affinity and specificity, the direct immobilization from crude mixtures while maintaining their catalytic activity. As a proof of concept for our self-immobilizing enzymes, a recently described one-pot enzymatic three-enzyme cascade, was adapted for the fluidic compartmentalised synthesis of *meso* diols. To this end, (*R*)- and (*S*)-specific ketoreductases were immobilized on superparamagnetic microparticles, which were loaded in a microfluidic packed bed reactor, thereby enabling fluidic production of the *meso* diol from a prochiral *CS*-symmetric diketone with an initial conversion of 73.6% (d.r. >99:1). For genetic encoded whole-cell immobilization *E. coli* strains were engineered which display orthogonal immobilization tags on their surface while heterologously overexpressing stereoselective ketoreductases. The latter strains gave high selectivities for specific immobilization onto complementary surfaces and also in the whole-cell stereospecific transformation of the diketone (d.r. >99:1). In recent works we further expanded the scope of self-immobilizing biocatalysts, enabling the production of binary self-assembling biocatalytic all-enzyme hydrogels.

## BTP089

### New insights into enzyme family GH9 - characterization of a novel endo- $\beta$ -glucanase

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**Introduction:** Endo- $\beta$ -glucanases hydrolyze  $\beta$ -glycosidic linkages in various glucans. Many endo- $\beta$ -glucanases hydrolyze different variations of glucans which reflects the difficulty of enzyme classification. Consequently, glycoside hydrolases were grouped in families based on sequence similarities. Glycoside hydrolase family 9 enzymes were described to act preferably on soluble or insoluble cellulose with lower side activities towards related polysaccharides such as xyloglucan, xylan or beta-1,3-1,4-glucans. In this study, a novel GH9 endo- $\beta$ -glucanase with extraordinary substrate specificity was identified and characterized.

**Material & Methods:** In this study, a biogas plant operating at 50°C was used as a source for isolation of metagenomic DNA. The ORF *bp\_cel9A* was predicted to encode a putative GH9 endo- $\beta$ -glucanase with low identity ( $\leq 55\%$ ) to annotated proteins. The ORF was cloned in an *E. coli* expression system and BP\_Cel9A was recombinantly produced, purified and characterized.

**Results & Conclusion:** The endo- $\beta$ -glucanase BP\_Cel9A hydrolyzed the  $\beta$ -1,3-1,4-linked barley beta-glucan at 30°C and pH 6.0. Lichenan and xyloglucan were decomposed up to 67% and 40%, respectively. The activity towards different substrates varied with different temperatures. Most GH9 glucanases act preferably on crystalline or soluble cellulose with only side activities towards related substrates. Nevertheless, The activity on CMC was extremely low ( $\leq 1\%$ ). The addition of calcium or magnesium ions enhanced the activity of BP\_Cel9A, especially at higher temperatures, whereas EDTA inhibited the enzyme. BP\_Cel9A is a calcium- and a magnesium-dependent enzyme with a distinct substrate spectrum. This enzyme exhibits great potential for mixed-linked glucan degrading process at moderate temperatures.

## BTP090

### Production of the low-caloric sweetener 5-Ketofructose in *Gluconobacter oxydans*

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High-sugar intake due to the consumption of high-sucrose/fructose-containing additives in the food industry are suspected to be a continuous risk factor for the development of obesity, cardiovascular disease and type 2 diabetes. As a consequence, there is a growing consumer demand for low-calorie, sugar-free foodstuff. Therefore, we focused on the microbial production of 5-keto-D-fructose (5-KF) as a promising sugar alternative, which is formed by membrane-bound fructose dehydrogenases (Fdh) in some *Gluconobacter* strains. 5-KF production was achieved by heterologous expression of the *fdh* genes in *Gluconobacter oxydans* in fructose-containing media resulting in a yield of about 95 %<sup>1</sup>. In addition, a new approach was tested for the production of the sweetener 5-KF by using sucrose as a substrate. The production of 5-KF from sucrose was performed by a two-strain system composed of the *fdh*-expressing strain and a *G. oxydans* strain that produced the sucrose hydrolyzing enzyme SacC. The strains converted 92.5% of the available fructose units into 5-KF. Also sucrose present in sugar beet extract as a low cost and renewable

substrate was converted to 5-ketofructose with a yield  $82 \pm 5$  %<sup>1</sup>.

## References:

[1] Siemen *et al.* (2017) Appl Microbiol Biotechnol doi: 10.1007/s00253-017-8699-1

## BTP091

### Engineering *Rhodobacter capsulatus* for the heterologous production of different terpene classes

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Terpenes constitute one of the largest and most diverse group of secondary metabolites and can be used for different applications such as biofuels, fragrances or pharmaceuticals. The phototrophic bacterium *Rhodobacter capsulatus* represents a suitable host for the production of terpenes, because it possesses an intrinsic MEP pathway to synthesize tetraterpenes and is able to form a large intracytoplasmic membrane system. Thus, *R. capsulatus* provides optimal properties for the production and storage of hydrophobic terpenes but has thus far not been biotechnologically exploited as a production host.

Here, we demonstrate the heterologous production of the monoterpene limonene, the sesquiterpene valencene, the diterpene casbene, the triterpene squalene and the tetraterpene b-carotene in *R. capsulatus*. In addition, it was investigated whether the terpene production can be further optimized by the coexpression of different terpene precursor genes. Remarkably, while expression of some of the terpene synthases already resulted in highest terpene titers in the *R. capsulatus* wild-type strain SB1003, in other cases, the coexpression of genes encoding the MVA pathway or the FPP synthase led to highest product titers (e.g. 324 mg/l for valencene). Finally, the relevance of the specifically employed terpene synthase for product titers was analyzed by heterologous expression of five pro- and eukaryotic squalene synthases. The expression of these synthases resulted in significantly different levels of squalene accumulation ranging from 5 mg/l up to 91 mg/l. We therefore clearly demonstrate here applicability of *R. capsulatus* for the heterologous production of terpenes from all basic classes as well as the relevance of individual synthase properties and metabolic engineering approaches.

## BTP092

### The yTRES-toolbox for rapid cloning, transfer and expression of biosynthetic pathways in bacteria

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High-value secondary metabolites are naturally produced in only low amounts. Their efficient production requires the transfer of entire metabolic pathways into suitable expression hosts evoking the need to develop effective tools for the cloning and heterologous expression of gene clusters in microorganisms.

Here, we developed a novel toolbox based on the transfer and expression system TREX<sup>1</sup> for straightforward expression of gene clusters in bacteria. First, the system was adapted to allow the cloning *via* homologous recombination in yeast to overcome handling difficulties of restriction endonuclease-based cloning. Effectivity was demonstrated by the rapid cloning and successful implementation of biosynthetic pathways in *Pseudomonas putida*<sup>2</sup>.

To obtain high flexibility within this yTRES-system, we moreover developed a toolbox concept consisting of changeable components that allow its adaption individually to different target pathways, hosts and experiments. Promoters, selection markers, and reporter elements as well as DNA sequences for chromosomal integration can easily be exchanged using designated recombineering sequences and homing endonucleases. Random integration can be exerted by applying the Tn5-transposition system to create strong constitutive production strains utilizing host promoters, enabling the identification of favorable integration sites<sup>3</sup>. Aiming for targeted utilization of such sites, integration vectors can be adapted to allow homologous recombination to integrate genes of interest directly downstream of a desired promoter.

In summary, the yTRES-toolbox offers effective strategies for the rapid generation of bacterial strains for the production of valuable compounds.

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[2] Domröse *et al.* 2017

[3] Domröse *et al.* 2015

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#### BTP093

##### Improvement of recombinant production of industrial relevant enzymes in *Coprinopsis cinerea*

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Enzymes do play a prominent role in the food industry. Some of them are extracted from animal or plant sources, but most enzymes are produced with genetically modified organisms, such as *Bacillus* sp. and *Aspergillus* sp.. Currently, almost all food relevant enzymes produced recombinantly are of bacterial or fungal origin. Nevertheless, higher eukaryotic sources like plants, animals and insects are getting of higher interest. However, the recombinant production of these enzymes from higher eukaryotes in bacterial and ascomycetous hosts may cause severe problems (post-translational modifications, proteolytic digestion etc.). Therefore, a eukaryotic expression system based on an edible mushroom as expression host for enzymes seems to be promising. *Coprinopsis cinerea* is an edible mushroom belonging to the phylum of basidiomycota and is already known as an expression host. Oxidases, like laccases or aryl-alcohol oxidases, have already been expressed successfully. In this study for the first time, we used *C. cinerea* as a host for recombinant production of homologous and heterologous peptidases, such as serine peptidases. Furthermore, we tried to improve the expression system using modification of an already known expression cassette with an alternative signal peptide, an additional Kozak consensus sequence or fusion of the gene of interest to an additional secretion domain (SUMO). Results showed a successful expression of the homologous serine peptidase able to hydrolyze the substrate Succinyl-Ala-Ala-Pro-Phe-p-

nitroanilide. In contrast, recombinant expression of heterologous peptidases in *C. cinerea* could only be confirmed by means of western blot analysis, whereas peptidase activity was not detectable.

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#### BTP094

##### Whole-genome sequencing and analysis of *Penicillium chrysogenum* MAT1-2 wild-type strain Pc3

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**Introduction:** The filamentous ascomycete *Penicillium chrysogenum* is the main industrial producer of the  $\beta$ -lactam antibiotic penicillin and is therefore of great economical and medical importance. All currently used industrial strains are derivatives from the MAT1-1 isolate NRRL1951, which was subsequently used for random mutagenesis to increase penicillin production yields. However, there is a lack of knowledge of the genomic variability of *P. chrysogenum* wild-type strains.

**Objective:** While genomes of diverse production strains were sequenced in the last decades, the genetic variability of *P. chrysogenum* wild-type strains is still unclear. To gain insights into the genetic composition and structural differences between wild-type strain Pc3 and industrial strains, the genomic sequence of Pc3 needs to be determined and investigated. Furthermore, the genome sequence of Pc3 is highly valuable, cause of its capability to complete a heterothallic sexual life cycle with industrial strains.

**Materials & methods:** The Pc3 genome was sequenced, using PacBio and Illumina sequencing platforms, and subsequently annotated. Furthermore, transcriptional (mRNA-seq) and comparative genomic analysis were performed.

**Results:** We assembled and annotated the 32.4 Mbp genome of Pc3. In addition, we predicted and partial analyzed the expression of 42 secondary metabolite gene clusters. Using comparative genomic analysis, we were able to identify structural and genetic variations to known strains.

**Conclusion:** Our work provides an insight into the genomic variability of *P. chrysogenum* and is a valuable resource for further functional analysis. In addition, it will contribute to our understandings of the secondary metabolism in wild-type and industrial strains.

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#### BTP095

##### Transcriptome analysis of the unrelated fungal $\beta$ -lactam producers *Acremonium chrysogenum* and *Penicillium chrysogenum* reveal Velvets important role during conventional strain improvement

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**Introduction:** *Acremonium chrysogenum* and *Penicillium chrysogenum* are the industrial producers of the  $\beta$ -lactam antibiotics cephalosporin C and penicillin G, which are used worldwide for the treatment of bacterial infections. Wild-type isolates from these taxonomically unrelated fungi were improved in conventional mutagenesis programs to reach economically relevant titers of  $\beta$ -lactam antibiotics. However,

there is still a lack of knowledge how the increased  $\beta$ -lactam production was achieved.

**Objectives:** We used RNA-seq analyses of wild-type and industrial strains of both species to address the question whether both fungi have undergone similar expressional changes during strain improvement. Furthermore, we investigated two mutants lacking Velvet, a global regulator of fungal secondary metabolism.

**Methods:** RNA-seq, identification of secondary metabolite clusters, expression quantification, and functional categorization.

**Results:** Improved strains of both fungi show common expressional adaptations, namely the upregulation of pathways supplying precursors and energy for  $\beta$ -lactam production and shutting down not required pathways or cellular functions. Furthermore, we elucidated Velvets regulatory network in the improved strains, containing approximately 50% of all secondary metabolite clusters. Most importantly, strain improvement and Velvet affect the expression of a large set of genes in a similar manner in both improved industrial fungi.

**Conclusion:** The major finding of our comparative transcriptome analysis is that strain improvement programs in two unrelated fungal  $\beta$ -lactam antibiotic producers affect similar metabolic pathways and cellular functions. Furthermore, we assign Velvet an important role during strain improvement in both organisms.

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#### BTP096

##### **Isolation of an electroautotrophic Knallgas-bacterium: Towards an aerobic, thermophilic electrosynthesis process for the production of polyhydroxyalkanoates (PHAs).**

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**Introduction:** Electroautotrophic microorganisms thrive using a cathode as electron and energy source and couple this to the simultaneous fixation of carbon dioxide. This process has the potential of providing a future technology using carbon dioxide as a feedstock and addressing the demand of storage solutions for excess electrical energy.

**Objectives:** From a thermophilic cathodic enrichment we isolated a novel electroautotrophic species belonging to the physiological group of the Knallgasbacteria. Using this strain as a model organism for electroautotrophic growth and a biocatalyst in a bioelectrochemical system, our aim is to establish a biotechnological process for the production of PHA from carbon dioxide and electrical energy.

**Materials & methods:** Strain EA-1<sup>T</sup> was isolated from hydrothermal systems. Enrichment was performed at 60°C under autotrophic conditions. Chronoamperometric experiments (-500 mV vs. SHE, graphite felt cathode) and Cyclic Voltammetry (CV) were conducted in minimal medium at pH 3.5 and 60°C. Electron micrographs of the cathode were taken. Nile Red staining was performed to investigate PHA-synthesis.

**Results & conclusions:** The isolate was found to be a novel species of the genus *Kyrpidia*. In an inoculated microbial electro-synthesis cell, current densities up to -95  $\mu\text{A}/\text{cm}^2$  were observed, in contrast to a sterile control showing a current density of -25  $\mu\text{A}/\text{cm}^2$ . Equally, the results of CV experiments indicated an electroactive cathodic biofilm. REM pictures of a cathode poised at -500 mV vs. SHE showed a distinct higher cell number than the micrographs of a control electrode. For a deeper understanding of the molecular mechanisms behind this process, transcriptomic approaches are in progress. Furthermore, a scalable bioreactor was developed.

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#### BTP097

##### **Engineering of a Non-natural Carboxylase for Synthetic Photorespiration**

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Photosynthetic capacity and therefore agricultural productivity is limited by a number of factors. One of the most striking problems is the natural inefficiency of RubisCO, the key enzyme of the Calvin-Benson cycle.

RubisCO catalyzes the carboxylation of ribulose-1,5-bisphosphate to form 3-phosphoglycerate (3-PGA). Unfortunately, RubisCO also catalyzes the non-productive incorporation of oxygen instead of carbon dioxide resulting in the formation of toxic 2-phosphoglycolate (2-PG). This process is called photorespiration. The 2-PG needs to be removed and recycled, because it acts as a strong inhibitor of several central carbon metabolism enzymes at micromolar levels. The recycling pathway for 2-PG is very energy-demanding: it involves many enzymes and cofactors, it releases ammonia and, as its main disadvantage, it also releases CO<sub>2</sub> again. It has been estimated that about 30 % of fixed carbon is lost due to this recycling pathway in C<sub>3</sub>-plants.

In this study, we were able to assemble a completely synthetic photorespiratory bypass that requires less energy and directly fixes CO<sub>2</sub> instead of releasing it. The key reaction of our bypass is catalyzed by an engineered carboxylase which is able to perform an unprecedented reaction not known to be part of any natural metabolic pathway. We were already able to improve the specific activity of the carboxylase by two orders of magnitude applying a rational design approach. To screen large libraries of carboxylase variants with even further improved catalytic efficiencies, we established an ultrahigh-throughput microfluidics assay. This enables us to test about 150,000 enzyme variants per hour.

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#### BTP098

##### **Quorum quenching compounds of entomopathogenic bacteria – a strategy to prevent bacterial biofilm formation**

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Bacterial biofilms cause huge problems in both healthcare and industry, thereby producing an enormous economic loss worldwide. The prerequisite for biofilm formation is bacterial communication, generally known as quorum sensing (QS). Under high cell density, bacteria start to organize in a biofilm by producing extracellular polymers to attach and grow on solid surfaces. Biofilms appear after a several time on nearly every wet surface, e.g. in freshwater supply, in seawater surfaces like on ship bodies, in pipelines, or on implants. Entomopathogenic bacteria of the genus *Photorhabdus* and *Xenorhabdus* produce a huge number of novel secondary metabolites. Here we show, that several of these metabolites exhibit a negative effect on biofilm formation of various bacterial species from different native biofilm habitats. We also verified that the natural compounds were not acting as simple bactericides, meaning neither affecting growth nor killing the cells. Therefore, we assumed that these natural compounds block QS of the target bacteria, a process that is referred to as quorum quenching (QQ). We tested the effect of 62 putative QQ-compounds on the biofilm formation of 30 different bacterial species. For most of those bacteria at least one QQ-compound could be detected that specifically blocked them in biofilm formation. We also observed that bacteria of equal biofilm habitats exhibited similar susceptibility towards the same QQ-compounds. These QQ-compounds had not only a negative effect on single-species biofilm formation, but also on those composed of multiple bacterial species. Summarizing, entomopathogenic bacteria represent an excellent reservoir of QQ-compounds that are putatively applicable as specific biofilm preventive agents.

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#### BTP099

##### Reporter constructs in *Corynebacterium glutamicum* for the analysis of CO<sub>2</sub>/HCO<sub>3</sub><sup>-</sup> induced regulatory response

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**Introduction:** *Corynebacterium glutamicum* is commonly employed in industrial scale bioreactors, e.g. for amino acid production. In the course of such cultivations, cells face a range of different carbon dioxide (CO<sub>2</sub>) availabilities, which vary with time (of an aerobically growing culture) and location inside the reactor. As the total pressure increases towards the bottom of the reactor, the local concentration of dissolved CO<sub>2</sub> as well as its related species HCO<sub>3</sub><sup>-</sup> and CO<sub>3</sub><sup>2-</sup> increase. It was shown, that elevated levels of CO<sub>2</sub>/HCO<sub>3</sub><sup>-</sup> manifested in an activation of DtxR, the master regulator of iron homeostasis (Blombach et al., 2013).

**Objectives:** To monitor the CO<sub>2</sub> induced response in *C. glutamicum*, we designed two constructs, which act as transcriptional reporters based on the activation state of DtxR.

**Methods:** In a first construct, *egfp* expression was repressed by active DtxR. A second construct comprised an additional semi-synthetic signal enhancer and eventually produced eGFP in the presence of active DtxR.

**Results & Conclusion:** Both constructs responded to high and low iron concentrations in the medium as expected from literature. To prove functionality regarding different partial pressures of CO<sub>2</sub> (pCO<sub>2</sub>), bioreactor fermentations were performed with the more sensitive reporter strain at standard and elevated pCO<sub>2</sub> in parallel. The biomass specific fluorescence at the beginning of the fermentation was identical at high and standard pCO<sub>2</sub> and split into high and low levels 6-7 hours after inoculation. Thus, biosensor activity mirrors the results of the initial transcriptional analysis

and provides a valuable tool to monitor a varying CO<sub>2</sub> partial pressure.

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#### BTP100

##### Engineering of quorum quenching enzymes

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**Introduction:** Quorum quenching (QQ) is a promising anti-virulence strategy. One possible QQ target is the quorum sensing signal. *Pseudomonas aeruginosa*, one of the leading nosocomial pathogens, uses the Pseudomonas quinolone signal (PQS; 2-heptyl-3-hydroxy-4(1H)-quinolone) besides *N*-acylhomoserine lactones to regulate its virulence. PQS is inactivated by the ring-cleaving dioxygenases HodC from *Arthrobacter* sp. Rue61a and AqdC from *Mycobacterium abscessus*. Unfortunately, these enzymes are either not thermostable (AqdC), or degraded by *P. aeruginosa* exoproteases (HodC). To be used in QQ therapy, those proteins need to be improved.

**Experiments:** HodC was fragmented by incubation with *P. aeruginosa* culture supernatant and analyzed using mass spectrometry. Residues within regions most susceptible to proteolytic cleavage were substituted by the corresponding amino acids of the AqdC sequence. For the protease stable but thermolabile AqdC, we aimed at solving its crystal structure, to enable the prediction of stabilizing amino acid substitutions by FRESCO. We also aim at generating HodC-AqdC hybrid enzymes. All protein variants were analyzed for stability (thermal shift assay) and catalytic activity.

**Results:** The exchange of three amino acids in a protease-susceptible helix of HodC resulted in 1.6-fold more undigested (active) protein, after incubation with proteases secreted by *P. aeruginosa*. The X-ray structure of AqdC was solved at 2.0 Å resolution. The melting temperature of AqdC was increased by 4.4 °C by two substitutions. We are currently constructing a chimaera, comprising the catalytic parts of AqdC and putative stabilizing parts of HodC. Knowing the structures of both dioxygenases, we can now use the full spectrum of bioinformatics to create stable QQ enzymes.

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#### BTP101

##### Crystal structures of different thermostable Penicillin G acylases from Gram-positive organisms

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**Introduction:** Penicillin G acylases (PGAs) are industrially important enzymes for the production of semi-synthetic β-lactam antibiotics as they catalyze the hydrolysis of penicillin G to 6-aminopenicillanic acid (6-APA) and the subsequent synthesis of penicillins.

**Objectives:** Thermal and mechanical stability of enzymes can be improved genetically. For the analysis of target amino acids within the enzyme, the crystal structure is of great interest. Further on, these stabilized crystals can be applied

for a long-term use to preserve integrity and activity of the enzyme of interest.

**Methods:** All PGAs were produced extracellularly by *Bacillus megaterium*. This allowed the purification directly from the cell-free supernatant by ion exchange chromatography followed by gel permeation chromatography. For activity determination a colorimetric enzyme assay was used. The thermostability of the enzyme was estimated by performing a thermal shift assay.

**Results:** New PGA enzymes from different *Bacillus* species were identified by database search based on *B. megaterium* PGA. Corresponding genes were introduced into *B. megaterium* plasmids, recombinantly produced and secreted. So far, two of these PGAs were active catalysts. They showed a strongly improved thermostability compared to the PGA from *B. megaterium*. The particle synthesis succeeded by crystallization using the sitting-drop vapor diffusion method. These crystals were also used for structure elucidation of the first Gram-positive PGA structures which can now be used for rational protein engineering approaches.

**Conclusion:** In summary, two new thermostable PGA variants were identified by a database search, produced and their molecular structure was elucidated.

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#### BTP102

##### Dynamics of a chain elongating reactor microbiome producing medium-chain carboxylates

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Microbial chain elongation (CE) is a secondary fermentation pathway in anaerobic digestion in which short-chain carboxylates are converted to medium-chain carboxylates (C6/C8) by reverse  $\beta$ -oxidation using e.g. lactate, a major constituent of ensiled crops, as electron donor. The investigation of CE bacteria in anaerobic fermentation, their relation to lactic acid bacteria (LAB) and the underlying microbial pathways were our objectives. The dynamics of a fermenting community was followed in a reactor with xylan and lactate as carbon sources. Four process stages were observed during 148 days reactor operation. After an adaptation time of 36 days, we achieved stable *n*-butyrate, *n*-caproate and *n*-caprylate productivities of 7.1, 8.1 and 1.8 g COD L<sup>-1</sup> d<sup>-1</sup>, respectively, at a period of 28 days (stage I). After a transition period, the process went to another stable stage (stage II), at which 57% more *n*-butyrate and 50% less *n*-caproate were detected. The microbial community dynamics followed the process development as determined by T-RFLP fingerprinting and amplicon sequencing of bacterial 16S rRNA genes. In stage I, LAB (*Lactobacillus*, *Olsenella*, *Atopobium*, *Aeriscardovia*, *Coriobacteriaceae*), potential C6/C8 producers (*Ruminiclostridium*, *Pseudoramibacter*) and the acetogenic *Syntrophococcus* predominated. During the transition, *Aeriscardovia* and *Pseudoramibacter* were lost. In stage II, the decreased abundance from 41% to 13% of *Ruminiclostridium* and the loss of *Pseudoramibacter* could be the reason for the lower C6/C8 productivity. The increased abundance from 20% to 33% of *Syntrophococcus* could be ascribed to its unique metabolism of ferulic acid, which is a component of xylan. Ferulic acid degradation was indicated by a higher concentration of phenylpropionic acid in stage II.

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#### BTP103

##### Generation of a thermo-resistant microbial Transglutaminase and studies on the thermal inactivation behavior

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The microbial Transglutaminase (mTG, EC 2.3.2.13) from *Streptomyces mobaraensis* is a versatile tool for industrial applications. The ability to crosslink proteins by generating isopeptide bonds is especially utilized in the food industry, improving the texture of meat. Additionally, novel applications are arising in the pharmaceutical industry for the production of antibody drug conjugates, or in the material industry as a composite in protein based films or glues. For this, harsh conditions can limit the applicability through inactivation of the wild type (wt) enzyme. Additionally, self-crosslinking leads to fast inactivation of the mTG, a problem which arises especially at elevated temperature. In order to improve the temperature resistance, seven previously identified amino acid positions affecting temperature resistance of mTG [1,2] were combined with each other using Golden-Gate cloning. 31 variants were produced, purified and inactivation kinetics were recorded at 60 °C. One variant, called BS16, with five substitutions exhibit an increased half-life at 60 °C by a factor of 19 compared to wt. BS16 changed the optimal reaction temperature from 55 °C to 60-65 °C. The inactivation energies (E<sub>a</sub>), determined by Arrhenius plots, showed an increase from 226 kJ/mol (wt) to 337 kJ/mol (BS16). To assess how the rigidity of the backbone and the side chains have changed, MD simulations were carried out. Interestingly, only minor influence on the amino acid fluctuation and a small decrease in rigidity for the variant BS16 compared to the wt was observed. In summary, our data implies that improved thermal resistance is predominantly caused by increased kinetic stability and provides new insights on the inactivation behavior between the wild type mTG and a variant called BS16.

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#### BTP104

##### Novel Enzymes From Metagenomes For The Biodegradation Of Polyethylene Terephthalate

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Polyethylene terephthalate (PET) is one of the most used polymers worldwide, with an annual production of more than 300 million tons worldwide. It accumulates in the environment and pollutes the oceans as nanoparticles. Due to its poor conversion in nature it is considered as an emerging threat for the environment. Further there is a high demand for developing very active enzymes for the modification of PET fibers used in textile and other industries. Therefore a detailed understanding of mechanisms and enzymes involved in biological breakdown of PET are important. By using combined enrichment and metagenome sequencing methods we were able to identify four novel organisms with the capability to actively hydrolyse PET-nanoparticles. Further deep metagenome sequence mining, involving a self-constructed Hidden Markov model (HMM) allowed the identification of eight conserved motifs connected to PET activity of hydrolases. Using this combined approach we have enriched the biodiversity of the known PET degrading bacterial phyla and displayed PET hydrolase distribution in a global context. Our data indicate that PET hydrolases occur in at least three other phyla besides the well-known Actinobacteria. The biochemical and sequence

data displaying this novel enzyme diversity are presented here. Currently, twelve enzymes are being characterized and tested for bioindustrial applications.

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#### BTP105

##### **Heterologous expression of a hyperthermophilic lipase from *Ignicoccus hospitalis* KIN4/I [Crenarchaeota] in *Pichia pastoris***

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The demand for new heat-stable lipases for industrial applications has increased considerably in recent years. The most thermostable lipases known to date almost exclusively originate from hyperthermophilic Archaea. However, the recombinant over-expression of archaeal enzymes often fails due to low yield, insolubility or misfolding; especially when attempted in common bacterial expression hosts such as *Escherichia coli*. This, in part, is attributable to numerous differences in the transcription and translation machineries of Bacteria and Archaea and thus, archaeal DNA often remains inaccessible to bacterial hosts.

The aim of this work was to successfully express and characterize Igni18, a putative metallohydrolase from *Ignicoccus hospitalis* KIN4/I, a hyperthermophilic crenarchaeon growing at an optimum temperature of 90 °C.

The gene *igni18* was expressed in the methylotrophic yeast *Pichia pastoris* (*Komagataella phaffii*) KM71H under the alcohol oxidase 1 promoter (AOX1). The process was scaled-up to a 2 L fermenter supplied with auto-induction medium. Ester chain length, pH and temperature optimum, as well as the stability towards metal ions, inhibitors, detergents, organic solvents and high temperature were determined by a para-nitrophenylester assay. A 3D model was calculated on the Robetta Protein Structure Prediction Server.

Fermentation of Igni18 resulted in yields of up to 10 mg/L. Igni18 showed lipase activity and formed stable homotrimers. Its high thermostability (46 h half-life at 90 °C) and its resistance towards methanol make Igni18 a very attractive candidate for biotechnological applications.

Moreover, *P. pastoris* was shown to be a suitable host for the expression of crenarchaeal genes, a source for hyperthermophilic biocatalysts yet to discover.

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#### BTP106

##### **Nutrition in root-fungi interactions.**

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Most terrestrial plants form associations with fungi which occur in the rhizosphere and colonise roots. These root colonisers can support plant growth and nutrition via different mechanisms: 1) They solubilise nutrients from inorganic sources which are usually not available for plants, 2) They release mineral nutrients from organic compounds and 3)

they transport nutrients and water along their hyphae towards the plant thereby crossing depletion zones around the roots and extending the rhizosphere to the mycorrhizosphere.

The major groups of root-colonising fungi are the arbuscular mycorrhizal fungi AMF (Glomeromycotina), fungi of the order Sebaciales (Basidiomycota), and the diverse form group of Dark Septate Endophytes DSE (Ascomycota). AMF are mainly involved in transporting Pi and N across depletion zones towards the roots. The representative fungus of the Sebaciales, *Serendipita indica*, improves P uptake of the plant to a high extent by solubilizing inorganic, but not organic P sources. P transport along *S. indica*'s hyphae, however, could not be detected. Experiments with two DSE fungi *Periconia macrospinosa* and *Cadophora* sp. show that they utilize organic N and P, and relieve Pi from inorganic sources and N from organic sources. In the greenhouse, they promote plant growth, but not through the transport of nutrients through their hyphae towards the plants. Their contribution to plant nutrition seems to be minor.

The three groups of root-colonizing fungi access different forms of nutrients and have different mechanisms by which they contribute to plant nutrition. They seem to have complementary roles which can be further investigated on the molecular level due to the availability of sequenced genomes of representative species.

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#### BTP107

##### **Investigating the molecular mechanisms of polyketide synthases**

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Polyketide synthases (PKSs) are highly modular multienzyme complexes producing a broad variety of valuable compounds such as antibiotics from simple acyl-CoAs and their  $\alpha$ -carboxylated equivalents. Despite decades of research, their biochemical and structural properties in terms of substrate specificity are still not completely understood.

Our aim is the understanding and engineering of an existing model system (6-deoxyerythronolide B synthase – DEBS) as well as establishing a novel *in vitro* system – the phoslactomycin synthase.

We have developed a novel chemo-enzymatic method for the synthesis of unnatural extender units and optimized methods for protein purification, *in vitro* enzyme assays and analysis via HPLC-MS to investigate the substrate specificity of DEBS.

Our chemo-enzymatic synthesis yielded an array of extender units harnessing the potential of enoyl-CoA carboxylases/reductases (ECRs). This library was used to screen for mutants of DEBS with altered or shifted substrate specificities, resulting in an unexpected high tolerance for different substrates and a very high robustness in terms of overall reactivity. Furthermore, we obtained hints for bottlenecks occurring downstream of the engineered modules in the assembly line.

These findings highlight the importance of a detailed structural understanding of how these enzymes act. Additionally, high-throughput methods might allow to screen

for the alteration of substrate specificity. A subsequent screen for the bioactivity of generated derivatives might give insight into new mechanisms of antibiotic activity. This could also be achieved by taking advantage of the single domains from different synthases, thereby generating chimeras capable of producing novel compounds.

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#### BTP108

##### **Analysis of fast intracellular NADPH accumulation kinetics in *C. glutamicum* using the genetically encoded sensor-probe mBFP\_Cg**

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**Backgrounds:** The Gram-positive *Corynebacterium glutamicum* is used in biotechnological applications which come along with high demands for intracellular NADPH. So far, these demands were mainly covered by genetic engineering of the pentose phosphate pathway. However, strains that excessively use the PPP often suffer from negative side-effects. The redirection of the carbon-flux toward the PPP by using a phosphoglucoisomerase-negative strain led to an improvement of *C. glutamicum*, but was accompanied by negative effects on growth and sugar uptake.

**Objective:** Kinetics of NADPH accumulation have not been investigated in bacteria so far, due to the lack of tools for fast online-analysis. The sensor probe mBFP\_Cg was created to solve this problem.

**Methods:** The protein mBFP was used for *in vitro* and *in vivo* assays. The protein was biochemically characterized and optimized using gene synthesis for its application in *C. glutamicum*, resulting in the protein mBFP\_Cg.

**Conclusions:** In addition to changes of its fluorescence properties upon presence of NADPH mBFP\_Cg possess enzymatic activity. This activity can be exploited to determine intracellular mBFP\_Cg levels, which is the prerequisite for the determination of the NADPH concentrations by mBFP fluorescence measurements. By this means fast NADPH accumulation was observed in *C. glutamicum* WT and *C. glutamicum*  $\Delta$ pgi, the final internal NADPH concentrations were as expected significantly higher in *C. glutamicum*  $\Delta$ pgi.

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#### BTP109

##### **Persulfide dioxygenase from *Acidithiobacillus caldus*: Identification of essential cysteine and hydrogen bond residues by mutagenesis and modification analysis**

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**Introduction:** Persulfide dioxygenases (PDO) are abundant in Bacteria but are also crucial for H<sub>2</sub>S detoxification in mitochondria. They catalyze the oxidation of glutathione persulfide (GSSH) with sulfite and reduced glutathione (GSH) as products. Despite intensive biochemical studies and X-ray structure analyses on various PDOs, the reaction mechanism of the enzyme class is still unclear.

**Objectives:** This study describes biochemical properties of the bacterial PDO from bioleaching bacterium *Acidithiobacillus caldus* (AcPDO), including inhibition

properties, melting points and an extensive mutational analysis. Preliminary X-ray crystallography results will be presented.

**Materials & methods:** The *A. caldus pdo* gene was heterologously expressed in *E. coli*. Enzyme kinetics were measured by colorimetric assays. Thermal unfolding was recorded by nanoDSF. The molecular mass of the PDO was determined by gel filtration and mass spectrometry (MS).

**Results:** The activity of the AcPDO was approximately 111 U/mg protein. Enzyme assays with GSH plus sulfur in the reaction mixture follow a Michealis-Menten kinetic, whereas incubation with GSSH resulted in a sigmoidal curve, suggesting positive cooperativity. Mutagenesis showed that residues in the predicted GSH/GSSH binding site and in the central hydrogen bond networks are essential for catalysis including the iron ligands. Two of five cysteines are also essential, both located in sulfide bridge distance on the surface of the AcPDO. MS analyses suggested that one Cys was S-glutathionylated.

**Conclusion:** The results suggest that glutathionylation was mediated by thiol:disulfide exchange from a pre-existing disulfide bridge in the as-isolated protein.

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#### BTP110

##### **Virus-X Project: Viral Metagenomics for Innovation Value**

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The virosphere represents the largest reservoirs of unknown genetic diversity in the biosphere and is being considered as the last big frontier of life. The viral gene pool is of enormous interest in terms of its potential utility and also from the perspective of functional dynamics of ecosystems and how viruses influence microbial evolution. Viruses may also provide the key to understand global diversity at the species and genome level. Much remains to be understood of virus-host interplay in nature.

The EU funded Horizon 2020 project Virus-X explores specifically the outer realms of this diversity by targeting the virosphere of selected microbial ecosystems such as hot springs and investigate the encoded functional variety of viral gene products. The project is driven by the expected large innovation value and unique properties of viral proteins, previously demonstrated by the many virally derived DNA and RNA processing enzymes. Due to the inherent challenges in gene annotation, functional assignments and other virus-specific technical obstacles of viral metagenomics, the Virus-X project specifically addresses these challenges in all parts of the discovery and analysis pipeline, from sampling extreme biotopes, through sequencing, innovative bioinformatics and annotation, selection of gene targets, cloning, expression and production, functional and structural characterization, to efficient production and commercialization of enzymes for molecular biotechnology. Although industrially driven, the project is also designed to be a vehicle for method development in the field of viral metagenomics.

The Virus-X project has been conducted at April 2016 and contains 14 research groups and company partners across Europe.

## BTP111

### Comprehensive Transcriptome Characterization of the Xanthan Producer *Xanthomonas campestris* pv. *campestris* B100 Using Next Generation RNA Sequencing

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*Xanthomonas campestris* pv. *campestris* (Xcc) is a plant pathogen and the producer of xanthan, a polysaccharide with a multitude of commercial applications as a thickening agent. The presented work aims at providing a better understanding of the mechanisms that govern xanthan biosynthesis on the transcriptional level. A comprehensive overview on the transcriptome of Xcc B100 was carried out applying transcription start sites sequencing and whole genome mRNA and small RNA profiling. In this study, the primary transcriptome of Xcc B100 was analyzed cataloguing its characteristic features at a single base pair resolution level. This analysis pointed out to the occurrence of antisense transcripts with a potential involvement in the regulation of xanthan. In addition, gene expression data were applied to enhance the accuracy of genomic feature prediction and facilitated the annotation of novel CDS and non coding RNA genes. Using whole genome mRNA profiling, differential transcriptomes of two Xcc B100 cultures obtained during the growth and stationary phases associated with xanthan biosynthesis was analyzed. During the stationary phase, 40% of genes were differentially transcribed where half of these genes were upregulated and further half was downregulated. Nucleotide sugar precursor genes of xanthan biosynthesis exhibited a transcription pattern that did not change while genes within the *gum* gene cluster, the major player of xanthan biosynthesis were differentially transcribed. The analysis of Xcc B100 transcriptome represents a foundation for genetic engineering studies aiming at enhancing the efficacy of industrial xanthan production and provides access to genetic blue prints of the molecular players that govern *Xanthomonas* metabolism and pathogenicity.

## BTP112

### From field to skin - Production of seco-pseudopterosin derivatives in *E. coli* grown on straw hydrolysate

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Seco-Pseudopterosins are diterpene glycosides, isolated from the Caribbean soft coral *Antillogorgia elisabethae*. Like Pseudopterosins, they show anti-inflammatory and wound healing activity and are promising drug candidates in cosmetics and pharmaceutical industry. Current production is derived from wild harvest and poses ecological and supply issues for an expanding market.

Key biosynthetic elements were identified from coral and other organisms using proteomic and genomic based methods. Wheat straw hydrolysate was used as sustainable carbon source for high cell-density cultivation.

By optimizing enzyme components and expression we were able to engineer an *E. coli* production host for valuable terpenoid compounds.

## BTP113

### *Synechocystis* sp. PCC 6803 as new hosts for cyclic plant triterpene biosynthesis

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Plant triterpenes form one of the most diverse groups of natural products. With more than 100 different types of basic scaffolds, they offer a structural diversity with enormous modification potential which can lead to versatile bioactive metabolites. The production of these substances has gained increasing attention for their attractive properties as pharmaceuticals, fuels, fragrances, food additives to benefit human health or to increase resistance to pathogens in crop plants. As an alternative to extraction from plants which is typically limited by low product abundance, the heterologous biosynthesis of plant secondary metabolites in microorganisms promises an efficient and environmentally friendly production option. In contrast to previously used heterotrophic organisms such as yeasts, photosynthetic cyanobacteria could be an economically more advantageous platform for the production of triterpene because the cost of the carbohydrate feedstock required for heterotrophic organisms can be more than half of the total production cost for making low value compounds. *Synechocystis* sp. PCC 6803 is used in this work for feasibility testing as an alternative triterpene production host and a potentially promising platform for the heterologous biosynthesis of cyclic plant triterpenes in the future.

## BTP114

### Understanding Substrate Specificity and Stereochemistry of Polyketide Synthases

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Polyketides are a class of natural products with an immense structural and functional variety. They exhibit a broad range of biological activities and find use in medical treatments and many more applications.

In modular polyketide synthases (PKS) the polyketide chain is assembled by sequential condensation of  $\alpha$ -carboxylated acyl-CoA thioesters. Standard acyl-CoAs are malonyl- and methylmalonyl-CoA, however more exotic acyl-CoAs such as ethylmalonyl- or 3-methyl-butylmalonyl-CoA are also reported to be incorporated into polyketides. The acyltransferase domain (AT) serves as a gatekeeper that specifically selects and activates an acyl-CoA for loading onto the growing polyketide. However, the mechanistic basis for this specificity is rather unknown.

In addition to the variation of acyl-CoA thioesters, polyketide diversity can also be attributed to the state of reduction and stereochemical configuration. Incorporation of branched acyl-CoAs leads to the formation of C-2-acyl-residues and the reduction of  $\beta$ -ketogroups by the ketoreductase-domain leads to the production of (3S)- or (3R)-hydroxyl groups. Dehydration of these hydroxyl groups forms double bonds, usually *trans*-, in rare cases also *cis*-bonds. The origin of *trans*-bonds is well reported, whereas the origin of *cis*-bonds is an ongoing research.

In this study the PKS of phoslactomycin is investigated. The specificity of the AT-domains which incorporate malonyl- and ethylmalonyl-CoA is determined. Phoslactomycin biosynthesis affords incorporation of two *cis*-bonds. Therefore we aim to characterize the dehydratase domains to know how their specificity has evolved. Our results will help in understanding polyketide synthases in more detail which will aid in the engineering and production of novel, bioactive compounds.

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### BTP115

#### Multispecies biofilm formation: Application of a novel cultivation device

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Biofilms are the main lifestyle of microorganisms and over the last decades coming more and more into the focus of scientific attention. Despite this, there is still a great complexity to analyze multispecies consortia. As matrix-enclosed communities biofilms containing a multitude of environmental niches. Under the aspect of co-culture-dependent enrichment of so far uncultured microorganisms we developed a microfluidic chip-system for the formation and analysis of biofilms. One of the core ideas of this new system is the fractionation of a natural three-dimensional biofilm into a two-dimensional structure to enable separated co-culture enrichment of previously uncultivable bacteria or archaea.

Within the fluidic chips we are able to generate biologically and physically induced gradients. The so formed biofilms are analyzed via newly developed robot assisted techniques like automated FISH, OCT analysis and an automatic  $\mu$ -accurate online sampling, which individually and in total allows a high spatiotemporal resolution of the cultured chips.

To provide validation through several experiments we analyzed for different inoculums of natural and synthetic coexisting species and their fractionation along the microfluidic flow. Beside separation via electron acceptor dependency and detoxification events we were also able to further isolate the first stable co-culture of an uncultivated member of the Thermoplasmatales and a nanoorganism belonging to the ARMAN (Archaeal Richmond Mine Acidophilic Nanoorganisms). Another area of application for the cultivation system is as a screening platform, which offers great potential for the search and optimization of novel strains for biotechnological applications.

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### BTP116

#### Towards Artificial Chloroplasts: The Design and Realisation of two Synthetic CO<sub>2</sub> Fixation Cycles

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Carbon dioxide (CO<sub>2</sub>) is an important carbon feedstock for a future green economy. To present a novel solution for the efficient conversion of CO<sub>2</sub> into multi-carbon compounds, we designed and realised two synthetic cycles that allow the continuous fixation of CO<sub>2</sub> *in vitro*: The Crotonyl-CoA-

Ethylmalonyl-CoA-4-Hydroxybutyryl-CoA (CETCH) cycle and the Hydroxypropionyl-CoA / Acrylyl-CoA (HOPAC) cycle.

Our synthetic pathways were drafted by metabolic retrosynthesis, are based on the efficient carboxylation reaction of enoyl-CoA carboxylase/reductase (ECR) and are more thermodynamically favourable than the Calvin-Benson-Bassham (CBB) cycle.

We demonstrated activity of both cycles by pooling enzymes, cofactors as well as NADPH and ATP regenerating systems *in vitro* and starting the reactions with propionyl-CoA and acetyl-CoA respectively. Samples were taken and measured by mass-spectrometry using C13 labeled bicarbonate to track the incorporation of CO<sub>2</sub> into glyoxlate, the primary fixation products of our synthetic pathways.

Here, we will present recent progress in improving the CETCH cycle with computer modelling and developing a robust variant of the HOPAC cycle. We will show the production of metabolically valuable compounds from the cycles' primary fixation products and present latest effort in developing our *in vitro* systems towards self-sustaining cycles with energy regeneration modules.

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### BTP117

#### Heterologous production and characterization of water dependent Steroid-C25-dehydrogenases: Mo-dependent key enzymes of anaerobic steroid catabolism

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Cholesterol C25 dehydrogenase (C25DH) belongs to the dimethyl sulfoxide reductase (DMSOR) family of molybdenum-containing enzymes and plays a key role in anaerobic cholesterol degradation (Warnke et al. 2017). The enzyme was so far only isolated from *Sterolibacterium denitrificans* Chol-1S and has an ( $\alpha\beta\gamma$ )-architecture (Dermer and Fuchs 2012). The  $\alpha$ -subunit contains the active site Mo-cofactor and one [4Fe-4S], the  $\beta$ -subunit contains three [4Fe-4S] and one [3Fe-4S] clusters and the  $\gamma$ -subunit harbors a heme *b* as cofactor. The enzyme catalyzes the stereospecific hydroxylation of cholesterol and cholecalciferol to a tertiary alcohol (Warnke et al. 2016). Hydroxylation of the latter results in the biotechnological valuable formation of calcidiol (25-OH vitamin D<sub>3</sub>), the circulating form of vitamin D<sub>3</sub> in the human body. In this work, we established a heterologous production system for C25DH in *T. aromatica*. By co-expression of the three structural genes together with a chaperone, we were able to heterologously produce four out of eight genetically encoded putative Steroid C25 dehydrogenases (S25DH) in *T. aromatica* in a soluble form. Kinetic analysis revealed that they were specific for different steroids with altered isoprenoid side-chain.

#### References:

[1] Dermer J, Fuchs G. 2012. *The Journal of biological chemistry* 287 (44), pp. 36905–36916.

[2] Warnke M, Jacoby C, Jung T, Agne M, Mergelsberg M, Starke R, Jehmlich N, von Bergen M, Richnow HH, Bröls T, Boll M. 2017. *Environmental microbiology* 19 (11), pp. 4684–4699.

[3] Warnke M, Jung T, Dermer J, Hipp K, Jehmlich N, Bergen M, Ferlino S, Fries A, Müller M, Boll M. 2016. *Angewandte Chemie (International ed. in English)* 55 (5), pp. 1881–1884.

## BTP118

### Bottlenecks in the whole-cell biotransformation process for the production of glass-forming ectoine derivatives

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**Introduction and objectives:** One of the most abundant osmolytes (compatible solutes) in nature is ectoine, a cyclic amino acid derivative. The formation of 5-hydroxyectoine is conducted by the ectoine hydroxylase EctD, a member of the non-heme-containing iron(II) and 2-oxoglutarate-dependent dioxygenases. Hydroxyectoine has the ability of glass formation, leading to high protein and cell stability which is responsible for desiccation survival. Heterologous overexpression of *ectD* of *Halomonas elongata* was shown to perform a whole-cell biotransformation process for the production of several hydroxylated cyclic amino acid derivatives with potentially useful glass-forming abilities. The aim of this project is to improve this biotransformation process.

**Results and conclusions:** We used *E. coli* BL21 as a whole cell biotransformation system, which expressed a pET-22b(+) plasmid-encoded ectoine hydroxylase *ectD* from *H. elongata*. It is known that EctD has a *K<sub>m</sub>* for both ectoine and oxo-glutarate in the mM range (5.7 mM and 4.8 mM, respectively). Whereas the intracellular concentration of ectoine is approximately 500 mM in *E. coli* BL21, the concentration of oxo-glutarate becomes critical. Oxo-glutarate levels in *E. coli* are usually below 1 mM, but are increased to approx. 4 mM under nitrogen-limiting conditions. We therefore applied a N-limited biotransformation process and investigated a number of potential limitations. From this it became apparent that N-limitation has an inhibitory effect on glucose uptake rate, reducing it to only 15 nmol / min x mg protein. We conclude that nitrogen-limiting conditions, required to increase cytoplasmic levels of oxo-glutarate, are at the same time a limiting factor for oxoglutarate-replacement.

## BTP119

### Extent of compatibility between stress tolerance and melanin production in dark septate endophytes (DSEs)

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Dark septate endophytes (DSEs) are a group of endophytic fungi that colonize the root tissues of host plant without causing disease symptoms. They are characterized by their morphology of melanized and septate hyphae. This group of root endophytes is a polyphyletic form-group of ascomycetes, and contains conidial as well as sterile fungi that colonize roots intracellularly or intercellularly. Our objectives in the current study are: (i) to study the responses of three model DSEs, *Periconia macrospinoso*, *Cadophora* sp. and *Leptodontidium* sp. to abiotic stress; salt and heat stresses, (ii) to identify the biosynthesis pathway for melanin production by using melanin inhibitors and (iii) to understand the relation between DSEs performance under abiotic stress and melanin biosynthesis.

Growth and morphology on different culture media subjected to heat and salt stresses suggested that these endophytes

have a high tolerance to abiotic stress. We found that 1,8-dihydroxynaphthalene (DHN) pathway was used for melanin biosynthesis in the current model DSEs and melanin content increased by the presence of salt stress. Based on different BLAST and alignments, genes which are speculated to be involved in DHN pathway were identified and their expressions were determined under salt and heat stress.

We conclude that DSEs used in this study can tolerate severe stress conditions but this tolerance might not necessarily be due to the presence of melanin in their hyphae. DSEs tolerance to abiotic stresses may serve to use them as a good biological tool in plant production systems increasing abiotic stress tolerance to different crops.

## BTP120

### Stereospecific reduction of substituted $\beta$ -keto-esters using whole cell catalysis and purified enzymes from yeasts

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**Introduction:** There is a need for stereospecific building blocks in pharmaceutical and fine chemicals syntheses. Several microorganisms (MO) and microbial enzymes have been shown to catalyze stereospecific reactions, which are difficult to achieve using chemical methods.

**Objectives:** The studied substrates in this work are substituted derivatives of ethyl acetoacetate, especially ethyl 2-phenylacetoacetate and ethyl 2-benzylacetoacetate (EB). First aim is to find suitable MO, which can be used for whole cell catalysis. The NADPH dependent reductase GRE2 from baker's yeast is able to reduce stereoselectively  $\beta$ -keto-esters. Therefore, second aim is the recombinant production of Gre2 from yeast and its homologues from other MO.

**Materials & methods:** Gre2 of baker's yeast and homologous genes were expressed in *E. coli* using pET-vectors. The enzymes were produced as fusion proteins with poly-His- and SUMO3-tags. For screening of enzymatic activities, decrease of NADPH absorbance was followed at 340 nm using 2,5-hexanedione as a substrate.

**Results:** The main proportion of fusion protein poly-His-SUMO3-GRE2 was obtained after homogenization in soluble form. The enzyme shows remarkable thermal stability; storage at 4°C for several weeks does not affect enzymatic activity. The same is true for several hours of incubation at 37°C in presence of dithiothreitol. Without further optimizations, GRE2 was able to convert the hydrophobic substrate EB in an aqueous setting.

**Conclusion:** GRE2 from yeast has a broad substrate spectrum including substituted derivatives of ethyl acetoacetate. However, the reaction conditions have to be optimized for a higher conversion rate of hydrophobic targets. The determination of yield, stereospecificity and enantiomeric excess is in progress.

## BTP121

### Increasing Mass Transfer In Syngas Fermentation: Effects Of Elevated Pressure

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Low productivities of bioprocesses using gaseous carbon and energy sources are usually caused by the low solubility of those gases (e.g. H<sub>2</sub> and CO). It has been suggested that increasing the partial pressure of those gases will result in higher dissolved concentrations and should therefore be helpful to overcome this obstacle. Investigations of the late 1980s with mixtures of hydrogen and carbon monoxide show inhibitory effects of CO partial pressures above 0.8 bar. We investigate growth and product formation of *Clostridium ljungdahlii* with carbon dioxide and hydrogen as sole carbon and energy sources, thus omitting inhibition by carbon monoxide. A 1.5 L glas bioreactor system is used to establish a baseline for growth at atmospheric conditions. Experiments at absolute process pressures of 1 bar, 4 bar and 7 bar are conducted in a high pressure batch stirred tank reactor (2.5 L) with constant amount of substance flow rate.

The product composition shifts from mainly acetic acid and ethanol to almost only formic acid at a total system pressure of 7 bar. By keeping the amount of substance flowrate constant instead of the volumetric gas feed rate when increasing the process pressure, the mass transfer coefficient decreases with increasing pressure. Nevertheless, with this strategy we increased the overall product yield of 7.5 times of what has been previously reported in literature with *Acetobacterium woodii*. After 90 h of cultivation at a total pressure of 7 bar a total of 4 g L<sup>-1</sup> of products is produced consisting of 82.7 % formic acid, 15.6 % acetic acid and 1.7 % ethanol.

The obtained results indicate that substrate gas partial pressures changed the product distribution of gas fermentation and enabled the biological conversion of H<sub>2</sub> and CO<sub>2</sub> to formate as main product.

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## BTP122

### Genetic engineering for optimisation of PHA production by *Pseudomonas putida* in nitrate containing media

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Pollution from non-recyclable petroleum-based plastic is a major problem of the global ecosystem. The great pacific garbage patch - the largest landfill in the world - consists of 60 - 80% plastic [1]. Since China has declared to ban its waste imports, the issue of how to deal with this problem has lately been raised. As an alternative to conventional plastics it is possible to use microbiologically synthesised biodegradable plastics, like polyhydroxyalkanoates (PHA). However, compared to petroleum-based plastics PHA production is still more expensive.

Therefore, we have been working on a more profitable process. As a natural PHA producer, *P. putida* KT2440 was used as a production strain. The intracellular accumulation occurs under nutrient-limited conditions. State-of-the-art processes depend on a depletion of nitrogen. Since this is not given in a lot of commonly used feedstock or promising

novel carbon sources like cyanobacterial sucrose, strains generating PHA in the presence of nitrate were created by genetic engineering. To achieve this, genes of the nitrate metabolism were knocked out using homologous recombination. The recombinant strains were then further investigated for growth behavior and PHA production. The advantage of these strains resides in the accumulation of PHA in otherwise nitrogen-unlimited nitrate media, paving the way for cultivation with potentially cheap nitrate containing carbon sources like cyanobacterial sucrose, as already conducted in previous studies [2].

In this study we show the production of PHA in the presence of nitrate under unlimited conditions using genetically modified *P. putida* strains.

#### References:

[1] Moore, C. J. (2008). *Environmental Research*, 108(2), 131–139.

[2] Löwe, Hannes, et al. (2017). *Biotechnology for biofuels* 10.1, 190.

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## BTP123

### P4SB – From Plastic waste to Plastic value using *Pseudomonas putida* Synthetic Biology

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Two hundred and seventy five million tons of plastic waste were produced in 2010 alone (Jambeck *et al.*, 2015), with Europe accounting for about 55 million tons/year. The environmental impact of these primarily fossil-based plastics has been broadly discussed. While the vast majority of these polymers are not biodegradable, their strength and light weight provide comparative advantages. Poly(ethylene terephthalate) (PET), for instance, has contributed significantly to reducing energy expenditure during transport, especially in the beverage industry. Due to its thermoplastic nature PET is also easy to recycle. However, recycled PET is of lower quality and current recycled PET products struggle to compete with virgin PET on price and quality, leading to an overall European recycling rate of less than 30%. Polyurethanes (PU), are used extensively in a wide range of applications including construction, transportation, furniture, and medicine. Since many PU types have a thermoset nature with covalent bonds, one of the main concerns for this product is the notable lack of end-of-life recycling (<5%). Hence, new ideas that give room for new incentives are required for the recycling of plastics.

We here propose a strategy that allows upcycling of plastic waste, by feeding degraded plastic as carbon source to microbial plastic producers (1). In detail, the enzymatic degradation of PET and the possibilities to produce PHA from the resulting molecules will be presented. In addition, synthetic biology possibilities to improve the bioplastic producing microbe, *Pseudomonas putida*, will be shown. Finally, the potential contributions to a more sustainable plastic industry will be discussed.

#### References:

[1] Wierckx et al. *Microb. Biotech.*, 2015, 8: 900–3

## BTP124

### Strategies for improvement of heterologous rhamnolipid production in *Pseudomonas putida*

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Rhamnolipids are the best characterized class of biosurfactants and feature a great potential for a wide variety of industrial applications. In contrast to conventional surfactants they possess a good biodegradability, biomedical compatibility and great stability against high pH-value, temperature and salt concentration. Moreover, they can be completely produced based on renewable resources, whereas classical surfactants are still produced on crude oil basis. To avoid disadvantages like pathogenicity and complex regulatory mechanisms in wild type rhamnolipid producers like *Pseudomonas aeruginosa*, we successfully established the heterologous rhamnolipid production in the non-pathogenic *Pseudomonas putida* KT2440 in the past.

Here the rhamnolipid production by *P. putida* was further optimized by the construction of a plasmid-based library of synthetic promoters. By screening the promoter library a set of 75 variants with various expression strengths was identified, resulting in a range from 22 to 334 mg/L of mono-rhamnolipids after expression of *rhlAB*.

The rhamnolipid synthesis was further increased by metabolic engineering, in which two strategies were carried out. First, relevant genes for the synthesis of educts for rhamnolipid production were coexpressed, what pointed out dTDP-L-rhamnose as the limiting factor. The second strategy comprised the construction of gene deletion mutants to abolish metabolic pathways in competition with the rhamnolipid synthesis, respectively providing more educts for rhamnolipid synthesis.

In the final step all applied strategies were combined to create a *P. putida* strain, which reached the highest rhamnolipid concentration of 882 mg/L, more than the *P. aeruginosa* PAO1 wild type under similar conditions.

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## BTP125

### Enhanced biotechnological production of antibacterial melleolides with *Armillaria ostoyae*

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Natural products are a tremendous source for drug discovery and development process, especially when it comes to antibiotics [1]. Usually low product yields hinder the development process [2]. Hence, methods for production of gram amounts of these molecules have to be established to ensure further development of promising compound classes.

In this study we report an optimized bioprocess for the production of antibacterial melleolides with a wild-type strain of *Armillaria ostoyae*. Maximum product titers in shake flask cultures reached 305 mg/L of melleolide B and 238 mg/L of melleolide E or 170 mg/L of melleolide C. Scale up was performed in 15 L stirred tank reactors with a working volume of 12 L and a fermentation process was established to produce melleolide B in gram scale.

The formation of side products could be prevented by adjustment of the pH level during the idiophase, which leads

to higher product titers of the main compounds and also eases downstream processing. Intensity of agitation, which causes hydromechanical stress, appeared to be the most critical parameter for product formation in stirred tank reactors.

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## BTP126

### Fine-tuned gene expression in *Pseudomonas putida* KT2440

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Industrial biotechnology is a rapidly growing area with the potential to replace chemical industry with economical and environmentally friendly processes [1]. Due to its metabolic versatility, the soil bacterium *Pseudomonas putida* KT2440 is used as an efficient cell factory for the production of high value products [2].

The fine-tuning of gene expression is an important method to improve the performance of the organism and to optimize the production [3]. Therefore, we constructed a synthetic promoter library of constitutive promoters for *P. putida* KT2440. We tested three different reporter systems for their suitability for the use in *P. putida* and established a simple and fast screening system. The red fluorescent protein MCherry allows the readout of the fluorescence at a high signal intensity unaffected by the organisms' high auto fluorescence. It also allows a stable monitoring of expression strength and a screening of the clones on agar plates by the colour of the colonies.

Our library consists of 20 synthetic promoters generated out of the homologous P<sub>gro</sub> and P<sub>tuf</sub> promoters as well as the heterologous P<sub>em7</sub> promoter by random mutagenesis. The promoters cover a broad range of expression levels from a 2.5-fold downregulation to a 25-fold upregulation. Thus, we are able to perform a precise gene regulation as a basis for the genetic enhancement of *P. putida*.

#### References:

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[2] Poblete-Castro I., Becker J., Dohnt K., Martins dos Santos V., Wittmann C. (2012) *Appl Microbiol Biotechnol* 93:2279-2290

[3] Hammer K., Mijakovic I., Jensen P.R. (2006) *Trends Biotechnol* 24(2):53-5

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## BTP127

### Enabling the valorization of guaiacol-based lignin: Integrated chemical and biochemical production of cis,cis-muconic acid using metabolically engineered *Amycolatopsis* sp. ATCC 39116

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Lignin is nature's second most abundant polymer and displays a largely unexploited renewable resource for value-added bio-production (Beckham et al., 2016). Novel lignin

valorization strategies provide a source of heterogeneous low-molecular-mass aromatics such as catechol, guaiacol, cresol and phenol to be utilized for bio-production. Here, we describe metabolic engineering of the actinomycete *Amycolatopsis* sp. ATCC 39116 for the conversion of guaiacol, catechol, phenol and o-cresol and true softwood lignin-hydrolysates into *cis,cis*-muconic acid (MA) (Barton et al., 2017). MA is an industrial chemical of recognized value and provides direct access to adipic acid and terephthalic acid, major building blocks of commercial plastics (Xie et al., 2014). From a metabolic viewpoint, MA is an intermediate of the  $\beta$ -ketoacid pathway, involved in aromatics catabolism.

We observed that *Amycolatopsis* sp. ATCC 39116 can utilize a broad spectrum of aromatics as the sole carbon source, such as catechol, guaiacol, phenol, toluene, *p*-coumarate, and benzoate. The microbe tolerated them in elevated amount and even preferred them over sugars. Subsequently, we developed a novel approach for genomic engineering of this highly challenging, GC-rich actinomycete. The successful introduction of conjugation and blue-white screening, using  $\beta$ -glucuronidase, enabled efficient tailored genomic modification within ten days. The successive deletion of two putative muconate cycloisomerases from the genome, successfully disrupted the  $\beta$ -ketoacid pathway downstream of MA and provided the mutant *Amycolatopsis* sp. ATCC 39116 MA-2, which accumulated 3.1 g L<sup>-1</sup> MA from guaiacol within 24 h, achieving a yield of 96%. The mutant was also capable to produce MA from a guaiacol-rich true lignin hydrolysate, obtained from pine through hydrothermal conversion. This provides an important proof-of-concept to successfully coupling chemical and biochemical process steps into a value chain from lignin polymers to industrial chemicals.

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## Poster Session 1

### Infection Biology (IBP)

16 April 2018 • 14:30–17:30

#### IBP283

##### The role of the Coenzyme A disulfide reductase Cdr in the defense against hypochlorite stress in the human pathogen *Staphylococcus aureus*

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**Introduction:** *Staphylococcus aureus* is an important human pathogen that causes life-threatening infectious diseases. The fast prevalence of methicillin-resistant *S. aureus* strains is a worldwide problem. An understanding of the adaptations to infection conditions can help to identify new drug targets. During infection, *S. aureus* has to cope with ROS and HOCl resulting in reversible thiol-oxidation of proteins [1].

**Objectives:** *S. aureus* utilizes the low molecular weight (LMW) thiol bacillithiol (BSH) which protects proteins against overoxidation and functions in redox-regulation under HOCl stress [1]. Apart from BSH, CoenzymeA (CoASH) is used as alternative LMW thiol that can be oxidised by ROS to form CoAS disulfides (CoAS<sub>2</sub>). *S. aureus* encodes for a CoAS<sub>2</sub> reductase (Cdr) that might be involved in reduction of CoAS<sub>2</sub>, but its exact role is unknown [2].

**Methods:** We constructed a *cdr* mutant to investigate the role of Cdr in the NaOCl defence using growth and survival assays as well as Brx-roGFP2 biosensor measurements.

**Results:** The *cdr* mutant was more sensitive to lethal NaOCl stress than the wild type. The S-bacillithiolation pattern was not affected by the *cdr* mutation in BSH-specific Western blots. Brx-roGFP2 biosensor measurements showed a significantly increased BSH redox potential in the *cdr* mutant compared to the wild type.

**Conclusion:** Our results indicate an impaired thiol-redox balance in the *cdr* mutant and suggest that the LMW thiol CoASH and Cdr play a role in the defence against NaOCl stress in *S. aureus*. Further studies will investigate the changes in the thiol-metabolome and redox proteome of the *cdr* mutant.

#### References:

[1] Chandrangu P et., al. Antioxid & Redox Signal, 2017

[2] delCardayr SB et., al. J. Biol. Chem, 1998

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#### IBP284

##### Real-time imaging of the mycothiol redox potential using the Mrx1-roGFP2 biosensor in *Corynebacterium glutamicum*

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<sup>2</sup>Center for Biotechnology (CeBiTec), Bielefeld, Germany

**Introduction:** Mycothiol serves as the major low molecular weight (LMW) thiol and functions in protein S-mycothiolation in *Corynebacteria* and *Mycobacteria*. The redox-regulation of S-mycothiolated proteins is controlled by mycoredoxin 1 (Mrx1) which acts in concert with MSH and the MSH disulfide reductase Mtr to reduce S-mycothiolated proteins.

**Objectives:** In *Mycobacterium tuberculosis*, a novel Mrx1-roGFP2 redox biosensor has been engineered for live-imaging of the MSH redox potential (EMSH) during infections.

**Materials & Methods:** Here we constructed a chromosomally integrated Mrx1-roGFP2 biosensor in *C. glutamicum* for dynamic measurements of the EMSH changes during the growth and in different mutant backgrounds with an impaired redox balance.

**Results:** The purified Mrx1-roGFP2 biosensor responds specifically to MSSM, but not to other LMW thiol disulfides *in vitro*. Treatment of *C. glutamicum* wild type cells with different oxidants lead to a fast Mrx1-roGFP2 biosensor response by 1.5 mM NaOCl stress while concentrations of 40 mM H<sub>2</sub>O<sub>2</sub> did not affect the MSH redox potential. This weak biosensor response to H<sub>2</sub>O<sub>2</sub> is in agreement with the high peroxide resistance of *C. glutamicum*. Monitoring the biosensor response along the growth curve confirmed a highly reduced EMSH during different growth phases in the wild type. In contrast, the probe was constitutively oxidized in the *mshA* and *mtr* mutants confirming an impaired redox balance in the absence of MSH and Mtr.

**Conclusion:** The stable integrated Mrx1-roGFP2 biosensor can be applied to quantify dynamic EMSH changes in *C. glutamicum* cells during the growth, in different mutants and under oxidative stress conditions.

## IBP285

### Impact of the BarA/UvrY two component system on fitness and competitiveness of *Escherichia coli* in urine

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<sup>1</sup>UKM / Institut für Hygiene, Microbial Genome Plasticity, Muenster, Germany

**Introduction:** The *E. coli* BarA/UvrY two component system (TCS) is known to be responsible for regulating many cellular processes including biofilm formation, virulence and carbon metabolism. At least some of this regulation is facilitated through the carbon storage regulatory (Csr) system, consisting of two proteins (CsrA, CsrD) and two sRNAs (CsrB, CsrC). Expression of these two sRNAs is directly affected by UvrY. We previously found that the *uvrY* gene that encodes a response regulator of the BarA/UvrY TCS is frequently mutated during growth in urine, which is known to be a carbon-limited medium.

**Objectives:** In order to confirm positive selection acting on *uvrY* during growth in urine, we (i) analyzed bacterial fitness and competitiveness in response to observed *uvrY* mutations, and (ii) we aimed at identifying metabolic pathways that could be involved in gaining of this advantage.

**Materials & Methods:** By cloning mutated *uvrY* alleles in an isogenic *E. coli* K-12 MG1655 background and conducting growth competition experiments we examined competitive fitness of *uvrY* mutants not only in urine, but also in other carbon-limited, carbon-rich or minimal media.

**Results & Conclusion:** We found that in all tested conditions, the *uvrY* deletion mutant as well as K-12 strains bearing positively evolved *uvrY* alleles from urine isolates, were performing better than the K-12 strain with a WT *uvrY* allele. This could be an indication of *uvrY* being under positive selection pressure. However, as the molecular mechanisms behind the observed phenotypes are still not determined, we are now aiming to examine the differences between these strains and the WT on the level of global transcription and translation.

## IBP286

### Biological Control of the date Palm tree borers, *Oryctes* spp. (Coleoptera: Scarabidae: Dynastinae)

M. Khalaf-Al-Juboori<sup>1</sup>

<sup>1</sup>Ministry of Science & Technology, Biological Control Dept., Baghdad, Iraq

The efficacies of the entomopathogenic nematodes (EPN), *Rhabdits blumi*, and the entomopathogenic fungi (EPF), *Beauveria bassiana* as a biocontrol agents against were determined date palm tree borers, *Oryctes* spp. (Coleoptera: Scarabidae: Dynastinae) in laboratory and field trials, during 2015 season. Laboratory results demonstrated that direct spray of 1000 infective juveniles (IJs) per ml of *R. blumi* on Arabian Rhinoceros Beetle, *Oryctes agamemnon arabicus* (ARB) larvae caused 71.67% and 15% mortality in the adults. While, treating the food source of the larvae (pieces of fresh tissue of the frond bases) with the same dose and period resulted in 48.33% mortality in larvae and 10% in the adults. Laboratory results also showed that using concentration 1×10<sup>9</sup> conidia/ml-1 of *B. bassiana* as direct spray of the ARB larvae, led to 66.7% and 60% as treatment of the food source. Field experiments results showed that injection of 50 ml per palm tree with a concentration of 1000 IJs/ml of *R. blumi* inflicted about 42% mortality in ARB larvae infested the tree. Meanwhile, injection 50 ml of 1×10<sup>9</sup> conidia/ml-1 of *B. bassiana* imposed 50% mortality in larvae.

Results of this investigation illustrate the possibility of using *R. blumi* and, *B. bassiana* as a biocontrol agents against palm borers in IPM programs.

**Keywords:** Entomopathogenic nematode, *Rhabdits blumi*, Entomopathogenic fungi, *Beauveria bassiana*, biocontrol, endophyte, Palm borers, *Oryctes* spp.

## IBP287

### Characterization of multiple secondary active transporters of the betaine/choline/carnitine transporter family in *A. baumannii*

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**Introduction:** The nosocomial pathogen *Acinetobacter baumannii* has become a major threat in healthcare institutions worldwide. This is due to the significant increase in antibiotic resistances and fostered by its extraordinary trait to cope with osmotic stress such as persisting for weeks on dry surfaces. Osmotic stress is characterized by loss of water which can be prevented by accumulation of compatible solutes. *A. baumannii* is equipped with an armada of genes encoding potential secondary active transporters of the betaine/choline/carnitine transporter (BCCT) family.

**Objectives:** We aimed to elucidate the function of the different BCCTs.

**Materials & methods:** Expression of BCCTs in *E. coli*, transport and mutant studies.

**Results:** Genome analyses of *A. baumannii* ATCC 19606 unravelled four potential BCCTs, BetT1, BetT2, BetT3 and BetTX and two additional BCCTs, BetTY and BetTZ, in strain AYE. Transport studies revealed that BetT1, BetT2 and BetTX are choline transporters, whereas BetT3, BetTY and BetTZ transport glycine betaine. BetT1, BetTX and BetTZ are osmo-independent. Competition experiments provide evidence that in addition BetTZ transports proline betaine, BetTY transports proline betaine and proline and BetTX transports carnitine. BetTX is essential for growth of *A. baumannii* with carnitine as sole carbon source.

**Conclusion:** Multiple osmo-dependent BCCTs mediate osmotic stress adaptation of *A. baumannii*. The osmo-independent BCCTs are suggested to play an important role in host adaptation.

## IBP289

### Transcriptional landscape of *Yersinia enterocolitica* O:8 and O:3

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Gastrointestinal diseases are cause of around 2.5 million deaths per year and therefore a problem concerning public health in all parts of the world. *Y. enterocolitica* is a major cause of gastrointestinal infections. In the human body, the bacteria cause symptoms associated with the gastrointestinal tract like diarrhea, enterocolitis or abdominal pain, but also reactive arthritis or mesenteric lymphadenitis.

Different isolates of *Y. enterocolitica* are divided into bioserotypes based on biochemical and immune reactive

properties. Diverse bioserotypes are able to cause clinical manifestations in humans. Most human infections in Europe are caused by bioserotype 4/O:3 (YeO:3). Bioserotyp 1B/O:8 (YeO:8) is less prevalent, although it shows a higher virulence in mice.

Aim of this study was to investigate differences in the transcriptional landscape of those two bioserotypes. It was shown previously that YeO:3 is better adapted to the porcine host and shows altered invasion and adhesion properties. The underlying mechanisms of this phenotype were further investigated. RNA-seq was performed in *Y. enterocolitica* 8081v, the most commonly used strain of YeO:8, and Y1, a clinical isolate that belongs to YeO:3. Samples of both strains were taken from *in vitro* cultures grown under different conditions either imitating environmental conditions or host body temperature and under different nutrient availabilities. We could create global maps of transcriptional start sites for *Y. enterocolitica*. Additionally, we were able to identify percentages of antisense transcripts at each condition as well as potential *trans*-encoded sRNAs. The comparative RNA-seq based approach allowed us to gain insights into the RNA expression pattern of each strain and differences between both strains.

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### IBP290

#### Identification of small peptides expressed under virulence-relevant conditions in the gastrointestinal pathogen *Yersinia pseudotuberculosis*

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A high number of gastrointestinal infections in human are caused by enteric pathogens like *Yersinia pseudotuberculosis*. *Y. pseudotuberculosis* infections lead to mild self-limiting gastrointestinal diseases commonly called yersiniosis.

The bacteria enter the body by contaminated food. After oral uptake, they travel through the gastrointestinal tract. In the ileum, *Yersinia* penetrates through the M cells of the epithelial cell layer into the Peyer's patches where they are confronted with the host immune system. To fight against the immune system, *Yersinia* synthesizes a special set of plasmid-encoded virulence factors including a type 3-secretion system (T3SS), effector proteins (*Yersinia* outer proteins; Yops) and the adhesin YadA as well as LcrF, the master regulator of virulence genes. Upon cell contact, *Yersinia* delivers the Yop effectors via the T3SS into the host immune cell. Inside the target cell, the Yops interfere with the host immune defense for example by preventing phagocytosis.

In a previous RNA-seq analysis, we demonstrated that *Yersinia* possesses more than 150 *trans*-encoded and antisense sRNAs (small RNAs) that were expressed under virulence-relevant conditions and during infection (Nuss *et al.*, 2015; Nuss *et al.*, 2017). Using a bioinformatics approach, manual screening and expression analysis of translational reporter fusions, we could identify and validate some of these small transcripts as potential dual-function sRNAs and/or small open reading frames (sORFs). In order to identify further sORFs, we are currently establishing the ribosome profiling (Ribo-Seq) approach to our system.

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### IBP291

#### Regulation of the major virulence activator LcrF by the *Yersinia pseudotuberculosis* carbon storage regulator system

M. Kusmierek<sup>\*1</sup>, J. Hoßmann<sup>1</sup>, R. Steinmann<sup>1</sup>, W. Opitz<sup>1</sup>, A. K. Heroven<sup>1</sup>, P. Dersch<sup>1</sup>

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The transcriptional regulator LcrF is the major activator of virulence genes required during the ongoing infection stage of *Yersinia*. These virulence factors comprise the *Yersinia* adhesin YadA and the Ysc-Yop T3SS with its secreted Yop effector proteins. Synthesis of LcrF is temperature-regulated on the transcriptional (YmoA regulator) and posttranscriptional (RNA thermometer) level.

Another environmental signal that further triggers *lcrF* expression is contact to eukaryotic cells. Moreover, previous data revealed that contact to host cells activates *lcrF* expression even at moderate temperatures. This fact indicates the presence of an unknown regulatory mechanism that enables upregulation of *lcrF* expression upon contact with the host cell in a temperature independent manner.

Recent data revealed that cell contact-dependent induction of *lcrF* expression requires CsrA. The global post-transcriptional RNA-binding protein CsrA was shown to repress *lcrF* transcription under non-inducing conditions. We were able to demonstrate that sequences in or close to the *yscW-lcrF* promoter region are necessary for CsrA to repress *lcrF* transcription. Furthermore, CsrA was found to be required for efficient *lcrF* translation under calcium depletion conditions (which mimic cell contact *in vitro*). Additionally, current experiments highlighted the crucial role of CsrA for the stabilization of the *lcrF* transcript under secretion conditions.

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### IBP292

#### Characterization of a novel ECF-type heme transporter in *Staphylococcus lugdunensis*

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Staphylococci are a major cause of healthcare associated infections. In general coagulase negative Staphylococci are described to be rather apathogenic but *S. lugdunensis* displays an elevated degree of virulence. During invasive disease iron is necessary for pathogen proliferation but very limited in the human body fluids. *S. lugdunensis* encodes iron-dependent surface determinants (*isd*). These gene products facilitate the acquisition of iron from human hemoglobin and heme during infection. Amongst the *isd* genes *S. lugdunensis* possesses an ABC transporter (Lha – *Lugdunensis* heme acquisition) from the energy coupling factor-type (ECF). This type of ABC transporters transport micronutrients and possess a substrate affinity in the nanomolar range. Heme as a substrate for these transporters was never described.

**Question:** We investigate the unique role of the Lha transporter of *S. lugdunensis* heme acquisition to overcome host derived iron limitation.

**Methods:** *S. lugdunensis* mutants deficient in various *isd* or *lha* genes were created. Phenotypic characterization in minimal media with defined iron sources were performed.

For biochemical analysis of the Lha transporter the substrate specific subunit (LhaS) was expressed and purified.

**Results:** Deletion of the of the *lha* genes resulted in a severe growth defect under iron limited conditions in the presence of heme as source of nutrient iron. Additionally, recombinant LhaS co-purified heme when cells were grown in heme containing environments suggesting heme binding capacity. Substrate affinity measurements are currently performed.

**Conclusion:** Our data suggests that Lha presents a novel heme specific ECF-type ABC transporter and thus a novel strategy of pathogens to overcome host induced iron limitation.

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### IBP293

#### Analysis of the RNA-based regulatory mechanisms controlling type III secretion systems – a crucial virulence system of bacterial pathogens

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The human pathogen *Yersinia pseudotuberculosis* evades the host immune response after translocation of the epithelial layer of the small intestine, which allows the pathogen to replicate efficiently in deeper tissues and cause disease.

Virulence regulation in *Yersinia* is strictly controlled and influenced by numerous environmental factors such as an increase in temperature (37°C) or attachment to host cells. This ensures an organized, precise expression of virulence factors necessary for infection establishment. Among those are proteins involved in the on-going infection stage such as the type III secretion system and its secreted Yop effector proteins that are encoded on the virulence plasmid pYV. Many of those are under the control of the plasmid-encoded transcriptional regulator LcrF, whose 5'-UTR undergoes a temperature-dependent opening of a thermo-loop leading to efficient translation of the *lcrF* mRNA.

One protein essential in this process is YopD, which together with YopB forms the translocation pore of the type III secretion system in the host cell membrane. Apart from this structural role, YopD was also shown to be involved in the regulation of LcrF and the global post-transcriptional regulator CsrA.

Interestingly, recent data suggests an interplay between different RNases and YopD. Among those are the endo- and exonucleases RNaseE and PNPase, which are part of a large multi-enzyme complex called degradosome. We currently investigate the role of different RNases and RNA metabolism in virulence properties such as the regulation of the type III secretion system in *Y. pseudotuberculosis*, and found that in particular RNaseE and PNPase affect LcrF synthesis in this pathogen.

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### IBP294

#### Molecular and functional analysis of the exotoxin CNF<sub>Y</sub> of *Yersinia pseudotuberculosis* YPIII during infection

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*Yersinia pseudotuberculosis* is a food-borne pathogen that causes gastroenteritis in humans. The *Y. pseudotuberculosis* strains YPIII and IP2666 of serotype O3 produce the cytotoxic necrotizing factor (CNF<sub>Y</sub>) toxin, a single-chain AB toxin that constitutively activates GTPases of the RhoA. In this study, we focused on the characterization of the functional domains of the CNF<sub>Y</sub> protein to elucidate its pathogenic mechanism during infection.

CNF<sub>Y</sub> deletions (CNF<sub>Y</sub>1-1014, 1-719, 1-526 and 1-443 amino acids) were constructed, deleting entire domains as predicted based on its functional homologue CNF<sub>1</sub> of *E. coli*. We found that only full length CNF<sub>Y</sub> (1-1014 aa) causes stress fiber formation and multinucleation in HEp-2 cells, which is due to the deamidation of RhoA at Gln63, whereas deletion of further C-terminal domains resulted in loss of catalytic activity. The results revealed amino acids 720-1014 as the catalytic domain of the CNF<sub>Y</sub> toxin and confirmed that the catalytically active amino acid is C866. Moreover, we followed the CNF<sub>Y</sub> protein delivery into the host cells during infection by using the reporter dye CCF4/AM and found that the deletion CNF<sub>Y</sub> 1-443aa cannot translocate into the host cell cytosol. Therefore, the region between amino acids 443-526 may be essential for the translocation of this toxin.

In summary, here we showed that CNF<sub>Y</sub> protein consists of different functional domains. The C-domain (720-1014aa) represents the catalytic part of the toxin and amino acid residues 443-526 are crucial for the toxin translocation. In further study, we will characterize binding domain and identify the host cell receptor involved in binding to CNF<sub>Y</sub>.

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### IBP295

#### Susceptibility of different respiratory epithelial cells to the cytotoxic effect of suilysin, the cytolysin of *Streptococcus suis*

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*Streptococcus suis* (*S. suis*) is a major cause of economic losses in the pig industry worldwide and an emerging zoonotic pathogen. *S. suis* can cause meningitis, septicaemia, pneumonia, endocarditis or arthritis. Suilysin (SLY) is a secreted toxin and one of the most important virulence-associated factors of *S. suis*. It belongs to the family of cholesterol-dependent cytolysins and can damage different cell types by lytic pore formation. Furthermore, it has been demonstrated to play an important role in the pathogenesis of *S. suis* infection and host-cell interaction *in vitro* and *in vivo*.

In the present study, we investigated the susceptibility of epithelial cells from different locations of the respiratory tract, either immortalized cell lines or cells of primary origin, to the cytotoxic effect of SLY.

We used the cell lines HEp-2 cells and NPT<sub>r</sub> cells, undifferentiated primary porcine tracheal (PTEC) and bronchial epithelial cells (PBEC), as well as differentiated PTEC and PBEC under air-liquid-interface (ALI) conditions. They were infected with a virulent *S. suis* 10 wild-type strain and a SLY-deficient mutant strain or were stimulated with the recombinant SLY (rSLY), respectively. Cytotoxicity was measured by LDH-release assay. Furthermore, we investigated the cell membrane-binding of rSLY using flow cytometry analysis and the cholesterol content of the

different cell types was determined by thin layer chromatography (TLC).

We observed a higher susceptibility of HEp-2 cells to the cytotoxic effects of SLY compared to NPT<sub>r</sub> as well as primary respiratory epithelial cells. Likewise, HEp-2 cells contained more cholesterol and showed the highest membrane-bound sulfolipin.

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#### IBP296

##### **PrsA2 of *Clostridium difficile* is an active parvulin-type PPIase that modulates virulence**

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*Clostridium difficile* is the main cause for nosocomial antibiotic associated diarrhea, and has become a major burden for the health care systems of industrial countries. Its main and extensively studied virulence factors are two large glycosylating toxins. In contrast, the contribution of other factors to disease development and progression are only insufficiently evaluated. Many bacterial PrsA-like peptidyl-prolyl-*cis/trans*-isomerases (PPIases) have been described in the context of virulence. Basing on this, we analyzed the PrsA2-homolog CD630\_35000 of *C. difficile* (CdPrsA2) enzymatically and phenotypically in order to assess its involvement *C. difficile* infection. For this, wild type CdPrsA2 and its several alanine-exchange mutants were recombinantly produced in *B. megaterium*. Recombinant CdPrsA2 had PPIase activity towards the substrate peptide Ala-Xaa-Pro-Phe with a preference for positively charged amino acids preceding the proline residue, and conserved residues in its enzymatic pocket were confirmed. Further on, a PrsA2 deficient mutant was generated in the *C. difficile* 630 $\Delta$ erm background using the ClosTron technology. Inactivation of *prsA2* resulted in a reduced germination rate in response to taurocholic acid, and in a slight increase in resistance to the secondary bile acids LCA and DCA. Furthermore, in the mutant the sporulation rate was not affected but the spores were more heat sensitive. Interestingly, in the absence of PrsA2 colonization of mice by *C. difficile* 630 was significantly reduced. Accordingly, here we show for the first time that CdPrsA2 is an active PPIase that acts as a virulence modulator by influencing crucial processes like sporulation, germination and bile acid resistance resulting in attenuated mice colonization.

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#### IBP297

##### **Transfer of Hematopoietic Stem Cells Improved Outcome of *P. aeruginosa* Lung Infection in a Cystic Fibrosis Mouse Model**

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Lungs of patients with cystic fibrosis (CF) are challenged by recurrent infections and chronic inflammation. In our current project, we focused on the question to what extent a *CFTR* based malfunction in professional phagocytes contributes to chronic lung infection and hyperinflammatory immune response. In transplantation experiments, we generated four classes of mouse chimeras (CF<sup>B6</sup>, CF<sup>CF</sup>, B6<sup>CF</sup> and B6<sup>B6</sup>) by intravenous transfusion of freshly isolated hematopoietic stem and precursor cells (HSPCs). We hereby were able to improve the genetically predisposed immune response of CF mice to airway infection with *P. aeruginosa*. Infection experiments revealed reduced lung bacterial numbers as well as increased survival in CF mice which were transplanted with wild type HSPCs (CF<sup>B6</sup>) compared to CF mice which received isogenic cells (CF<sup>CF</sup>). Cytokine analysis of macrophages harvested from infected lungs by bronchoalveolar lavage fluid (BALF) showed reduced levels of inflammatory cytokines in CF<sup>B6</sup> mice as well. Vice versa experiments in B6<sup>CF</sup> chimeras and B6<sup>B6</sup> controls did not display significant differences in the clinically apparent infection. However, looking at the cellular level, we detected different activation profiles of macrophages of B6<sup>CF</sup> and B6<sup>B6</sup> mice. Infection experiments showed an improved outcome in chimeric CF<sup>B6</sup> mice due to genotype conversion in the *Cftr* locus after HSPC transplantation. In contrast, *in vitro* phagocytosis assays with wild type and CF macrophages displayed no differential phagocytic activity. Albeit cellular mechanisms still have to be identified, the transfer of HSPCs significantly improved the immune response towards *P. aeruginosa* infection in CF mice and therefore may even be a therapeutic approach in treating lung infections in CF patients.

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#### IBP298

##### **Uptake, Survival and TNF Response of a *Mycobacterium abscessus* ssp. *abscessus* Rough Colony Type Isolate Obtained from a Cystic Fibrosis Patient in Murine Bone Marrow Derived Macrophages**

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**Question:** *Mycobacterium abscessus* ssp. *abscessus* (MAB) is a rapidly growing non-tuberculous mycobacterium causing skin, soft tissue and lung infections in animals and humans. MAB virulence has been linked to the morphological switch from the smooth (S) to a rough (R) colony type. It is still unclear why the R-type is more virulent than the S-type. R-type strains have been shown to multiply more rapidly in human monocytes and to induce hyper-proinflammatory responses. In the present study, uptake, survival and TNF response of a clinical cystic fibrosis R-type MAB isolate was investigated in murine bone marrow derived macrophages (BMDM) and compared to BMDM infected with a MAB-S strain and the non-pathogenic *M. smegmatis* (MSM).

**Methods:** Growth of MAB-R (clinical isolate) and MAB-S (DSM44196) were obtained as a single cell suspension by stirring culture and OD<sub>600nm</sub> was monitored. BMDM were infected at an MOI of 10 with logarithmically grown MAB strains or MSM (strain mc<sup>2</sup>). Uptake and survival of the MAB

strains were determined by plating. TNF expression was quantified by qRT-PCR and FACS.

**Results:** Compared to MAB-S, MAB-R exhibited retarded growth. At similar OD the bacterial cell numbers of MAB-R were considerably lower. MAB-R was phagocytosed by BMDM in a higher number than MAB-S and MSM, however, the ingested bacilli were mostly cleared within a few hours after infection. In contrast, MAB-S was able to survive and proliferate. MAB-R infection of BMDM strongly induced TNF expression whereas the TNF level in MAB-S infected cells was low.

**Conclusion:** Unlike other MAB R-type studies in human macrophages, the strong induction of TNF by the clinical MAB-R strain presented here was not associated with the intracellular multiplication in murine BMDM.

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### IBP299

#### Role of PGRS domain of *Mycobacterium tuberculosis* PE\_PGRS protein in ER-stress mediated apoptosis

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The genome of *Mycobacterium tuberculosis* (*M.tb*), the causal organism of Tuberculosis (TB), encodes unique protein family known as the PE/PPE family present exclusively in the genus mycobacterium, with unexplored functions. PE\_PGRS proteins interact in both ways during pathogenesis either by modulating host cell responses or by impacting the pathogen itself. PE\_PGRS5 was found to be highly expressed in lung tissues during a proteomics study. In a high-throughput mutant study, the enrichment of *M.bovis* BCG PE\_PGRS mutants (PE\_PGRS5, 28, 44, 59) in acidified phagosomes was observed; pointing to the role of PE\_PGRS proteins in the arrest of vacuole acidification thus maximizing the intracellular survival. The diverse functional roles of different PE\_PGRS proteins in *M.tb* infection thus points towards the importance of highly variable PGRS domain in the survivability of mycobacteria during macrophage infection. We describe the functional significance of PGRS domain of Rv0297, a member of this family. *In-silico* analyses revealed the presence of intrinsically disordered stretches and putative ER localization signals in the PGRS domain of Rv0297. The PGRS domain aids in ER localisation of Rv0297. The Rv0297 protein of *M.tb* leads to generation of ROS and NO and consequent apoptosis of host macrophages. These results implicate a hitherto unknown role of the PGRS domain of PE protein family in ER stress-mediated cell death.

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### IBP300

#### Quality control and standardization of diagnostic tools for enzootic bovine leucosis

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**Introduction:** Enzootic bovine leucosis (EBL) – is a chronic contagious disease of tumor origin, caused by the oncogenic virus. EBL is registered on all continents.

**Objectives:** For identify diseased animals OIE recommends provide in vivo diagnosis, based on the detection of

antibodies in AGIT and by ELISA. In order to do diagnostic work accurately it is very important to use diagnostic tools with proper quality, which requires the use of standards. Our goal was: work out the manufacturing technology of National Standard (NS) which are calibrated against International Standards.

**Materials & methods:** For manufacturing the NS we used the positive serum came from the naturally infected cattle and negative serum - from healthy cattle. For validation of NS we used panel of lyophilized samples of blood serums from the International Reference Laboratory and Reference Standard Antigens from companies «Pourquier» and «Synbiotics». Reactions were conducted according to the classic methods.

**Results:** Have been developed the technology of manufacturing and have prepared the Set of NS for EBL, that consists of freeze-dried positive (weak-positive in dilution 1:10 in AGID) and negative serums. Validation of NS was conducted in OIE Reference Laboratory for EBL, PIWet, Poland. The results of independent research of NS revealed their sensitivity and specificity. In order to verification the quality of commercial EBL kits we are always using the NS. This allows to accurately and objectively assessing the quality of diagnostic tools.

**Conclusions:** Developed and implemented NS for quality control of kits for detection of antibodies to the pathogen of EBL. The use of high quality diagnostic tools contributed to the fact, that epizootic situation in respect of EBL in Ukraine significantly improved.

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### IBP301

#### Oxidative stress in depolarized cells and its effects on persister formation in *Escherichia coli*

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Persister cells are phenotypic variants that are metabolically inactive and therefore highly drug-tolerant. Thus, persister cells contribute to survival under stressful conditions by a bet-hedging strategy. Bacteria are believed to enter a persistent state by the means of chromosomal toxin-antitoxin (TA) systems. Many toxins from these systems corrupt essential cellular functions to inhibit growth and induce persistence.

The *tisB/istR-1* TA locus is linked to persistence upon treatment with DNA-damaging agents [1]. The TisB toxin is a small hydrophobic protein that depolarizes the inner membrane [2,3]. Transcription of *tisB* is induced upon DNA damage, which increases the likelihood for individual cells to become depolarized and suffer from ATP depletion [4]. Here we present evidence to show that TisB-induced depolarization increases the levels of reactive oxygen species (ROS), and that both antioxidants and oxidative stress regulons support TisB-dependent persister formation.

We hypothesize that ROS formation plays a crucial yet Janus-faced role during the establishment of a persistent state: Firstly, being a major mediator passing on the message to eventually enter the persistent state. Secondly, ROS are likely one culprit responsible for the mortality rate during TisB-induced poisoning.

#### References:

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### IBP302

#### Host adaptation of *Streptococcus equi* subsp. *zooepidemicus*

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**Introduction:** *Streptococcus equi* is commonly isolated from bacterial infections in animals, particularly horses. Whereas *Streptococcus equi* subsp. *equi* is essentially confined to equids, causing strangles, *S. equi* subsp. *zooepidemicus* (SEZ) is found in a wide range of animals and in humans, where it colonizes mucosal surfaces but is also associated with a wide variety of diseases.

**Objectives:** The aim of the study was the phenotypic and genotypic comparison of SEZ isolates of equine and human origin to identify characteristics of zoonotic isolates.

**Materials & methods:** A collection of SEZ isolates was phenotypically plasma absorption assay with subsequent anti-fibrinogen and anti-fibronectin Western blot quantification of fibrinogen (Fg) binding by flow cytometry measurements and genotypically sequencing of the genes *szp* and *szm* (both encoding surface anchored proteins) characterized.

**Results:** Western blots of the plasma absorption assay showed a significant difference between SEZ isolates of equine and human origin regarding the binding of Fg. Furthermore, flow cytometry measurements using FITC-labelled equine and human Fg showed that human isolates bound higher amounts of human Fg than equine isolates. In contrast, no significant differences in the binding of equine Fg were observed. Sequence analysis of the *szm* revealed a clustering of most human isolates into two clusters. These isolates showed the strongest binding activity to human Fg.

**Conclusion:** Binding of human Fg is a common phenotype in human but much less so in equine isolates of SEZ. Furthermore, sequencing results indicated that only SEZ isolates with a particular *szm* are associated with infection of humans.

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### IBP303

#### Synthetic oligomers as inhibitors of *Pseudomonas aeruginosa* virulence factor lectin LecB

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*Pseudomonas aeruginosa* is an opportunistic pathogen causing severe nosocomial infections and life-threatening infections in immunocompromised persons often followed by high resistance towards antibiotics. *P. aeruginosa* produces an array of different virulence factors, including lectins LecA and LecB. LecB is a fucose-binding tetrameric protein related to the bacterial adhesion to host cells, biofilm formation and the disruption of epithelial barrier. Since lectins perform their function by sugar binding, a new therapeutic approach in progress, anti-adhesion therapy, is focusing on disarming these virulence factors by developing high-affinity glycomimetic ligands that interfere with natural ligand binding.

A first ligands were designed as glycooligo(amidoamines) functionalized with one to six  $\alpha$ -L-fucose units with additional variation of the spacing between fucose units along the oligomeric backbone. Oligomers were obtained by solid phase polymer synthesis through stepwise assembly of tailor-made building blocks and conjugation of the sugar ligands via copper(I)-catalyzed azide alkyne cycloaddition. The inhibition potency of glycooligo(amidoamines) was assessed by modified Enzyme-Linked Lectin Assay (mELLA) developed for quantitative analysis of LecB binding by the use of anti-LecB antibodies. Our results revealed three-valent fucose oligomers with two and three spacers as the most potent LecB competitive inhibitors, with respectively 57 and 60 times higher relative potencies to LecB natural ligand  $\alpha$ -L-fucose and respectively 32 and 33 higher relative potencies to  $\alpha$ -L-methyl-fucose. In agreement with mELLA results, these two oligomers showed slight inhibitory effect on biofilm formation in micromolar range in biofilm formation assay.

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### IBP304

#### A reference compendium of proteomic responses for classification of novel antibiotics against *Pseudomonas aeruginosa*

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Proteomic responses to antibiotics provide information on mechanism-related protein modifications and differential protein expression<sup>1</sup>. In this study a 2D-PAGE-based reference compendium of proteomic responses to clinically utilized antibiotics for *P. aeruginosa* was established that, in combination with global analysis tools, allows the rapid classification of novel compounds with regard to bacterial antibiotic response and antibiotic mode of action. *P. aeruginosa* PA01 was grown in minimal medium and stressed with sub-lethal doses of drugs. Newly synthesized proteins were labeled with L-[<sup>35</sup>S]-methionine, separated by 2D PAGE and visualized by autoradiography. Protein spot intensities were quantified and "marker proteins" (two-fold increase after antibiotic treatment) were identified by LC-MS/MS. Proteomic profiles of *P. aeruginosa* were compared mathematically based on marker protein induction factors using principal component analysis and CoPR (Comparison of Proteomic Responses) similarity scoring. This analysis revealed distinct sets of marker proteins for each antibiotic class that mirror the mode of action and enhance our understanding of the physiological stress response.

## References:

[1] Bandow *et al.* 2003 Antimicrob Agents Chemother 47(3):948-55

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### IBP305

#### Investigations of the repercussions of photodynamic inactivation on *Bacillus atrophaeus* under the influence of biological relevant ion

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As already predicted by Alexander Fleming, the misuse of antibiotic treatment caused antibiotic resistant microorganisms, like methicillin resistant *Staphylococcus aureus* (MRSA) which may harm people worldwide. As antibiotic treatment is not as effective as it used to be in the therapy of certain bacterial infections, it is inevitable to focus on other methods to treat these kinds of infections. One alternative method is the photodynamic inactivation of bacteria (PIB). PIB is based on positively charged dye molecules (called photosensitizer) that attach to bacterial cells. Absorbed energy, which is generated by exposing the photosensitizer to visible light, is transferred to molecular oxygen resulting in the production of singlet oxygen. This kills effectively cells via irreversible oxidation. In contrast to antibiotics for this treatment no development of resistances in microorganisms has been observed. Previous examinations with different photosensitizers showed that various ions have a negative effect on photodynamic inactivation, as the sensitivity of the bacteria is reduced and the photosensitizers cannot attach to the cells. To examine the effects of e.g. monovalent and divalent ions during PIB, the experiments were carried out with TMPyP as photosensitizer (a protophorphyrin derivate) and *Bacillus atrophaeus*, as a model of Gram-positive bacteria. Several ions were added to get insights into hindering or rather stimulating influences. We were able to show that higher concentrations of the ion dilutions had an inhibiting effect to TMPyP, which was reduced by diluting the ion concentration. Especially divalent ions and NaHCO<sub>3</sub> showed the most inhibiting effects. Their implications on possible applications in human medicine will be discussed.

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### IBP306

#### *In silico* analysis and heterologous expression of Clp proteins of *Chlamydia trachomatis*

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Bacterial Clp proteases are crucial for protein quality control and play important roles in the proteolysis of regulatory proteins thereby guiding developmental processes like competence, sporulation and differentiation [1]. In pathogenic bacteria, Clp proteases further modulate expression of virulence factors or host-bacteria interactions [2]. Therefore, Clp proteases have attracted considerable attention as antimicrobial targets in recent years. Here, we investigate the Clp protease of *Chlamydia trachomatis*, an obligate intracellular bacterial pathogen that is one of the most prevalent causes for sexually transmitted diseases in the US and blinding trachoma in developing countries [3]. Due to its obligate parasitic nature, *C. trachomatis* has evolved in a distinct manner adapted to exploit host cells which is reflected by its specialized life

cycle, and it may be assumed that Clp also plays a role in this process. *C. trachomatis* encodes two putative *clpP* genes, *ctclpP1* and *ctclpP2*, in two separate operons. In contrast, most bacteria contain only one *clpP* gene and in case of mycobacteria two copies are located in a bicistronic operon. *In silico* analyses revealed high sequence similarities of *ctclpP2* to *clpP* genes of *E. coli* or *B. subtilis* whereas *ctclpP1* showed significantly less similarity to previously described *clpP* genes including *ctclpP2*. Nonetheless, both ctClpPs possess conserved catalytic triads required for enzymatic activity. Biochemical analyses using purified ClpP proteins will help to elucidate the operating mode of these multi-component proteolytic machineries in chlamydia.

## References:

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[2] Frees, et al., Int J Med Microbiol, 2014. 304(2): p. 142-9  
[3] Peeling & Brunham, Emerg Infect Dis, 1996. 2, 307-19

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### IBP307

#### Chelocardin and Chlorotoniil A/B as novel therapeutics for the treatment of *Clostridioides difficile* infections

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**Introduction:** Chelocardin (atypical tetracycline) and chlorotoniil A and B (macrolides) are anti-infective substances that have regained attention during the last years. However, there is little knowledge on their exact mode-of-actions, their global impact on bacterial physiology and the systemic effects in patients.

**Objectives:** We will analyze the transcriptomic and proteomic response of *Clostridioides difficile* to chelocardin and chlorotoniil derivatives. Moreover, we will apply a meta-omics approach to analyze systemic effects of a chelocardin or chlorotoniil derivative on the composition and functional capacity of the gut microbiome of piglets.

**Material & methods:** Minimal inhibitory concentrations (MIC) were determined in *C. difficile* isolates by the broth dilution method in BHI medium (24 h, 37 °C). Shock experiments were performed in a synthetic medium (CDM) for selected strains and substances.

**Results:** Chlorotoniils exerted growth inhibition on *C. difficile* isolates from human and porcine origin at relatively low concentrations compared to the approved macrolide tylosin (0.1 - 6.4 µg/ml vs. 2 - >128 µg/ml). Of note, chlorotoniils were just as active against strains that showed significantly increased MIC values for tylosin. This leads to the hypothesis that no cross-resistance exists. Also MIC values of the chelocardins were low for *C. difficile* isolates (2 - 4 µg/ml).

**Conclusion & Outlook:** Chelocardins and chlorotoniils might be new treatment options for *C. difficile*. In ongoing experiments, we will analyze the effects of selected chelocardin and chlorotoniil derivatives on the transcriptome and proteome of *C. difficile in vitro* and we will investigate the impact of one derivative on the gut microbiome of piglets using multi-omics technologies.

## IBP308

### Functional characterization of a lipid anchored protein in *Staphylococcus aureus*

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*Staphylococcus aureus* is a human commensal bacterium whose lifestyle can become pathogenic, especially in immunocompromised patients, which can lead to skin infections, endocarditis, pneumonia etc. Nowadays the ability of *S. aureus* to form biofilms and the increasing incidence of antibiotic resistant variants cause serious problems not only in hospitals. Therefore, it is essential to understand the biology of this bacterium. However, the bacterium still expresses many proteins with unknown functions. Discovering the functions of these proteins might have the potential to find possible new targets that can be used for novel pharmaceutical strategies. In this study, we characterize the functionally unknown protein NWMN\_0364, which is lipid-anchored on the outside membrane and contains two PepSY domains that are assumed to have protease inhibitory functions. To estimate the potential function of this hypothetical protein, we generated a deletion mutant of the *S. aureus* Newman strain and analysed the extracellular proteins with a GeLC-MS/MS approach. With this method, we identified 149 proteins that are secreted or surface associated. Among them, the amount of 18 proteins was significantly induced in the mutant strain for at least 1.5 fold. Interestingly, murein hydrolases were found, which are important for the metabolism of the cell wall as well as virulence factors that are regulated by the two-component system SaeRS. Moreover, in the deletion mutant we found 3 *de novo* synthesized proteins, which are two substrate binding proteins of ABC transporters, important for Ni, Co and Zn uptake and the sortase, that connects proteins to the cell wall. Further analysis will clarify a possible role of NWMN\_0364 in cell wall metabolism and/or activation of the SaeRS regulatory system.

## IBP309

### Human Von Willebrand factor, a new adhesion-co factor of *Streptococcus pneumoniae*

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**Introduction:** *Streptococcus pneumoniae* is a main causative agent for community acquired pneumonia, associated with a high amount of cardiovascular complications such as major adverse cardiac events (1). These complications are associated with an imbalanced coagulation and are characterized by a severe, life threatening disease progress. In our studies we are

interested in *S. pneumoniae* interaction with human von Willebrand factor (VWF), mediating coagulation.

**Objectives:** Objectives of this study were focused on the functional characterization of VWF in pneumococcus interaction with the human endothelium and the identification of the enolase as VWF-binding protein.

**Materials and Methods:** Recruitment of VWF to the bacterial surface was determined by binding analyses with iodinated VWF, flow cytometry studies and in cell-culture infection assays. Dot spot-assays and western blot analysis were performed for molecular characterization of VWF-enolase interaction. Determination of binding kinetics were done by surface plasmon resonance and microscale thermophoresis. Also a microfluidic flow chamber model was established, using endothelia cells in order to simulate the situation in blood flow.

**Results:** *S. pneumoniae* is directly recruiting VWF on the surface via the surface exposed enolase and subverts this interaction for adhesion to the vascular endothelium even at high flow rates.

**Conclusion:** VWF-mediated endothelial cell adhesion offers a model for vascular colonization by pneumococci thereby promoting the hemostatic imbalance leading to severe cardiovascular complications.

## References:

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## IBP310

### Identifying genetic determinants related to antibiotic resistance in clinical isolates of *P. aeruginosa* by Transposon-Sequencing

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*Pseudomonas aeruginosa*'s ability to acquire new antimicrobial resistances during infection makes it a very challenging bacterium for current diagnostics and therapies. In a previous study, clinical isolates from different clinics were collected and analyzed concerning their antibiotic resistance determinants. Some resistance phenotypes could not be correlated to known markers. Beyond RNA sequencing analysis, ceftazidime resistance of the further analyzed clinical isolates could neither be explained by acquired enzymes, efflux pump overexpression nor intrinsic overexpression of *ampC*. To identify putative novel antibiotic resistance determinants, we use the high-throughput sequencing technology transposon sequencing (Tn-Seq).

Tn-Seq tracks changes in the composition of transposon mutant libraries grown under selection by ceftazidime at sub-MIC level. The Himar I C9 *Mariner* transposon mutant libraries were generated using selected ceftazidime resistant clinical isolates. Mapped reads resulting from sequencing of the transposons' flanking regions determined which of the transposon mutants are depleted after sub-MIC selection and lack an essential gene conferring antibiotic resistance.

Using RNA-Seq as well as standard screening methods, many key genetic determinants of antibiotic resistances were identified. Focusing on ceftazidime resistance Tn-Seq draws on and is used for identifying novel resistance related genetic

regions. We are the first group establishing Tn-Seq technology in clinical isolates.

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### IBP311

#### Transcriptional regulation of the c-di-GMP binding protein FlgZ and its role in the regulation of motility in *Pseudomonas aeruginosa*

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The second messenger c-di-GMP is an important bacterial signaling molecule mediating the regulation of motility versus sessility. High intracellular levels of c-di-GMP are associated with a sessile lifestyle within biofilms while low levels of c-di-GMP promote motility and virulence. The c-di-GMP signal is translated into a cellular response by c-di-GMP effectors. One prominent class of c-di-GMP effectors are PilZ domain containing proteins, which have been linked to the regulation of flagella-driven motility and exopolysaccharide production. In this study, we analyzed the transcriptional regulation of *flgZ* and the functional role of its gene product in *P. aeruginosa*.

*FlgZ* is the third gene within the *flgMNZ* operon. Global gene expression analysis indicated that transcription initiation of the *flgMNZ* genes is driven by the alternative sigma factors RpoF and RpoN. We identified an alternative transcriptional start site (TSS) that is located downstream of the annotated start codon of *FlgZ*. Confirmation of this alternative TSS by 5'RACE and analysis of the protein length lead to the re-annotation of the translational start site of the *FlgZ* protein. In a *flgZ* mutant we observed an improved swimming and swarming motility, while overexpression of *flgZ* showed a negative influence on these flagellar mediated types of motility. We generated a motile suppressor mutant, which did not show the inhibitory effect of *flgZ* overexpression. This mutant harbors a mutation in a gene whose gene product functions as a polar anchor. We are currently analyzing the involvement of the anchor protein in the coordination of the *FlgZ* mediated impact on motility. Our results help us to shed light on the complex regulation of motility versus sessility in the opportunistic pathogen *P. aeruginosa*.

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### IBP312

#### Human lung tissue explants, a powerful infection model for *Legionella pneumophila*

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Legionnaires' disease is a severe pneumonia caused by inhalation of *Legionella pneumophila* containing aerosols. We developed and applied a novel infection model for Legionnaires' disease comprising living human lung tissue explants (HLTEs). Human lung specimens were obtained from patients undergoing lobe- or pneumectomy because of lung cancer. To model molecular, cellular and histological events of *L. pneumophila* infection, tumor-free pulmonary tissue samples were incubated with reference strains, clinical isolates, mutants or purified bacterial virulence factors. Our results show that the HLTE model allows characterizing tissue damage, bacterial localization, dissemination and growth kinetics, and the host's molecular response. Moreover, it is suitable for diverse proteome and transcriptome-based analyses. We conclude that HLTEs with

their multitude of cell types and extracellular components are well suited for a comprehensive investigation of extra- and intracellular pathogenicity mechanisms of *L. pneumophila* at a unique level of complexity.

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### IBP313

#### Etiology of crown gall of rhododendron in Germany

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Crown gall disease occurs on various ornamental plants and may cause high economic losses in plantations and nurseries worldwide. Different *Rhizobiaceae* species carrying tumor-inducing (Ti) plasmid are recognized as causal agents of disease. Although crown gall of rhododendron (*Rhododendron* spp.) has been recorded before, little is known about the pathogen causing this disease. Therefore, the objective of this study was to characterize bacteria associated with crown gall disease of rhododendron.

Rhododendron plants showing crown gall symptoms were collected in commercial nursery located in northern Germany in 2017. Aerial tumors were observed on shoots, mostly at cutting injuries, as well as on leaf petioles. Bacteria were isolated from tumor tissue and representative strains were differentiated by ERIC-PCR. Phylogenetic analysis based on *recA* gene sequence suggested that isolated strains belong to the novel phylogenetic lineages within the genera *Agrobacterium* and *Rhizobium*. However, only one group of *Rhizobium* sp. strains induced tumors on inoculated sunflower and tomato plants. Pathogen was re-isolated from tumor tissue and re-identified by using ERIC-PCR. Tumorigenic strains recovered from rhododendron, as well as strains re-isolated from inoculated plants were positive in PCR targeting *virD2* gene of Ti plasmid. Preliminary analysis suggested that strains analyzed possess unusually large Ti plasmid, differing from those described so far.

The results obtained indicated that bacterial populations inhabiting tumors on rhododendron are genetically diverse. However, only a relatively genetically homogenous group of *Rhizobium* sp. strains was pathogenic. Additional investigations, including plasmid sequencing, are ongoing in order to further characterize the pathogen.

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### IBP314

#### Genetic diversity and prevalence of *Bartonella* species in small rodents and their ectoparasites in Lithuania

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The bacteria of the genus *Bartonella* are obligate parasites associated with mammals and transmitted by arthropods vector. The aim of the present study was to investigate the prevalence and genetic diversity of *Bartonella* spp. in small mammals and their ectoparasites from Lithuania. A total of 430 small mammals representing nine species were captured with live-traps and 151 fleas representing eight species were collected from rodents during 2013-2014. Multilocus sequences analyse of partial *gltA*, *rpoB*, *groEL* genes and *ITS* region were used for *Bartonella* spp.

identification. *Bartonella* DNA was detected in 23.7% of small rodents: in 33.2% of *Apodemus flavicollis*, 23.7% of *Micromys minutus*, 15.5% of *Myodes glareolus*, 12.5% of *Microtus oeconomus*, and in one specimen of *M. arvalis*. *Bartonella* was detected in *Megabothris walkeri*, *M. turbidus*, *Ctenophthalmus agyrtes*, *Ct. uncinatus* and *Hystrihopsylla talpae* fleas with overall prevalence 29.1%. Sequence analysis of *Bartonella* DNA from positive samples indicated the presence of *Bartonella grahamii*, *Bartonella taylorii* and *Bartonella* sp. belonging to the *Bartonella rochalimae* group. *B. grahamii* genotypes were detected in five small rodent species and three flea species, while *B. taylorii* genotypes in two rodent species and five flea species. *Bartonella* genotype belonging to the *B. rochalimae* group was detected in *M. glareolus* and *M. turbidus* fleas.

Keywords: *Bartonella*, rodents, ectoparasites, fleas, Lithuania

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### IBP315

#### How commensal staphylococci adapt to our immune system

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In Gram-positive bacteria, lipoproteins (Lpp) are major players in alerting our immune system. The question is whether commensal staphylococcal species differ in immune activation from a non-commensal species. Here we show that the TLR2 response of *S. aureus* and *S. epidermidis* is almost ten times lower than that of *S. carnosus*. The major reason for this is the different modification of the lipid moiety of the Lpp. In *S. aureus* and *S. epidermidis* the N-terminus of the lipid moiety was acylated with a long-chain fatty acid (C17), while in *S. carnosus* it was acylated with a short-chain fatty acid (C2). The long-chain N-acylated Lpp, recognized by TLR2-TLR1 receptors, silenced both innate and adaptive immune response, while the short-chain N-acetylated Lpp of *S. carnosus*, recognized by TLR2-TLR6 receptors, boosted it. Here, we unraveled a new mechanism of immune adaptation by commensal staphylococci based on long-chain fatty acid N-acylation of Lpp.

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### IBP316

#### Pathological changes observed in *Mycoplasma mycoides* infected caprine and bovine Precision-cut lung slices (PCLS)

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**Introduction:** Respiratory infections in ruminants, caused by *Mycoplasma* species, lead to considerable economic losses. Two closely related important pathogens are *Mycoplasma mycoides* subsp. *mycoides* (*Mmm*), the causative agent of contagious bovine pleuropneumonia (CBPP), and *Mycoplasma mycoides* subsp. *capri* (*Mmc*), which causes pneumonia, mastitis, arthritis and septicemia in goats.

PCLS are an organotypic tissue model, reflecting the microanatomy of the respiratory tract. Since they comprise widely varying cell types that may respond differently to the same stimulus, PCLS are better suited to mimic the natural host than cell lines.

**Objective:** Characterization of bacterial adhesion to host cells, host specificity, cell tropism and cytotoxicity.

**Materials & methods:** Lungs were obtained from healthy slaughtered adult cattle and goats. Accessory and cranial lobes were used for the preparation of PCLS. Slices were infected with freshly grown *Mycoplasma* strains for 4 h or 24 h. After removal of unbound bacteria, slices were further incubated and samples were preserved every 24h. Slices were investigated using H/E staining, immunohistochemistry and fluorescent microscopy. The infection burden was monitored by plating out of bacteria and qRT-PCR. Tissue samples from experimentally infected animals were provided by Dr. J. Jores.

**Results:** Infection of bovine and caprine PCLS with *Mmm* and *Mmc* revealed (i) a reduced adhesion of *Mmm* in caprine PCLS, (ii) the colonisation of the sub-bronchial tissue by *Mmc* and (iii) a destruction of the ciliated epithelium by *Mmc*.

**Conclusion:** The PCLS system is a suitable model to study host pathogen interaction in the tested *Mycoplasma* species.

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### IBP317

#### Surface coating with D-amino acids impairs biofilm formation of *Clostridium difficile*

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Biofilm formation is a natural process of bacteria in which microbial cells adhere to each other and surfaces within a self-produced matrix of extracellular polymeric substance. Biofilms protect bacteria against adverse environmental influences or chemical agents e.g. antibiotics. *Clostridium difficile*, the causative agent of irritable bowel diseases, is also able to form biofilms. Biofilm formation together with other factors like sporulation, antibiotic resistance, and adherence to epithelial cells or toxin production can play an important role in gut colonization and thus survival of this pathogen in this environment.

The aim of this study was to analyze biofilm formation by *C. difficile* isolates, which differ in virulence. Furthermore, the influence of D-amino acids to the adhesion on surfaces was determined *in vitro*. Overnight grown cultures of *C. difficile* 630 and VPI 10463 strains were transferred to uncoated or with D-amino acids coated 24-well plates and cultured up to 72 h. The biofilm formation was quantified by crystal violet staining and visualized by fluorescence microscopy after live-dead staining.

Both strains exhibited similar biofilm formation after 24 h. However, after 72 h the high virulent strain VPI 10463 showed a significantly reduced biofilm. In general, coating of the well surfaces with D-amino acids had a negative effect on biofilm formation of *C. difficile*. From all D-amino acids analyzed, D-phenylalanine most effectively impaired biofilm formation of strain 630 but not of strain VPI 10463. We conclude that under the conditions tested, D-amino acids are able to inhibit biofilm formation of *C. difficile* but more effectively in the low virulent strain.

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### IBP318

#### Proteomic investigation of the pneumococcal kinase and phosphatase couple StkP/PhpP

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Due to the worldwide rise of resistance to antibiotics in pneumococci the understanding of its physiology is of increasing importance. In this content, the analysis of the pneumococcal proteome is helpful as data about protein abundance in *Streptococcus pneumoniae* may provide an extensive source of information to facilitate the development of new vaccines and drug treatments.

It is known, that protein phosphorylation on serine, threonine and tyrosine residues is a major regulatory post-translational modification in pathogenic bacteria. Hence, it is of particular interest to gather precise qualitative and quantitative information about the phosphoproteome of pneumococci.

In our study, the unencapsulated D39 strain, a kinase ( $\Delta$ stkP) and phosphatase ( $\Delta$ phpP) mutant were analysed in a label-free global proteome quantification experiment. Additionally, the phosphoproteome was investigated using SILAC followed by enrichment of phosphorylated peptides with titan dioxide.

The morphological characterisation of all strains by electron microscopy revealed abnormal cell division and cell separation in both mutants. Label-free data point out several protein groups with contrary regulation in  $\Delta$ phpP and  $\Delta$ stkP: for example, proteins involved in cell division, peptidoglycan biosynthesis, DNA replication and pyrimidine metabolism are higher abundant in  $\Delta$ phpP but lower abundant or not changed in  $\Delta$ stkP. Proteins belonging to fatty acid biosynthesis are regulated oppositely. These results already support the assumption that the PhpP and StkP are forming a functional signalling couple.

Commonly identified phosphorylated proteins include cell division proteins FtsZ and DivIVA. Furthermore, phosphorylation sites in some ABC-transporters and cation transporters were identified so far.

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### IBP319

#### Zebrafish models for early *in vivo* evaluation of anti-infective natural products

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The zebrafish (*Danio rerio*) is a known ornamental fish for home aquaria that has emerged as a powerful model organism for *in vivo* research of human diseases. Approximately 70 % of human proteins have a functional orthologue in zebrafish. Easy and cheap maintenance, high fecundity of parent animals, rapid egg development, and its transparency bring significant advantages for the usage of this organism.

The aim of this project is to characterize early-stage compounds that were discovered in our continuous efforts of isolating novel anti-infective compounds from microbial sources (mainly myxobacteria) in zebrafish *in vivo* models. Using zebrafish as a tool will allow us to rapidly identify potential candidate molecules already at an early stage of the discovery pipeline. Special emphasis is given to toxicity models and to infection models with the pathogen *Staphylococcus aureus* to assess *in vivo* efficacy of novel antibacterial compounds. Such models are of particular interest for qualifying early lead structures within a program on the chemical optimization of chlorotoniols, potent macrolide antibiotics that inhibit *S. aureus* and other pathogens in the low nanomolar range. First results of the *in vivo* profiling of chlorotoniols and some other natural products are presented.

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### IBP320

#### Sigma proteins X and Y of *Mycobacterium indicus pranii* alter host immune responses: A moonlighting function

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In spite of availability of BCG vaccine, TB caused by *Mycobacterium tuberculosis* is still a matter of concern due to limited knowledge about its virulence determinants and regulatory mechanisms which controls mycobacterial gene expression. An in-depth study of protein families involved in regulation of mycobacterial gene expression can lead to proper control of the pathogen. Sigma family, an important player of transcription initiation process, and are suspected to have a role in regulatory mechanism of *Mycobacterium*.

*Mycobacterium indicus pranii* (MIP), a non-pathogenic mycobacterial species is a strong immune modulator as evident from its ability to modulate cell mediated immune responses in *M. leprae* patients. It also shares antigens with *M. tuberculosis* (*M.tb*). A MIP based anti-leprosy vaccine, trade marked as "Immuvac", is already in use and clinical trials for its suitability as an anti-TB vaccine are underway.

Present study suggests the moonlighting functions of MIP sigma proteins in immune modulation. Our finding suggests that protein X is good B-cell and T-cell antigen in mice model. It generates significant level of antibodies against itself in immunized mice. Also, there is significant increase in the number of multi-functional CD4+ cells double positive for IFN-g and TNF- $\alpha$  in restimulated splenocytes from immunized mice. Protein Y also shows some T-cell antigenic immunogenic properties by inducing TNF- $\alpha$  and IFN-g significantly but the B-cell response was weak. We measured the secreted levels of different cytokines in supernatant of re-stimulated splenocytes and found that protein X induces the level of IFN-g, TNF- $\alpha$ , IL-6 in dose dependent manner.

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### IBP321

#### Artificial increment of intracellular NADH confers ciprofloxacin resistance and decreases the formation of reactive oxygen species (ROS) in *Pseudomonas aeruginosa*

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**Introduction:** The metabolic plasticity displayed by *Pseudomonas aeruginosa* endows this pathogen with several mechanisms required to surpass bactericidal treatments. However, the killing mode of antimicrobials and the mechanisms underlying its resistance in this bacterium are yet not fully understood. Recent theories suggest a common mechanism of antimicrobial killing by inducing the hyperoxidation of NADH in the respiratory chain, leading to the formation of ROS.

**Objective:** To manipulate the intracellular concentrations of NADH/NAD<sup>+</sup> to study this effect in the context of antibiotic killing and antibiotic resistance in *P. aeruginosa*.

**Methods:** The coding sequence of the NADH oxidase from *Streptococcus pneumoniae* (Nox) and the NADH-producing formate dehydrogenase from *Candida boidinii* (FDH1) were overexpressed into *P. aeruginosa* PA14. Both enzymes were fully functional in this bacterium.

**Results and conclusions:** A first antibiotic screening showed that the *FDH1*-overexpressing strain resisted higher concentrations of ciprofloxacin (CIP). That was, however, not the case for the *nox* strain. Transcriptional profiles of both overexpressing bacteria showed antagonistic effects. The oxidation of NADH by *nox* induced the overexpression of energy scavenging traits, general stress factors, chemotaxis and virulence. The synthesis of NADH by *FDH1* caused opposite effects, down-regulating genes related to energy production, general stress, chemotaxis and virulence. Surprisingly, the genes related to the detoxification of ROS were also downregulated, suggesting that the rise in NADH levels does not necessarily increase ROS formation. Moreover, overexpression of *FDH1* caused the up-regulation of antibiotic resistance genes, which could help to explain the CIP resistant phenotype.

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### IBP323

#### Proteome analysis of *Streptococcus suis* under stress conditions and in host-pathogen interaction

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**Introduction:** *Streptococcus suis* is a Gram-positive bacterium which causes serious infections in swine, such as meningitis, septicemia, endocarditis and pneumonia. As an emerging zoonotic agent *S. suis* is able to induce meningitis and sepsis as well as toxic shock-like syndrome in humans. At present, distinct 35 serotypes are identified, based on their capsular polysaccharides [1]. Serotype 2 and 9 are the most prevalent serotypes in European countries, followed by serotype 7, 8 and 3 [2]. The mechanisms involved in the pathogenesis and virulence are only partially resolved. A better understanding of this system is needed to improve knowledge regarding potential virulence factors.

**Objectives:** By analyzing and comparing the proteomes of different *S. suis* serotypes cultivated under various physiological conditions, virulence factors can be identified. They may constitute potential components of new multi-component vaccines.

**Materials & methods:** We analyzed cellular and cell wall proteomes at different stages of serotype 2, 9 and 7 comparing the growth in nutrient-rich medium and cerebrospinal fluid (CSF) of clinically healthy swine using Q Exactive™ Plus and data-independent acquisition (DIA) mass spectrometry workflow.

**Results:** The proteome analysis rely on a DIA approach using an in-house spectral library. Preliminary mass spectrometric data reveal roughly 60 proteins as more abundant in CSF than in nutrient-rich medium.

**Conclusion:** Our experiments demonstrate differentially expressed proteins in CSF, when compared to THB media.

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### IBP324

#### Novel digital image analysis for automated bacterial cell and colony counting

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**Introduction:** The adhesion of bacteria is a widespread phenomenon and a crucial step in colonization of host organisms. Bacterial adhesion to organic material like cell lines and heart valves or non-organic material like prostheses causes high risk for chronic infections. In order to determine the presence of bacterial cells adhered to different materials, the analysis of attached bacteria is of high interest.

Particularly, the manual cell count within bacterial colonies is difficult to ascertain.

**Objectives:** Using automated digital image analysis, the aim was to develop a tool to recognize and count bacterial cells stained with fluorescence *in situ* hybridization (FISH) adhered to cell lines. We intended to apply different algorithms enabling the determination of cells within diverse sizes of bacterial colonies.

**Material & Methods:** We enhanced our VideoScan technology, which is based on fully automated fluorescence microscopy. Using specific mathematical algorithms, we developed a novel tool for the detailed analysis of bacterial cells adherent to mammalian cells e.g., FindMaxima for the detection of FISH-stained bacteria, basic focussing, a stitching strategy and a specific watershed transformation for cell separation.

**Results:** With the novel tool, we were able to detect and count FISH-stained bacteria and to determine the amount of bacterial cells within bacterial colonies. Thereby, *Escherichia coli* were incubated on the human epithelial cell line HEp-2. Adherent bacteria were stained with FISH protocols using FISH probe EUB338 conjugated with a red fluorophore.

**Conclusion:** Automated digital image analysis of bacterial cells and colonies is a promising alternative for time-consuming manual methods of analysing adhesion of bacteria to host cells.

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### IBP325

#### Survival and exit from dormancy in *M. tuberculosis*

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Currently, *M. tuberculosis* is the deadliest pathogen. One of the reasons is its increased ability to persist in a non-replicating, metabolically quiescent and drug tolerant state that nonetheless allows it to regrow and potentially trigger active tuberculosis. In order to understand which bacterial factors are crucial for latency we worked with an *in vitro* carbon-starvation model that renders *M. tuberculosis* antibiotic tolerant and resembles the non-replicating or latent state *in vivo*. We performed high throughput genomic screens and transposon insertion sequencing using a library that covers the entirety of the non-essential genome. Among the most interesting hits was *rv1363c* an Mce associated protein that is conserved among many Mycobacterial species and most likely plays a role in fatty acid metabolism. A deletion of the gene decreases fitness during carbon-starvation, though its over-expression enhances survival dramatically. In addition, *rv1363c* over-expressers react much faster and more uniformly to favorable conditions after starvation, lacking the lag phase observed in wildtype cells. A combination of Transcriptomics and Metabolomics suggests that *rv1363c* links fatty acid metabolism with sigma factor cascades and leads thus to global changes within the bacterial cell. *Rv1363c* over-expressers also show higher tolerance as well as slower kill kinetics to rifampin during carbon-starvation. We believe our work helps to improve our understanding of regulatory processes in dormant *M. tuberculosis* cells and might even have the potential to shift drug tolerance during dormancy.

### IBP326

#### Staphylococcal cyclic dipeptides: a tale of pathogen and host

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Cyclic dipeptides are a heterogeneous class of molecules produced by bacteria, fungi and humans. They exhibit a variety of functions, ranging from antiviral, antibacterial or antifungal functions to anti-tumor or inter-species communication signaling molecules. Most cyclic dipeptides belong to the class of 2, 5-diketopiperazines and have been described as a class of Quorum Sensing molecules in bacteria with potential role in inter-kingdom signaling.

*Staphylococcus aureus*, a prominent human pathogen as well as a human colonizer, produces three cyclic dipeptides: phevalin, tyrvalin and leuvalin, also known as aureusimines. Aureusimines belong to the class of monoketopiperazines and are synthesized by the non-ribosomal peptide synthase AusA which needs priming by the phosphopantetheinyl transferase AusB for activation. While the role of aureusimines in staphylococcal virulence is still unclear, they have been shown to exert effects on different types of host cells, both professional and non-professional phagocytes. Moreover, staphylococcal mutants lacking AusA or AusB show differences upon interaction with the host.

Here we further characterize mutants lacking AusA and AusB. We discuss potential new functions for the cyclic dipeptide phevalin for host-pathogen interplay as well as inter-species communication. Additionally, we dissect the role nutrient availability has on aureusimine production and we examine the transcriptional regulation of the *ausAB* operon.

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### IBP327

#### Chemotaxis in *Clostridioides difficile*

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*Clostridioides difficile* is an increasing problem in modern healthcare environments, where it is a major cause of hospital-associated diarrhoea and membranous colitis. The ability of *C. difficile* to navigate its way through the mucus rich environment of the lower intestine necessitates the ability for motility. This in turn requires a form of taxis for directed movement. *C. difficile* possesses a single predicted methyl-accepting chemotaxis protein (MCP), which in other organisms acts simultaneously as the sensory unit and trans-membrane signal transducer. It is the central receptor that allows directed movement. Here, we try to elucidate which exact chemical stimuli can be sensed by *C. difficile* in general, and its MCP in particular. Since chemotaxis has not been described for *C. difficile* before, we started to establish a chemical in plug assay that allows a fast and broad screening of various putative chemoattractants. From a collection of 20 amino acids, we found positive reactions for

alanine, cysteine, isoleucine, and proline. In addition, fructose and glucose could repeatedly be demonstrated to cause attraction in various *C. difficile* clades. However, the chemical in plug assay is known to be prone to false positive results [Li et al., 2010]. To verify our putative chemoattractants, we are in the process of designing a quantitative assay based on capillaries, and adapt it for our anaerobic organism. Moreover, the specificity of identified ligands for MCP binding will be confirmed with the aid of a MCP deletion mutant that we recently generated. As motility and toxin production are closely linked in *C. difficile*, shining light on how directed movement is facilitated is an important step in increasing our understanding of *C. difficile* infections.

#### References:

[1] Li et al.: The chemical-in-plug bacterial chemotaxis assay is prone to false positive responses. BMC research notes, 2010

#### Acknowledgment:

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#### IBP446

##### The role of posttranscriptional control mechanisms during the infection of *Ustilago maydis* using a KH domain protein as an example

S. Sankaranarayanan\*<sup>1</sup>

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Posttranscriptional regulation at the level of RNA biology is very important for pathogenicity of eukaryotic microorganisms. Fungi secrete, for example, small RNAs to interfere with the immune response of the host. Moreover, in *Candida albicans* as well as in *U. maydis* the transport of mRNAs along the cytoskeleton is important for infection. In the later example local translation on endosomes is needed for the correct formation of infectious hyphae. These secrete a number of effector proteins during infection that are needed to orchestrate pathogenicity.

Key factors are RNA-binding proteins (RBPs) that regulate defined steps of mRNA expression, like their stability and translation. Thereby RBPs determine spatio-temporal gene expression. At present a number of RBPs are known in pathogenic fungi but the target mRNAs and the underlying mechanisms of regulation are not well understood. Loss of the multi-KH domain protein Khd4, for example, results in loss of pathogenicity. In the last years intensive progress has been made with next generation sequencing techniques for transcriptome wide views. E.g. ribosome profiling allows to determine when and how efficient mRNAs are translated. Aim of this project is to use these new methods to identify the function of RBPs like Khd4 during pathogenic development.

## Mini Symposium Cybergenetics – At the interface between living and non-living regulatory systems (FG RSV)

16 April 2018 • 17:30–19:30

#### RSV-FG01

##### Communicating with and controlling biology via biofabrication, synthetic biology, and microelectronics

W. E. Bentley\*<sup>1</sup>

<sup>1</sup>University of Maryland, College Park, MD, United States

We are developing tools of "biofabrication" that enable facile assembly of biological components within devices, including microelectronic devices, that preserve their native biological function. By recognizing that biological redox active molecules are a biological equivalent of an electron-carrying wire, we have developed biological surrogates for electronic devices, including a biological redox capacitor that enable bi-directional "electron" flow. We have also turned to synthetic biology to provide a means to sample, interpret and report on biological information contained in molecular communications circuitry. Finally, we have developed synthetic genetic circuits that enable electronic actuation of gene expression. That is, using simple reconstructions, one can apply voltage on an electrode and directly actuate genetic responses and associated phenotypes. This presentation will introduce the concepts of molecular communication that are enabled by integrating relatively simple concepts in synthetic biology with biofabrication. Our presentation will show how engineered cells represent a versatile means for mediating the molecular "signatures" commonly found in complex environments, or in other words, they are conveyors of molecular communication.

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#### RSV-FG02

##### FluidFM as emerging tool for force-controlled single-cell manipulation and force measurement

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There is increasing interest to manipulate and analyze single cells, e.g. to uncover cell-cell interactions, investigate single cell behavior in complex consortia, or quantify single-cell adhesion forces. Fluidic force microscopy (FluidFM) addresses the demand for novel single-cell research, by combining atomic force microscopy with microfluidics via microchanneled cantilevers.

Pressure application through the microchannels allows controlled liquid release as well as aspiration, whereas force spectroscopy allows monitoring of the occurring forces. Real time feedback of cell indentation and membrane perforation enables the non-destructive insertion of the probe tip inside a cell. In this manner, soluble molecules can be injected into single cells, as well as quantitatively extracted from cells while preserving viability and physiological context.

By aspiration, single cells can also be immobilized reversibly on the cantilever and be used for precise measurements of adhesion forces. However, the characterization of adhesion forces of single bacteria in their natural environment remains challenging, due to the small forces involved as well as demanding surface topographies. We demonstrate a combination of FluidFM with a modular system of

functionalized colloids to quantify otherwise inaccessible adhesion characteristics.

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### RSV-FG03

#### Design and Engineering of Cybergenetic Systems

M. Khammah\*<sup>1,2</sup>

<sup>1</sup>ETH Zurich, Biosystems Science and Engineering, Basel, Switzerland

<sup>2</sup>ETH Zurich, Basel, Switzerland

Among the possible applications enabled by synthetic biology is the design and engineering of feedback control systems that steer the dynamic behavior of living cells in real time. Such controllers can be implemented on a computer and interfaced with living cells especially engineered to sense control inputs and respond to them. Alternatively, the control systems may themselves be genetically engineered into living cells as networks of biomolecules that achieve feedback function when interfaced with endogenous networks. We refer to the set of methods to design and build such control systems and the resulting technology as *Cybergenetics*—a genetics era realization of Norbert Wiener's cybernetics vision. In this talk, we present our ideas on the design and synthesis of cybergenetic control systems and discuss the main theoretical and practical challenges in their design and implementation. We also explain the potential impact such cybergenetic systems can have on industrial biotechnology and medical therapy.

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### RSV-FG04

#### Interrogating cellular dynamics with computer aided dynamics in individual bacteria

C. Guet\*<sup>1</sup>

<sup>1</sup>Institute of Science and Technology Austria (IST Austria), Vienna, Austria

In order to understand biological processes at a fundamental level we need to be able to perturb in a controlled fashion and in real time individual biological processes at the level of the single cell. It is only then that we dream of actually engineering biology in meaningful and safe ways. We developed a platform that allows parallel and real-time interfacing of intra-cellular generated signals with a computer that in turn feeds back control signals to individual growing bacteria.

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## Mini Symposium

### Systems biology approaches in environmental microbiology – Dealing with complex communities (FG EMV)

16 April 2018 • 17:30–19:30

#### EMV-FG01

##### Mathematical tools for analysing systems of Ordinary Differential Equations

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Systems of Ordinary Differential Equations (ODEs) are standard models of complex biological systems. Typically, these systems are high dimensional, have many unknown parameters and cannot be solved explicitly except in trivial cases.

In this talk I will discuss mathematical methods to analyze a system of ODEs with respect to properties of biological relevance - such as persistence (non-extinction), switching behavior and multistationarity - without having to fix parameters or perform numerical analysis. The methods draw on many areas of mathematics, such as graph theory, dynamical systems theory and algebraic geometry.

Biological applications often require surveying different mathematical models or proposing models with prescribed qualitative features that might be built experimentally. Simple analysis, ideally algorithmic, is therefore essential for usability. I will give examples of methods and applications to models of real biological systems, such as gene transcription and cell signaling. In many cases, the analysis reveals insight that might be interpreted biologically.

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#### EMV-FG02

##### Strategies for Modeling of Large-Scale Metabolic Models of Microbial Communities

S. Koch\*<sup>1</sup>, D. Benndorf<sup>2</sup>, F. Kohrs<sup>2</sup>, P. Lahmann<sup>2</sup>, U. Reichl<sup>3</sup>, S. Klamt<sup>1</sup>

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<sup>3</sup>Max Planck Institute Magdeburg, Bioprocess Engineering, Magdeburg, Germany

Microbial communities play a major role in ecology, medicine and various industrial processes. A challenge in modeling microbial communities is the large number of organisms involved which results in complex stoichiometric networks. Here, we introduce an approach to handle this complexity. It relies on compartmented models and the concept of balanced growth [1, 2]. First, we construct and validate stoichiometric models of the core metabolism of the organisms. In a next step, we compute bounded elementary flux vectors (EFVs) [3] for each model and reduce them to their overall stoichiometry. Selected EFVs fulfilling a species-level optimality criterion serve as reactions for the community model.

To illustrate our approach, a reduced model was established consisting of nine organisms including *Escherichia coli*, *Clostridium acetobutylicum*, *Acetobacterium woodii*, *Propionibacterium freudenreichii*, *Syntrophobacter fumaroxidans*, *Syntrophomonas wolfei*, *Desulfovibrio vulgaris*, *Methanococcus maripaludis*, and *Methanosarcina barkeri*. These organisms are representatives for typical degradation steps of anaerobic digestion. The reduced model is analyzed with standard methods of constrained-based modeling and compared to non-reduced community models as well as to experimental data. Product yields and ratios of a chemostat enrichment culture grown on ethanol are well reflected with the model. For glucose as a substrate, the expected ratio of approximately 50% CH<sub>4</sub> and 50% CO<sub>2</sub> in the biogas as well as an anti-correlation between acetate and methane yields is obtained.

#### References:

[1] Khandelwal et al. (2013) PLoS One 8: e64567.

[2] Koch et al. (2016) Biotechnology for Biofuels 9: 17.

[3] Urbanczik (2007) IET Systems Biology.;1(5):274-9.

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## EMV-FG03

### A marriage made in soil – combining individual based models with metabolic networks to quantify bacterial life in heterogeneous habitats

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Notwithstanding the dynamic and inhospitable conditions, soil hosts unparalleled diversity of bacterial life. Genetic diversity and metabolic versatility are key to their success, enabling exploitation of diverse growth strategies and a wide range of resources. Each bacterial cell responds to the local microscale conditions, giving rise to complex metabolic landscapes that affect bacterially-mediated processes ranging from nutrient cycling to soil greenhouse gas emissions. The heterogeneous microscale may trigger fundamentally different growth strategies even for the same species that, in turn, adds to the diverse chemical landscape. Most mathematical models cannot capture such versatility and local adaptation, calling for a more nuanced representation of bacterial metabolism. We report bacterial growth strategies and adaptive metabolic activity based on flux balance analysis using reduced models of genome-scale metabolic networks. These are integrated into a mathematical framework that considers individual bacterial cell dispersal and interaction with nutrient diffusion fields in a spatial context. The inclusion of aqueous phase configuration at the cell scale offers unprecedented opportunities to simulate bacterial life in complex habitats with diffusional and dispersal constraints to multispecies bacterial communities.

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## EMV-FG05

### Individual-based metabolic modeling of heterogeneous microbes in complex communities

J. Zimmermann<sup>\*1</sup>

<sup>1</sup>Christian-Albrechts-University Kiel and University Hospital Schleswig-Holstein, Medical Systems Biology, Kiel, Germany

All metazoan hosts exist in close association with colonizing microorganisms. Additionally, microorganisms themselves can form complex communities. In order to investigate the "rules of engagement" for the establishment of such communities, we recently presented a framework called BacArena for modeling metabolic interactions in cellular communities. In a typical simulation, you first create an environment of defined size and with certain substrate concentrations. To determine metabolic fluxes in each organism, we are using genome-scale metabolic models, which are available in abundance now. We also account for e.g. cell movement, duplication, chemotaxis, or lysis. Altogether, a simulation provides an *in silico* growth experiment in which diverse cells compete and cooperate in space and time. Afterwards, global properties (e.g. an increase of butyrate concentration) can be tracked down to individual cells and metabolic pathways. Furthermore, exchanges, e.g. cross-feeding, between organisms can be identified. By this means, our approach can provide insights into functional reasons behind global community properties. Beside an introduction to BacArena, I will talk about recent extensions to simulate microbiota in a colon-like environment.

#### References:

[1] E. Bauer\*, J. Zimmermann\*, F. Baldini, I. Thiele, C. Kaleta "BacArena: Individual-Based Metabolic Modeling of Heterogeneous Microbes in Complex Communities", *PLoS Computational Biology* (2017) 13, 1-22. \* Equal contribution.

## EMV-FG06

### Synergistic coevolution speeds up molecular evolution

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<sup>1</sup>University of Osnabrück, Department of Ecology, Osnabrück, Germany

<sup>2</sup>Max Planck Institute for Chemical Ecology, Research Group Experimental Ecology and Evolution, Jena, Germany

Ecological interactions are key drivers of genome evolution. Parasitic interactions for example have been shown to increase the rate of molecular evolution through a process of recurrent natural selection for adaptation and counter-adaptation. In contrast, the genomic consequences of a synergistic coevolution remain poorly understood. We addressed this issue by experimentally coevolving two amino acid auxotrophic *Escherichia coli* strains, whose growth depended on a reciprocal exchange of amino acids. Populations of prototrophic wild type cells that evolved under the same conditions served as controls. The obligate by-product interaction showed initially poor growth in coculture, yet rapidly evolved towards cooperative cross-feeding within less than 150 generations. The growth enhancement of auxotrophic consortia was significantly increased relative to cultures of prototrophic cells, suggesting that synergistic coevolution increased the rate of adaptation. Derived cocultures of auxotrophic genotypes produced significantly more of the exchanged amino acids than their evolutionary ancestors and derived prototrophs, which is consistent with an increased cooperative investment of auxotrophs into their respective partners. Resequencing the genomes of isolated clones identified a significantly increased number of mutations in auxotrophic relative to prototrophic genomes. Finally, comparing mutation rates of derived and ancestral genotypes corroborated that auxotrophic genotypes have indeed evolved increased rates of molecular change, presumably to generate increased numbers of cooperative phenotypes. Taken together, our results suggest that similar to host-parasite interactions, also a synergistic coevolution can increase the rate of molecular evolution.

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## Mini Symposium

### Hygienic impact of wastewater on the environment (FG WAV)

16 April 2018 • 17:30–19:00

#### WAV-FG01

##### Transient pollution of urban surface waters by wastewater

H. C. Selinka<sup>\*1</sup>, F. Kubek<sup>1</sup>, S. Beyer<sup>1</sup>, C. Rien<sup>1</sup>, L. Weise<sup>1</sup>, R. Gnirss<sup>1</sup>, R. Szewzyk<sup>1</sup>

<sup>1</sup>German Environment Agency, I1.4 Microbiological Risks (UBA), and Berliner Wasserbetriebe, Berlin, Germany

In conventional wastewater treatment plants human viruses are normally reduced by about 2-3 logs and subsequently released to ambient waters. In addition, in urban areas, heavy rainfall events may lead to combined sewer overflows with significant transient faecal pollution of surface waters.

Within the multibarrier-approaches suggested by the WHO, microbial and viral loads can be further reduced in wastewater treatment by establishing additional effluent disinfection steps, like UV treatment, ozonation, hydrogen peroxide or microfiltration treatments.

We tested the presence of bacteriophages, human adenoviruses, noroviruses and other human viruses and their removal efficiencies. Such Log reduction values are important tools for health-based microbial risk assessments,

but vary due to operational and environmental conditions. Therefore, appropriate additional monitoring indicators and validation procedures for multi treatment unit processes have to be established and harmonized.

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### WAV-FG02

#### Test procedure for the retention of viruses by ultrafiltration

B. Hamsch\*<sup>1</sup>, P. Lipp<sup>1</sup>

<sup>1</sup>DVGW-Technologiezentrum Wasser, Karlsruhe, Germany

Most ultrafiltration membranes have a nominal pore size of 20 nm according to the information of producers. Thus, they also should retain viruses in the size range of 20 to 100 nm. Requirements for UF-membranes are given in the technical rule DVGW W 213-5 consisting in a 4 Log removal for particles in the size range of 20 – 30 nm. A standardized procedure to test this requirement was not given so far.

The goal of a DVGW-funded project was to elaborate a procedure that could be used for testing the efficiency of UF-membranes. Within the research project, it was decided to test the UF membranes by dosing MS2-phages as surrogates for enteropathogenic viruses. As test water a water with ground water quality was used which had a very low fouling potential and as such gave worst case conditions for the test.

The spiking concentration was set to 10<sup>5</sup> -10<sup>7</sup> pfu/mL and effluent concentration was analyzed in 100 mL, giving a detection limit of 10<sup>-2</sup> pfu/mL. Thus, a maximum removal of at least 7 Log could be detected. The procedure was tested in single fibers, laboratory modules and technical modules.

The chosen procedure guaranteed a good reproducibility and could clearly show differences in membranes and modules. In the beginning, producers delivered laboratory modules with very low removal efficiencies. An influence of the production procedure was given. A variation of production conditions improved the removal efficiency considerably.

As the production conditions show a considerable effect on the results of the test, it was decided that the virus removal always had to be tested in technical modules, produced in the large scale production process.

The newly developed test procedure will be included in the technical rule DVGW W 213-5.

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### WAV-FG03

#### Dissemination of antibiotic resistant bacteria via urban wastewater

T. Schwartz\*<sup>1</sup>

<sup>1</sup>Karlsruher Institute für Technologie (KIT-Campus Nord), Institut für Funktionelle Grenzflächen (IFG), Karlsruhe, Germany

Antibiotic resistance is one of the major challenges to modern health care, as antibiotic resistance mechanisms, often transferrable between different bacteria, render once life-saving drugs ineffective. It is hard to dispute the connection between the widespread use of antibiotics and the rise of antibiotic resistance. Hospitals are known to harbor antibiotic-resistant bacteria that are often acquired by patients while receiving treatment (nosocomial infections), but infections are increasingly also acquired in the community (community-acquired infections). While antibiotics use in hospitals and the community are a large portion, more

than 70% of antibiotics are estimated to be used in meat production. Wastewater treatment plants (WWTPs) have been identified as "hotspots" for the transfer of antibiotic resistance genes (ARGs) between different bacteria, often through mobile genetic elements (MGEs), and are typically not capable of completely removing antibiotics, antibiotic resistant bacteria, antibiotic resistance genes in the treatment process. Thus, different advanced wastewater treatments processes were investigated for their microbiological removal potential. The first process utilized ozone to treat wastewater after biological treatment. The second was a UV-treatment. The consecutive application of ozone followed by UV-radiation was the third investigated process. An ultrafiltration as well as an activated carbon filter followed by sand filtration for comparison represented the most recently studied advanced wastewater processes. What is not well understood is the fate of these determinants after discharge from WWTPs plus their release via untreated wastewater into the environment and what impact they have on various ecological communities, including human.

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## Mini Symposium

### The molecular basis of symbiosis (FG SIV) 16 April 2018 • 17:30–19:30

#### SIV-FG01

##### Molecular mechanisms of intracellular coral-algal symbiosis

L. Hambleton\*<sup>1</sup>

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Many animals establish symbioses with microorganisms to gain an ecological advantage. A remarkable example is the endosymbiosis between corals and dinoflagellates (genus *Symbiodinium*), which provide photosynthetically fixed nutrients to enable coral survival in nutrient-poor tropical oceans. Many reef-building corals acquire symbionts during planula larval stages from the environment anew each generation. To date the molecular mechanisms underlying symbiosis establishment are poorly understood mostly because corals are not suitable as model systems. Here I will present our advances in developing larvae of *Aiptasia*, a marine sea anemone, as a tractable model to dissect fundamental aspects of symbiosis establishment at the mechanistic level. I will summarize our currently available resources and experimental toolkit for *Aiptasia*, and present one ongoing projects in more detail. The project addresses how key nutrients are transferred between the two partners. Specifically, we use the Niemann-Pick type C (NPC2) proteins and their role in sterol transfer from symbiont to host as a paradigm for the metabolic exchange that is a prerequisite for the biodiversity and productivity of the whole coral reef ecosystem.

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#### SIV-FG02

##### Metabolic coupling in bacteria

S. Shitut\*<sup>1,2</sup>, T. Ahsendorf<sup>3,4</sup>, S. Pande<sup>4</sup>, C. Kost<sup>1,2</sup>

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Bacteria within communities display a wide range of interactions that have either a positive or negative impact on their growth. Cross-feeding is one such interaction that has a positive effect on either one (commensalism) or both (mutualism) individuals. By exchanging metabolites, bacteria

can save metabolite biosynthesis cost and hence enhance growth as a consortium resulting in the spread of cross-feeding genotypes in nature. However, it is unclear what physiological effects arise from metabolite exchange between two individuals. How does the metabolite-producing cell cope with the loss of the metabolite upon interacting with a recipient? Using a synthetically designed cross-feeding system in *Escherichia coli* that exchange amino acids we aim to answer this question. We show an uptake of amino acids by an auxotrophic recipient from a prototrophic donor cell through nanotubes in coculture. Upon quantifying internal amino acids pools, fluctuations were observed in the amino acid levels in both genotypes. Interestingly, this change in internal amino acid pool in the donor cell triggered amino acid production by delaying the feedback inhibition of amino acid biosynthesis. Taken together, our results show how a loss-of-function mutation (auxotrophy) can lead to a functional fusion through metabolic coupling of two independent cells, much like that seen in host-symbiont associations.

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### SIV-FG03

#### Transcriptomic insights into animal-bacteria interactions in marine chemosynthetic symbioses

B. Yuen<sup>\*1</sup>, J. Petersen<sup>1</sup>

<sup>1</sup>University of Vienna, Division of Microbial Ecology, Vienna, Austria

Lucinid clams form a highly stable symbiosis with one type of sulphur-oxidising bacteria, the success of which has enabled their colonisation of marine environments across the globe. The specificity of this partnership presents a unique opportunity to study the molecular basis of animal-bacteria crosstalk in a natural yet simple system. Despite this, our understanding of how lucinids regulate interactions with their endosymbionts lags far behind progress in unravelling the functional role of the symbiont. We work with the clam *Loripes orbiculatus* and its intracellular symbiotic bacteria to explore the molecular dialogue underpinning this symbiosis. The evolutionarily conserved role of innate immunity in both beneficial and harmful interactions with bacteria makes it a prime candidate for mediating crosstalk between lucinids and their endosymbionts. To investigate this, we are developing two new molecular approaches that exploit the natural specificity of the symbiosis for a particular organ - the gill. First, we are using RNA-Seq to compare the transcriptome profiles of the *L. orbiculatus* symbiont-hosting gills versus the symbiont-free organs. Work is ongoing to identify and characterise the transcripts specifically enriched in the gills, and therefore potentially involved in mediating host-symbiont crosstalk. Second, we have developed methods to dissociate individual cells from the symbiont-hosting organ, which will enable us to investigate the transcriptomic profile of the specialised bacteria-hosting cells using single-cell RNA-Seq technologies. These efforts will lead to novel insights into the molecular and cellular processes underpinning the maintenance of this beneficial endosymbiosis.

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### SIV-FG04

#### Developmental interdependence of *Hydra viridissima* and *Chlorella*: a window into the evolution of symbiotic interactions

J. Bathia<sup>\*1</sup>, M. Hamada<sup>2</sup>, K. Schroeder<sup>1</sup>, T. Bosch<sup>1</sup>

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<sup>2</sup>Ushimado Marine Institute, Okayama University, Okayama, Japan

The symbiotic relations between a eukaryotic host and microbes has been known for decades. These relations vary in degree of interdependence from loose associations (facultative symbiosis) to strong associations (obligatory

symbiosis) with later being a result of vital and mutual coordination of the cellular machineries and cell signaling in the host and symbiont. The underlying molecular mechanisms of this interdependence are still unclear. The fresh water polyp *Hydra viridissima* with its unicellular algae *Chlorella* represents an informative model to study the molecular basis of symbiosis. It can be assembled in 3 grades of interdependence: (i) Aposymbiotic *Hydra* without any intracellular algae (ii) *Hydra* with foreign symbiont (*Chlorella variabilis* NC64) and (iii) *Hydra* with the native *Chlorella* sp. A99. To get an insight into the interactions, transcriptomic analysis was carried out to identify the genes differentially expressed among the three symbiosis systems and 10 differentially expressed genes (DEGs) were identified in host in presence of native *Chlorella* sp. A99. Remarkably, the same set of genes was responsive when aposymbionts were treated with maltose or inhibition of algal photosynthesis pointing to dependence of host on algal metabolism. One of the DEGs codes for glutamine synthetase (GS) which together with absence of some important nitrogen metabolism genes in native *Chlorella* suggests that glutamine produced by host GS might be the nitrogen source for the symbiont. This is supported by improved *in vitro* growth of algae by addition of glutamine to the culture media. Together with genomic analysis and genetic manipulation, this study will elucidate molecular nature of symbiosis and establish a general understanding of mechanics behind other cases of symbiosis.

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### SIV-FG05

#### Polysaccharide Degrading Potential in the Hindgut of Higher Termites

M. Vera<sup>\*1</sup>, V. Hervé<sup>1</sup>, S. Griesel<sup>1</sup>, A. Brune<sup>1</sup>

<sup>1</sup>Max Planck Institute for Terrestrial Microbiology, RG Insect Gut Microbiology and Symbiosis, Marburg, Germany

The symbiotic digestion of lignocellulose by higher termites involves the cooperation between the activities of the host and its bacterial microbiota housed in the enlarged hindgut compartments. Previous studies have shown a consistent decrease of *Fibrobacteres* and an increase in *Clostridia* as the diet of the host termite changes from sound wood to humus. This likely reflects the differential availability of polysaccharides and suggests distinct carbohydrate-degrading capabilities between members of the two clades, both of which have been implicated in the degradation of cellulose and hemicelluloses in the rumen and the colon of mammals. To overcome the lack of isolates for any of the abundant lineages, we investigated their functional roles in termites guts using an *in silico* approach. We reconstructed the genomes of 589 lineages of bacteria and archaea from metagenomic libraries of the hindgut of eight higher termites. A classification of the carbohydrate-active enzymes in 112 draft genome belonging to *Fibrobacteres* or *Clostridia* revealed a distinct and potentially complementary repertoire in each of the major lineages. The abundance and distribution of putative cellulases, xylanases, and other glycoside hydrolases of different families indicated a functional specialization of *Lachnospiraceae* and *Ruminococcaceae* in hemicellulose degradation. Moreover, the apparent lack of cellulosomes in the *Ruminococcaceae* lineages associated with termites stands in marked contrast to those found in ruminants and other mammals. Physiological characterisation of a novel strain of *Lachnospiraceae* isolated from the termite gut further supports our findings.

## SIV-FG06

### Chemical and biological lessons from symbiotic microbes of fungus-growing termites

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<sup>4</sup>University of Copenhagen, Department of Biology, Jena, Germany

<sup>5</sup>Forestry and Agriculture Biotechnology Institute, Department of Microbiology and Plant Pathology, Pretoria, South Africa

**Introduction:** *Macrotermitinae* cultivate a mutualistic food fungus for nourishment in so called "fungus gardens", a nutrient-rich environment prone to exploitation. Symbiotic bacteria presumably defend the fungus garden by secretion of antimicrobials, which prevent growth of garden parasites and other invading species.

**Objectives:** We performed a functional, genomic and chemical analysis of symbiotic microbes of fungus-growing termites to study their role as defensive symbionts.

**Methods:** We isolated and phylogenetically analyzed the fungal mutualist, abundant and putative defensive microbes, as well as putative parasitic fungi. We assessed their bioactivities using ecological and pharmaceutically relevant bioassays against human-pathogenic microorganisms, and entomopathogenic fungi. Co-cultivation studies led to the identification novel metabolites involved within the systems. Bacterial strains with high antifungal activity were chemically analyzed and full strain description was pursued. The produced antimicrobial secondary metabolites were characterized using HPLC/LC-HRMS/NMR.

**Results:** Two gut-associated *Actinomadura* strains were defined as new and fully described. *Actinomadura* sp. RB29 produced new tropolone alkaloids (rubterolone A-F) and genome mining revealed the putative biosynthetic pathway. Co-cultivation studies revealed new secondary metabolites produced by the stowaway fungus *Pseudoxylaria* sp. X802. Analysis of the fungal mutualist revealed a rich metabolome presumably mediating termite husbandry.

**Conclusion:** Termite fungiculture is a rich source of new microbial species and novel natural products with unique chemical scaffolds.

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## Mini Symposium

### Fungal development in space and time (FG FBV)

16 April 2018 • 17:30–19:30

#### FBV-FG01

##### Checkpoints and feedbacks of circadian transcription in *Neurospora crassa*

M. Brunner<sup>\*1</sup>

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Circadian clocks modulate rhythmic expression of a large number of genes and thus generate the potential to control biochemical, physiological and behavioral functions in a time-of-day specific manner. Eukaryotic circadian clocks are composed of a network of interconnected positive and

negative feedback loops that produce rhythmic expression and modification of one or more clock proteins. The frequency (*frq*) gene is a key element of the circadian clock of *Neurospora*. Expression levels of *frq* RNA and FRQ protein oscillate in a circadian fashion. Expression of *frq* is controlled by the heterodimeric transcription factor White Collar Complex (WCC), which activates clock-controlled expression of *frq* by modulation of transcriptional bursting. FRQ assembles with casein kinase 1a (CK1a) and FRQ-Interacting-Helicase (FRH) forming the FFC complex. FRH stabilizes FRQ and serves as a sensor of the metabolic states of *Neurospora*. The FFC is a scaffold that interacts transiently with WCC. It inactivates the circadian transcription factor via phosphorylation by CK1a and thereby inhibits synthesis of *frq* RNA and FRQ protein in a negative feedback loop. In the course of a day FRQ is progressively hyperphosphorylated in cis by bound CK1a. Hyperphosphorylated FRQ releases CK1a and is thereby functionally inactivated and degraded, resulting in relieve of the negative feedback.

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#### FBV-FG02

##### Rapid adaptation of signaling networks in the fungal pathogen *Magnaporthe oryzae*

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<sup>1</sup>Johannes Gutenberg-Universität (JGU) Mainz, Mikrobiologie und Weinforschung, Mainz, Germany

Evolutionary adaptation of living organisms is commonly thought to be the result of processes that acted over long periods of time. The following research is motivated by recent observations that microorganisms are able to rapidly adapt to new environments and establish stable phenotypes by natural selection, even within few generations. We found the filamentous rice blast fungus *Magnaporthe oryzae* to rapidly rewire signal transduction required for osmoregulation in several independent "loss of function" (*lof*)-mutants of the High Osmolarity Glycerol (HOG)-pathway upon exposure to salt stress. Adaptation resulted in stable mutants being restored in osmoregulation arising as individuals outgrowing from the salt-sensitive *lof*-mutants. These "adapted" strains memorize the restored osmoregulation even if they were cultivated under unstressed conditions for several weeks. Interestingly, the major compatible solute produced upon salt stress by these "adapted" strains was found to be glycerol in contrast to arabitol in *Magnaporthe* wildtype strains. These findings lead to the hypothesis that stable adaptation-events under continuously environmental stress enable *M. oryzae* to rapidly rewire signaling networks. To address the hypothesis, we aim to identify the molecular mechanisms and characterize associated factors and signaling pathways which enable or prevent adaptation. We will combine sequencing data from genomics and transcriptomics with modern quantitative (phospho)-proteomics. Furthermore, reversed molecular genetics will be used to validate the candidate genes found to promote or constrain rapid evolutionary adaptation. Consequently, our research will open the door to investigate the relationship of phenotypic and genetic evolution in eukaryotic microorganisms.

### FBV-FG03

#### Velvet domain protein VosA represses the zinc cluster transcription factor ZtfA regulatory network for *Aspergillus nidulans* asexual development, oxidative stress response and secondary metabolism

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The NF- $\kappa$ B-like velvet domain protein VosA (viability of spores) binds to more than 1,500 promoter sequences in the filamentous fungus *Aspergillus nidulans*. VosA inhibits premature induction of the developmental activator gene *brlA*, which promotes asexual spore formation in response to environmental cues, such as light. VosA represses a novel genetic network controlled by the *ztfA* gene for the nuclear zinc cluster transcription factor A. ZtfA function is antagonistic to VosA, because it induces the expression of early activator genes of asexual differentiation, such as *flbC* and *flbD* as well as *brlA*. The ZtfA controlled network promotes asexual development and spore viability, but is independent of the fungal light control. ZtfA interactions with the RcoA transcriptional repressor subunit suggest additional inhibitory functions on transcription. ZtfA links asexual spore formation to the synthesis of secondary metabolites including emericellamides, austinol as well as dehydroaustinol and activates the oxidative stress response of the fungus. The fungal VosA-ZtfA regulatory system of transcription includes a VosA control of the *ztfA* promoter, common and opposite VosA and ZtfA control functions of fungal development and several additional regulatory genes. The relationship between VosA and ZtfA illustrates the presence of a convoluted surveillance apparatus of transcriptional control, which is required for accurate fungal development and the linkage to the appropriate secondary metabolism.

### FBV-FG04

#### *Thecaphora thlaspeos*: Smut fungal effectors mediate asymptomatic colonization of Brassicaceae

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<sup>3</sup>Ludwig-Maximilians-Universität, Biology Genetics, Munich, Germany

Biotrophic fungal plant pathogens are able to balance their virulence and form intricate relationships with their hosts. In some cases, this leads to systemic colonization over long timescales without interference with plant development. How plant-pathogenic endophytes manage to establish and maintain such sustained systemic infection and what determines these types of interactions and host specificity remains largely unknown.

*Thecaphora thlaspeos* is a relative of the well-studied grass smut *Ustilago maydis*, and it is the only known smut fungus adapted to Brassicaceae hosts including the model species *Arabidopsis thaliana*. Its capability to overwinter with perennial hosts and its systemic infection of the entire plant are unique

characteristics among smut fungi. We could show that *T. thlaspeos* has a typical smut genome with a set of candidate effector genes that comprises common smut and unique members. For three candidates, we have functionally proven effector activity.

Besides effector candidates a highly upregulated orthologue of the *U. maydis* virulence factor Pit1 was identified in *T. thlaspeos*. Interestingly, the effector protease inhibitor Pit2 from the same effector cluster, which is crucial for tumour formation in *U. maydis*, is missing in *T. thlaspeos*. This offers the opportunity to study Pit1 virulence function independently of Pit2.

The transcriptional response of *Ar. hirsuta* towards *T. thlaspeos* infection lacks activation of key salicylic acid-dependent signalling-related genes suggesting *T. thlaspeos* to distinctly balance its virulence during biotrophic growth. In the future we aim at analysing transcriptome wide transcriptional changes induced by infection in *A. thaliana* and *A. alpina* to compare plant responses in different host species.

### FBV-FG05

#### Volatilom of the mushroom *Agrocybe aegerita* during different developmental stages

A. Orban<sup>\*1</sup>, M. Rühl<sup>1</sup>

<sup>1</sup>Justus-Liebig-University Gießen, Institute of Food Chemistry and Food Biotechnology, Gießen, Germany

For ages, mushrooms are a valued source for nutrition and appreciated for their unique as well as delicious flavours. The black poplar mushroom *Agrocybe aegerita* is a well-known mushroom, favoured for its pleasant aroma. Previous studies determined the aroma profile of cultivated and collected fruiting bodies of *A. aegerita*. Nevertheless, there is no information about changes in the profile of volatile organic compounds (VOC) during development of *A. aegerita* fruiting bodies and little for mushrooms in general. Therefore, we decided to establish a system allowing the measurement of volatiles at different developmental stages.

The tested *Agrocybe aegerita* wildtype-strain AAE-3 was grown in modified crystallizing dishes. VOC were collected directly in the headspace by solid phase microextraction (SPME). The volatiles were analyzed with GC-MS. Compounds were identified by comparing mass spectra with data from the NIST14 database and matching determined retention indices with published ones. Furthermore, authentic standards were used for identification.

In the headspace of the mycelium, mainly alcohols and ketones, like 1-octen-3-ol, 2-methyl-1-butanol, acetone and cyclopentanone, were detected. The composition of the VOC changed drastically with the occurrence of fruiting bodies and during sporulation. Here, sesquiterpenes, especially  $\Delta^6$ -protoilludene,  $\alpha$ -cubebene and  $\delta$ -cadinene, were the dominant substances. After sporulation, the amount of sesquiterpenes decreased, while additional VOC, mainly octan-3-one, appeared. The results of the present study revealed that the VOC profile of mushrooms is not static, but is changing considerably depending on the fungal life stage.

## FBV-FG06

### **BRO-1 interacts with the SO protein and is essential for germling communication and fusion in *Neurospora crassa***

H. Haj Hammad<sup>\*1</sup>, M. R. Schumann<sup>1</sup>, A. Fleißner<sup>1</sup>

<sup>1</sup>Institut für Genetik, Technische Universität Braunschweig, Braunschweig, Germany

Colony initiation of filamentous fungi commonly involves fusion of germinating vegetative spores. Studies in *Neurospora crassa* revealed an unusual cell-cell communication mechanism mediating this process, in which the fusion partners alternate between two physiological stages, probably related to signal sending and receiving. This "cell dialog" involves the alternating, oscillatory recruitment of the SO protein and the MAK-2 MAP kinase module to the apical plasma membrane of growing fusion tips.

We identified BRO-1, the homolog of the mammalian ALG-2-interacting protein X (ALIX), as a new factor promoting germling interaction and fusion. Alix is an ESCRT accessory component and has a main role in regulating the biogenesis of exosomes and the secretion of extracellular vesicles. In *N. crassa*, BRO-1 is essential. In a conditional mutant, suppression of the expression of the bro-1 gene results in a  $\Delta$ so-like phenotype, including the lack of chemotropic interactions and subsequent fusion. Subcellular localization and live cell imaging revealed that BRO-1-GFP is recruited to the tips of the interacting germlings in a dynamic, oscillating manner, such that high signal intensity of BRO-1-GFP in one tip correlates with low signal intensity at the tip of the fusion partner. Co-localization experiments of BRO-1 and SO revealed that both proteins co-localize in the described dynamic manner at the growing, interacting cell tips.

We hypothesize that BRO-1 plays a role in signal secretion during cell – cell communication. Future analysis of its molecular function will greatly contribute to our understanding of the unique "cell dialog" mechanism and the molecular bases of fungal cellular communication.

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## FBV-FG07

### **Analysis of Clp1-dependent UPR-modulation in *Ustilago maydis***

N. Pinter<sup>\*1</sup>, K. Heimel<sup>1</sup>

<sup>1</sup>Georg-August-Universität Göttingen, Institute for Microbiology and Genetics, Göttingen, Germany

The unfolded protein response (UPR) is conserved eukaryotic signaling pathway to counteract endoplasmic reticulum (ER) stress through extensive restructuring of the secretory pathway. During the life cycle of the biotrophic fungal phytopathogen *U. maydis* the UPR is inactive during saprophytic growth but specifically activated after successful penetration of the host. We identified the central UPR-regulator Cib1 (Clp1 interacting bZIP1) to physically interact with Clp1, an important developmental regulator of development inside the host plant. Both genes are crucial for pathogenic development and deletion strains are arrested after penetration of the leaf surface. Cib1-Clp1 complex formation results in stabilization of both Clp1 and Cib1 and alters the transcriptional program of the UPR, rendering cells hyper-resistant towards ER-stress. Transcriptional profiling of Clp1-mediated UPR modulation was used to understand the principles of long-term UPR activation throughout the host-pathogen interaction. Gene deletion analysis identified a previously uncharacterized UPR target gene, being specifically required for biotrophic growth of *U. maydis*. While growth under axenic conditions and ER- stress resistance is

not affected, deletion strains are avirulent in infection assays. Our results suggest that a single protein-protein interaction regulates fungal development and modulation of the UPR pathway and by that aligns effector gene expression, secretion, and execution of developmental programs during the biotrophic interaction between *U. maydis* and *Zea mays*.

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## Mini Symposium

### **The role of viable type material in the light of alternative species concepts in prokaryotic taxonomy (FG ISV) 16 April 2018 • 17:30–19:00**

#### ISV-FG01

##### **Uncultivated Bacteria and Archaea in need of their own taxonomy**

R. Amann<sup>\*1</sup>, R. Rossello-Mora<sup>2</sup>, K. Konstantinidis<sup>3</sup>

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<sup>3</sup>Georgia Institute of Technology, Atlanta, GA, United States

Most Bacteria and Archaea have not been cultivated, but are today readily detected and studied by the tools of molecular biology. There is no supervised nomenclature for uncultivated prokaryotes since the Bacteriological Code requires them to be studied and deposited as pure cultures. A provisional *Candidatus* status was created in the early 1990s. It was not widely accepted, likely due to its data poorness (microscopy, 16S rRNA, FISH). In the past decade metagenomics and single cell genomics have facilitated data-rich characterizations of uncultivated microbes, however, standards were missing and the nomenclature mostly remained alphanumeric (NS3, SUP05, SAR92 etc.). In parallel, genomics made its way into the description of cultivated species, e.g., the average nucleotide identity is replacing DNA-DNA hybridization in species discrimination. There are accepted 16S rRNA and average amino acid identity values for the classification of higher taxa, which can also be applied to genomic bins obtained from yet uncultivated microbes. We therefore see a possibility to create one taxonomy for all Bacteria and Archaea, not restricted to what can be cultivated today. Its descriptions should be formalized and extensive. These would use proper Linnean nomenclature, obtain taxonomic priority, and be strongly based on high-quality genomic bins. Phenotypic information on the uncultivated species could be obtained by microscopic and single cell methods, and environmental metadata. Furthermore, the physiological potential would be predicted based on genomic information, which would also serve as voucher. Extending taxonomy to the uncultivated microbes would bring order into chaos and greatly improve communication. It is by no means meant to undermine efforts for cultivation.

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#### ISV-FG02

##### **Valid species description of novel nitrite-oxidizing bacteria: A challenge for microbiologists**

E. Spieck<sup>\*1</sup>

<sup>1</sup>University of Hamburg, Department of Microbiology and Biotechnology, Hamburg, Germany

Nitrification is a key process for N-removal in natural as well as man-made habitats and protects living organisms from toxic nitrite. Continuous discovery of novel species and surprising new features demonstrated that the entire diversity and metabolic potential of nitrifying bacteria has still not been uncovered. Due to their low energy yield,

chemolithoautotrophic nitrifying bacteria are slow-growing and therefore hard to isolate and maintain in the lab. Especially microorganisms performing the second step of nitrification – nitrite-oxidizing bacteria (NOB) – are often neglected despite their ecological importance. Reference cultures are required to confirm novel metabolic functions predicted from genome annotations. However, only some representatives are stored in public culture collections (*Nitrobacter*, *Nitrococcus*), since standard preservation methods are mostly not suitable for this functional group. Stable growth is hampered by the sensitivity against high substrate concentrations and long-lasting lag-phases. In 2013, a modified cryopreservation method [1] opened the door for the storage of further pure cultures (*Nitrospina*, *Nitrospira*). Nevertheless, NOB are phylogenetically heterogeneous and cultivation strategies have to be adapted for new enrichments. Detailed knowledge about physiological optima is requisite to obtain reproducible growth.

This presentation will give insights into the problematics of the cultivation of NOB and the difficulties to deposit them at culture collections to validly describe new species. If possible at all, the preservation of these fastidious bacteria for the scientific community requires high effort and man-power.

#### References:

[1] Vekeman et al., 2013: SAM 36(8): 579-84.

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#### ISV-FG03

##### On the taxonomy of microbial dark matter

J. Overmann\*<sup>1</sup>

<sup>1</sup>Leibniz Institute DSMZ, German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany

Despite decades of cultivation and isolation attempts, only about 12,000 species of *Bacteria* and *Archaea*, representing 0.1 - 0.001% of the estimated global number of species, have so far been validly described. Even more significantly, laboratory cultivation has returned predominantly isolates from four bacterial phyla, whereas only 10% of the described species are affiliated to the 29 other bacterial and archaeal phyla that contain cultivated representatives, and no single isolate is available for 85 phyla. This pronounced bias of cultivation approaches towards a few taxonomic groups has profound effects on our current understanding of bacterial diversity. At the same time, bacterial genome sequences become available at a faster rate than newly described species. It has therefore been concluded that establishing taxa for not-yet-cultured microorganisms based on similarity levels of their genome sequences represents the only realistic approach for a consistent classification of microbial diversity. Thus, it was suggested to establish valid species names for groups of closely related genome sequence types and deposit just these sequences as type material in databases. Such a novel approach has important implications regarding (1) the stability of nomenclature, (2) stability of genome sequences as a reference system, (3) the effects of assembly and binning procedures, (4) the criteria for delineating species, (5) *in silico* functional predictions, and (6) functional biodiversity research, which will be discussed in this talk.

## Mini Symposium

### A quality and diagnostical view on Food microbiology (FG FMV)

16 April 2018 • 17:30–19:30

#### FMV-FG01

##### Honor Thy Lodgers? - The Microbiome of the Built Environment of Humans

M. Egert\*<sup>1</sup>

<sup>1</sup>Furtwangen University, Institute of Precision Medicine, Villingen-Schwenningen, Germany

The microbiome of the built environment (BE) of humans and its complex mutual interactions with the human occupants represent a new and highly interdisciplinary research field, comprising very basic as well as highly applied aspects. The BE has been existing for ~ 20.000 years and is estimated to cover up to 6% of the global ice-free land surface [1]. It is characterized by very fluctuating environmental conditions and sharp gradients of physicochemical parameters (temperature, pH, nutrients, antimicrobials etc.), which are supposed to significantly shape the structure and function of the microbial communities residing there [1]. The significance of the BE microbiome for human health and well-being is far from being understood. However, in view of an increasingly aging population and a steadily growing share of medical care at home, this significance definitely needs to be investigated in more detail. While it is clear that entry and dispersal of obligate, e.g. food-borne pathogens have to be tightly controlled, the role of thousands of (probably) non or facultative pathogenic species is obscure. Current research on the BE is rather structure-oriented, i.e. focused on microbiome composition; future studies should also address microbiome functionality (pathogenicity, antibiotic resistance, malodor production etc.) in order to better understand the hygienic relevance of the BE microbiome. Our own research in this field is focused on spectacles, kitchen sponges [2], washing machines [3], and holy water basins in Roman Catholic churches [4].

#### References:

[1] Martin et al. (2015). Trends Ecol. Evol. 30: 223.

[2] Cardinale et al. (2017). Sci. Rep. 7: 5791.

[3] Egert et al. (2017). SOFW Journal 143 (4): 44.

[4] König et al. (2017). J. Water Health 15: 823.

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#### FMV-FG02

##### Sinner or Saint - Evaluation of the microbiological quality of domestic appliances

D. Bockmühl\*<sup>1</sup>

<sup>1</sup>Rhine-Waal University of Applied Sciences, Faculty of Life Sciences, Kleve, Germany

The microbiology of domestic appliances, such as automated coffee machines and dishwashers, has hardly been regarded in the past, although most devices provide a suitable environment for microbial growth. Like externally introduced microorganisms, the microbial communities colonizing domestic appliances have to be considered as potential contaminants of food and food contact surfaces. Moreover, for the sake of sustainability, there's a trend towards lower temperatures, which is tried to be compensated by other parameters, such as longer program durations. This principle has been known for long and is supported by the concept of

the Sinner circle, although its impact on the microbiological efficacy of a cleaning process is not fully understood yet. To be able to evaluate the putative hygiene risks resulting from these issues and to develop adequate solutions, there is a need for comprehensive investigations on the microbiological status as well as on the hygienic efficacy of the relevant appliances.

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### FMV-FG03

#### **Persistent spore formers constitute a widespread phenomenon in dairy food production lines**

E. Doll<sup>\*1</sup>, A. Dettling<sup>1</sup>, S. Scherer<sup>1</sup>, M. Wenning<sup>1</sup>

<sup>1</sup>*Technische Universität München, Lehrstuhl für Mikrobielle Ökologie, Freising, Germany*

Spore forming bacteria are of particular relevance in food products as they may constitute a health risk or serve as hygiene indicators. However, many food products such as extended shelf life (ESL) milk or milk powder are manufactured without UHT treatment and spores are not fully eliminated. In case of ESL milk psychrotolerant spore formers are of particular interest, as they lead to premature spoilage. For milk powder thermophilic spore formers are critical as they are able to proliferate over the production process.

As spores are introduced into the process via the raw material, but may also be a result of recontamination events, detailed knowledge on contamination routes and influence of process parameters on the development of spore counts is needed to set up effective prevention measures.

To unravel reasons for elevated spore counts we analysed the correlation of spore counts and microbiota of raw material and resulting end products for several production lines of ESL milk as well as milk and whey powder. Analyses of biodiversity of spore formers in the raw material were performed using culture-dependent techniques and the change in microbiota expressed as cell counts and species composition were monitored over the production processes. In all cases, the raw material turned out to have only minor impact, because the spore counts were exceptionally low. Instead, occurrence of identical strains in end products over several production batches was determined and a persistence of strains in production lines over several months was demonstrated.

Consequently, prevention strategies to limit spore counts and recontamination events largely need to focus on plant hygiene rather than spore counts of raw material.

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### FMV-FG04

#### **IHO - Organisation and Services; Disinfection and their uses in the professional sector**

N. Neubauer<sup>\*1</sup>

<sup>1</sup>*IHO-Industrieverband Hygiene und Oberflächenschutz, Verband, Frankfurt, Germany*

The German Industrial Association for Hygiene & Surface Protection (IHO) – headquartered in Frankfurt am Main – represents the interests of German manufacturers of cleaning and disinfection products for industrial and institutional applications. Business fields of the 54 IHO member companies include products for healthcare, large-scale catering and laundries, cleaning of buildings and the food and metal industries. The IHO members employ over 7,000 staff in this country. They achieve annual sales of ca. 1 billion euros, reflecting a market share of around 90%. The

membership largely consists of small and medium-sized enterprises with an above-average research & innovation ratio.

IHO work in Europe takes place within the International Association for Soaps, Detergents and Maintenance Products (AISE). At the national level, the IHO has close links with the German chemical industry association Verband der Chemischen Industrie (VCI).

The IHO celebrated its 25th anniversary in 2017, looking back on a successful quarter of a century. Services for IHO members comprise, inter alia, the organising of working groups and the listing of effective disinfectants for various uses according to the state of the art. Furthermore, the IHO finds solutions for practical issues like exposure measuring by product users in special fields of application.

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### FMV-FG05

#### **Does bacterial growth influence the prediction accuracy of bioburden measured by fluorescence spectroscopy?**

C. Grimmmler<sup>\*1</sup>, H. Schmidt<sup>1</sup>

<sup>1</sup>*University of Bayreuth, Bioanalytical Sciences and Food Analysis, Kulmbach, Germany*

Conventional control of the bioburden of meat using colony counts (TVC) is time consuming and allows only for a random monitoring. Non-invasive spectroscopic methods like vibrational and fluorescence spectroscopy have been shown to be promising methods for a real-time assessment of the microbial status of meat using partial least-squares regression (PLSR). However, correlations reported so far were performed with rather small numbers of independent samples, and bioburden was shown to be strongly linked to storage time of the meat. It was therefore not clear how fit the prediction models were for practical application.

The aim of this work was to evaluate the robustness of prediction models for bioburden based on fluorescence spectra. To increase biological variance and variance in colour, a larger number of minced meat samples (n=80) was collected from 15 different sources using minced pork and mixed minced pork and beef. The samples were stored at 2°C for up to 6 days and fluorescence spectra were measured with a handheld device (freshdetect,  $\lambda_{exc} = 405$  nm). Total viable mesophilic plate counts (TVC) were measured in parallel.

The validation of previous models with independent samples performed differently and was depending on the data sets which were used. A systematic evaluation of the predictive ability of models calculated according to the storage time showed that the prediction accuracy of a model improved for bacterial growth rates  $>0.5$  log TVC/day and for bioburden levels  $>5.0$  log TVC/g. With increasing growth rates the correlation between bioburden and fluorescence spectra increased to  $R^2=0.8$ . Results suggest that prediction models are based on a mean bacterial growth rate implying exponential growth where high metabolic rates and high oxygen consumption are reflected in the fluorescence spectra decreasing prediction errors to 0.5-0.2 log TVC/g (RMSECV).

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### FMV-FG06

#### **Relevance of species identification for risk assessment of *Bacillus cereus* sensu lato group members**

M. Wenning<sup>\*1</sup>, C. Huptas<sup>1</sup>, S. Scherer<sup>1</sup>

The *Bacillus cereus* sensu lato group comprises nine species including the two human pathogens *Bacillus anthracis* and *Bacillus cereus*. *Bacillus thuringiensis* is widely used as a natural herbicide and *Bacillus mycoides* and *Bacillus weihenstephanensis* are known as food spoilers.

The species in this complex are closely related which leads to substantial difficulties in species discrimination and reliable species identification by easy-to-handle technologies is still not possible. Because some species have been defined based on few or even only one specific characteristic (e.g., production of BT toxin by *B. thuringiensis*), these characteristics need to be confirmed in order to affirm species identity. This leads to insecurities in the evaluation of risks, as particular risks are commonly associated to species identity.

Recent analyses of the phylogeny and distribution of virulence factors among members of *B. cereus* s. l. based on whole-genome sequences of 281 strains indicate that the species definitions currently used in this group clearly contradict the genomic phylogenetic relationships. *B. thuringiensis* cannot be distinguished from *B. cereus*, and *B. mycoides* and *B. weihenstephanensis* form one species. The same applies for the distribution of virulence factors such as toxin genes that are present in multiple species, but may also be absent in a large number of strains of a single species.

Consequently, risk assessment based on species identity urgently needs to be re-considered and the taxonomy of the *B. cereus* s. l. complex needs to be revised and adapted to the most recent phylogenetic data. Since this process will involve species of common interest such as *B. thuringiensis* or *B. cereus*, it may lead to severe irritations outside the scientific community.

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## Short Lecture

### Biotechnology 2

17 April 2018 • 08:00–10:00

#### BTV09

##### C<sub>4</sub> dicarboxylic acid metabolism in *Clostridium autoethanogenum*

R. Breitkopf<sup>1,2</sup>, A. T. Wichlacz<sup>1</sup>, H. E. L. Williams<sup>1</sup>, N. P. Minton<sup>1</sup>, K. Winzer<sup>1</sup>

<sup>1</sup>University of Nottingham, Nottingham, United Kingdom

<sup>2</sup>Hans-Knöll-Institute, Biotechnikum, Jena, Germany

The acetogenic bacterium *Clostridium autoethanogenum* possesses the inherent ability to produce acetate and ethanol during growth on industrial waste gases such as carbon monoxide and carbon dioxide using the Wood-Ljungdahl Pathway (WLP) for carbon fixation. With the urgent need to reduce greenhouse gas emissions and produce chemicals and fuels in a more sustainable manner, this bacterium has attracted considerable interest over recent years. Yet, our understanding of its metabolism remains incomplete.

Here, we report on the utilisation of C<sub>4</sub> dicarboxylic acids by *C. autoethanogenum*. Fumarate, malate and oxaloacetate were found to serve as sole sources of carbon and energy, whilst succinate could not be utilised. Fumarate breakdown was studied in more detail, using an *in vivo* nuclear magnetic resonance (NMR) approach as well as mutational analyses.

*C. autoethanogenum* was grown in pressure-resistant NMR tubes within a Bruker AV(III) 800 MHz NMR spectrometer for 72 hours with spectra obtained every sixty minutes. Use of 2,3-<sup>13</sup>C<sub>2</sub>, 1,4-<sup>13</sup>C<sub>2</sub> and fully labelled fumarate revealed that its breakdown included reactions of the incomplete tricarboxylic acid cycle and the WLP. The reducing equivalents gained from oxidative breakdown of fumarate to acetate were used to partially re-assimilate the CO<sub>2</sub> that was liberated during that process (decarboxylation of malate and pyruvate). Hence, fumarate was almost exclusively converted to acetate.

Our work suggests that under the employed conditions *C. autoethanogenum* prefers WLP-dependent acetate formation, rather than ethanol generation, as a means to re-oxidise reduced cofactors and to conserve further energy.

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#### BTV10

##### Role of arginine biosynthesis in salt stress adaption of *Clostridium acetobutylicum*

M. Lehmann<sup>\*1</sup>, H. Bahl<sup>1</sup>, R. J. Fischer<sup>1</sup>

<sup>1</sup>University of Rostock, Division of Microbiology, Rostock, Germany

The Gram-positive bacterium *Clostridium acetobutylicum* is well known for its ability to produce solvents like butanol, a next-generation biofuel. Lignocellulosic biomass used in biobutanol production is often pre-treated with sodium hydroxide to break up complex carbohydrates into sugars usable for fermentation. In consequence, the resulting hydrolysate usually contains high sodium concentrations inhibiting butanol production and thus limiting the use of these low-cost feedstocks. The aim of this study was to analyse the adaptation of *C. acetobutylicum* to salt stress. We observed a positive effect of arginine on solvent production in NaCl-containing media. To investigate the role of this amino acid, a regulator for arginine biosynthesis was disrupted using the Clostron group II intron-based gene inactivation system. In a synthetic medium with 300 mM NaCl, the knockout mutant showed a 33% higher growth rate ( $0.176 \pm 0.025$  vs.  $0.132 \pm 0.032$  h<sup>-1</sup>) and produced 85% more butanol ( $8.92 \pm 0.26$  vs.  $4.83 \pm 1.58$  g/l) compared to the wildtype. Thus, we conclude that arginine might play an important role in salt stress adaptation of *C. acetobutylicum*.

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#### BTV11

##### Engineering *P. putida* for Efficient Production of Bioplastics in Phototrophic Mixed Cultures with *S. elongatus cscB*

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At the moment, most bioprocesses are dependend on carbon source derived from crops like sugar cane or corn. In order to produce these carbohydrate containing crops, extensive agricultural area is needed. Due to ethical and ecological issues, new feedstocks for Industrial Biotechnology have to be found. Cyanobacteria and eukaryotic algae are promising candidates for the production of new carbon sources using the flue gas CO<sub>2</sub> with potentially higher areal productivities, less demand for fresh water and nutrients than traditional crops [1]. Herein we report the cultivation of *Pseudomonas putida* KT2440 fed by sucrose produced from light and CO<sub>2</sub> by transgenic *Synechococcus elongatus cscB* in mixed culture. Wildtype *P. putida* KT2440 is not able to metabolize sucrose. By introducing genes from a so far uncharacterized sucrose

operon from *P. protegens* pf-5 into *P. putida*, it was possible to produce polyhydroxyalkanoates (PHA), biodegradable plastics, in one step from cyanobacterial sugar in this mixed culture. Sugar production rates of 0.32 g L<sup>-1</sup> d<sup>-1</sup> could be reached that were in turn used to produce up to 24 mg L<sup>-1</sup> d<sup>-1</sup> PHA in a 1.8-L photobioreactor. To achieve more relevant productivities and final titers, both bacterial species have subsequently been spatially separated in different compartments to guarantee a large area for photosynthesis and a small volume for PHA production. In summary, sucrose produced by *S. elongatus* was used to fuel growth of *P. putida* in a defined mixed culture to produce industrially relevant bioplastics. This modular approach will be expanded on other products available for *P. putida* in the future, making them quasi carbon-neutral and thus more sustainable for future generations.

#### References:

[1] Song et al. (2016) Appl Microbiol Biotechnol 100:7865–7875

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#### BTV12

##### Establishing a technology platform for the conversion of waste to platform chemicals and electrical energy

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**Introduction** Currently, the recycling and reduction of waste is a major international topic. Therefore, we concentrate on the usage of organic waste streams like pure cellulose from paper and cardboard and vegetable waste as substrates for biotechnological production.

**Objectives** One aim is to establish a highly efficient anaerobic community to convert organic waste to volatile fatty acids (VFAs). These will be used on the anode site of a microbial electrolysis cell as carbon and electron source. The remaining organic carbon serves as substrate for a subsequent aerobic production of valuable platform chemicals. Furthermore, a methanogenic biofilm at the cathode will be used to produce a biogas consisting of high percentages (> 90%) of methane.

**Material and methods** Up to now, the most suitable conditions are on the one hand, pH 6 - 7 and 30°C for the fermentation of paper waste, and pH 6 at 60°C for the vegetable waste. Both fermentates contain mainly the VFAs acetate, propionate and butyrate and can therefore serve as substrate for microbial electrochemical cells.

**Results and conclusion** Depending on the inoculum and usage of a methanogenesis inhibitor, acetate and butyrate are degraded and the excess of electrons is transferred to the anode at different coulombic efficiencies. At the cathode, these electrons could be used for the abiotic formation of hydrogen and the subsequent production of methane via the addition of carbon dioxide. Propionate is a rather recalcitrant carbon source under anoxic conditions and, if any, slowly degraded. It can be purified from the fermentate via membrane filtration. The resulting propionate containing solution is used as substrate for genetically optimized *E. coli* strains to generate different platform chemicals under oxic conditions.

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#### BTV13

##### Waste to value - Production of the bioplastic poly(3-hydroxybutyrate) (PHB) and its precursor 3-hydroxybutyrate (3-HB) from waste-gas

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**Introduction & Objectives:** The biopolymer poly(3-hydroxybutyrate) (PHB) is a fully biodegradable polymer and an attractive alternative to petroleum-based plastics. Synthesis gas (CO, H<sub>2</sub>, CO<sub>2</sub>) is a low-cost substrate for fermentations processes and a large-quantity waste gas in steel mill industry. We aimed for construction of gas-utilizing acetogens that produce PHB and its monomeric compound 3-hydroxybutyrate (3-HB).

**Materials and Methods:** The acetogens *Clostridium ljungdahlii* and *C. coskatii* were genetically engineered using two different plasmid-based production pathways for PHB and 3-HB. Recombinant strains were cultivated under heterotrophic and autotrophic conditions. Subsequently, production of 3-HB was analyzed using high performance liquid chromatography and PHB by various techniques such as gas chromatography, microscopy, nuclear magnetic resonance spectroscopy, differential scanning calorimetry, and gel permeation chromatography.

**Results:** Recombinant strains of *C. ljungdahlii* and *C. coskatii* produced considerable amounts of PHB. PHB production in cells was visualized by microscopy. Thermal properties clearly showed all characteristics of PHB with high molecular weight. In contrast, successful synthesis of 3-HB was exclusively observed with engineered *C. coskatii*. Both, PHB and 3-HB proved to be stable products that can be further extracted, purified, and processed for a wide range of applications.

**Conclusion:** Production of the biopolymer and its precursor using recombinant acetogens opens the possibility of producing biodegradable plastic materials from cheap (waste) gases with low variability and large availability compared to other waste substrates.

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#### BTV14

##### Hydrogen storage using acetogenic bacteria as biocatalysts: Hydrogen-dependent CO<sub>2</sub> reductase from a thermophilic Acetogen

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**Introduction:** In times of climate change and increasing CO<sub>2</sub> emissions there is a strong need for alternative renewable energy sources. However, the energy needs to be stored and transported. Hydrogen is an ideal energy carrier that can be produced from renewables in a CO<sub>2</sub>-neutral process by electrolysis. A recently discovered hydrogen-dependent CO<sub>2</sub> reductase (HDCR) from the mesophilic acetogenic Bacterium *Acetobacterium woodii* [1] is a promising biocatalyst for storage of hydrogen in the form of liquid formic acid.

**Objectives:** To expand the temperature range and applicability of HDCRs by searching for enzymes from thermophiles.

**Materials & methods:** Genome analyses revealed HDCR genes in the thermophilic acetogenic bacterium *Thermoanaerobacter kivui*. The enzyme was purified from cell paste of *T. kivui* grown on pyruvate to apparent homogeneity and analyzed.

**Results:** The HDCR purified from *T. kivui* contained four subunits, a hydrogenase subunit and a formate dehydrogenase subunit in addition to two small iron-sulfur containing subunits. The enzyme complex catalyzed H<sub>2</sub>-dependent CO<sub>2</sub>-reduction as well as formate oxidation to CO<sub>2</sub> and H<sub>2</sub> with high rates. The enzyme is orders of magnitude more efficient in the direct hydrogenation of CO<sub>2</sub> than the best recently known chemical catalysts. Furthermore, the enzyme is thermostable and showed the highest catalytic activity at 60 - 70 °C.

**Conclusion:** The HDCR of *T. kivui* is much more active than the already known enzyme from *A. woodii* and has superior stability properties. The enzyme catalyzed H<sub>2</sub>-dependent CO<sub>2</sub>-reduction as well as the reverse reaction and is thus a promising candidate for future applications in hydrogen storage and/or hydrogen production from formic acid.

#### References:

[1] K. Schuchmann and V. Müller (2013), *Science* 342:1382-1385

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#### BTV15

##### Recombinant production of benzylsuccinate synthase and 2-(methyl-naphthyl)succinate synthase

I. Salić<sup>1</sup>, J. Heider<sup>1</sup>

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The pathway of anaerobic toluene degradation is initiated by a radical-type fumarate addition reaction, catalyzed by glycyl radical enzyme benzylsuccinate synthase (BSS). It has been found that benzylsuccinate synthase is a member of several related fumarate-adding enzymes (FAEs), which are involved in the anaerobic biodegradation of various hydrocarbons, both aromatic and aliphatic. Another example is 2-(methyl-naphthyl)succinate synthase (MNS) – a member of FAE family, which is predicted to catalyze a similar radical type fumarate addition reaction to 2-methyl-naphthalene. In our study we have produced recombinant BSS from *Thauera aromatica* K172 and MNS from the 2-methyl-naphthalene degrading culture N47 and show data on the first structure-function relations of FAE.

We established a near-homologous overexpression system of active wild type recombinant BSS in *Azoarcus evansii*, purified and characterized the wild type enzyme and some mutated variants. We tested the conversion of several substrates, including toluene, cresol and the xylene isomers. Remarkably, we found that a single mutation replacing an isoleucine valine, allows the enzyme to convert all xylene isomers, which are not recognized by wild type BSS. In addition, we have purified non-activated BSS and MNS for further structural characterization of the enzyme complexes by X-ray crystallography and spectroscopy.

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#### BTV16

##### Glycosynthases: Biocatalytic synthesis of glycosides

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<sup>2</sup>Forschungszentrum Juelich, Institute of Bio- and Geosciences IBG-1: Biotechnology, Juelich, Germany

The synthesis and analysis of naturally occurring glycosidic compounds can help towards the discovery and development of new bioactive compounds. Chemical synthesis and modification of complex glycosides can be very difficult requiring many synthetic steps. However, new genetically modified glycosidases, namely "glycosynthases", which are void of hydrolytic activity can catalyze selective glycosidic bond synthesis with high yields using activated glycosyl donors such as glycosyl fluorides, azides, or oxazoline structures.<sup>1</sup>

We are working on the identification and characterisation of glycosidases, their mutagenesis towards new glycosynthases, and possible applications in organic synthesis. To achieve this various methods in the area of genetic engineering, biochemistry (biocatalysis, screening) and organic chemistry (compound synthesis, analysis) are combined.

We have produced new glycosynthases, developed an activity assay, which allowed the comparison of the synthetic potential of psychrophilic, thermophilic and mesophilic mutant glycosidases, and demonstrated the possibility of glycodiversification of flavonoids by glycosynthases.<sup>2</sup>

Glycosynthases show a high potential for glycoside synthesis and with the development of new mutants, more and more synthetic problems will be overcome.

#### References:

[1] Hayes, M.R.; Pietruszka, J. *Molecules* **2017**, *22*, 1434.

[2] Hayes, M.R.; Bochinsky, K.A.; Seibt, L.S.; Elling, L.; Pietruszka, J. *Biotechnol.* **2017**, *257*, 162.

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## Short Lecture

### Environmental Microbiology and Ecology 2

17 April 2018 • 08:00–10:00

#### EMV09

##### Microbial communities in soil hotspots and the opportunity to use quantitative DNA-SIP data for carbon turnover models

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In the soil hotspots detritosphere and rhizosphere, fresh plant-derived C is degraded by a complex microbial community. However, C turnover models have barely considered different life strategies and substrate preferences of microbes. Quantitative DNA-SIP data offers detailed insights into the contribution of active key players to the C-turnover in soil hotspots. We propose that this information can be used to improve the description of microbial diversity, composition and processes in C turnover models.

We present 2 case studies: i) We determined the C-flow from differently aged maize litter into bacteria and fungi of an arable soil by applying <sup>13</sup>C-labeled and unlabeled maize litter

on top of soil cores. A reciprocal transplantation of the litter allowed us to track the C-flow at the early (0-4d), intermediate (4-12d) and late stage (28-36d) of decomposition. ii) Wheat was cultivated on undisturbed soil cores in a greenhouse and fumigated with  $^{13}\text{C-CO}_2$  to label rhizosphere bacteria utilizing plant-derived C in topsoil (0-20 cm) and subsoil (20-80 cm). We determined the  $^{13}\text{C}$ -atom fraction excess in the DNA of each OTU by quantitative DNA-SIP and sequencing of bacterial 16S rRNA and the fungal ITS region and used the data as a quantitative estimate for substrate usage.

In both experiments, copiotrophs (Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria) were key players in the  $^{13}\text{C}$ -utilizing bacterial community, whereas oligotrophic phyla (Acidobacteria) exhibited almost no  $^{13}\text{C}$ -enrichment. Differences in substrate quality through time and soil depth significantly influenced the degree of C-turnover and the microbial key players. Our data can be used to augment C-turnover models with phylogenetically resolved  $^{13}\text{C}$ -substrate usage related to specific life strategies.

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#### EMV10

##### **Anaerobic oxidation of methane at low temperatures in subaquatic permafrost environments**

M. Winkel<sup>1,2</sup>, S. Liebner<sup>2</sup>, A. Sepulveda-Jauregui<sup>1</sup>  
K. Martinez-Cruz<sup>1</sup>, J. Mitzscherling<sup>2</sup>, J. Heslop<sup>1,3</sup>, P. P. Overduin<sup>4</sup>  
C. Knoblauch<sup>5</sup>, F. Horn<sup>2</sup>, D. Wagner<sup>2</sup>, K. Walter Anthony<sup>1</sup>

<sup>1</sup>Institute of Northern Engineering, Water And Environmental Research Center, Fairbanks, AK, United States

<sup>2</sup>Deutsche GeoForschungsZentrum GFZ, Helmholtz Zentrum Potsdam, Geomicrobiology, Potsdam, Germany

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<sup>5</sup>Institute of Soil Science, Hamburg, Germany

Subaquatic permafrost in marine and lacustrine systems represent natural chronosequences of high organic matter sediments that thaws over long timescales and can help with understanding future processes in a warming climate. The thawing organic matter introduces microbial substrates that degrade and can produce large amounts of greenhouse gases such as  $\text{CO}_2$  and  $\text{CH}_4$ . Anaerobic oxidation of methane (AOM) in subaquatic permafrost has been suggested to occur but the responsible microbial communities and rates are unknown.

We investigated submarine permafrost sediments of the Siberia Laptev Sea and sediments from the thaw bulb of a thermokarst lake in Central Alaska. We measured methane concentrations and its corresponding stable isotopes, performed stable isotope tracer experiments, and analysed the microbial community with molecular techniques (Illumina sequencing, qPCR and CARD-FISH).

Stable isotope signatures of methane showed oxidation at certain sediment depths. Depending on different fractionation factors, modeled AOM rates in these depths potentially consume 41-100% of the methane *in situ*. Stable isotope tracer incubations for AOM at the same depth showed rates of up to  $3 \text{ pmol cm}^{-3} \text{ d}^{-1}$ . We identified anaerobic methanotrophic archaeal assemblages related to *Methanoperedenaceae* (ANME-2d) and ANME-2a. Quantification of the methyl co-enzyme reductase subunit A (*mcrA*) specific for ANME-2d revealed between  $0.5$  to  $6.7 \pm 0.1$  to  $1 \times 10^5$  copy numbers  $\text{g}^{-1}$  wet weight.

In summary, we show that anaerobic methanotrophic archaeal assemblages are responsible for AOM in deep

subaquatic permafrost sediments at near  $0^\circ\text{C}$  temperatures. This is a central advance of processes that mitigate the permafrost carbon-climate feedback, which have major impacts on the global methane cycle.

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#### EMV11

##### **Hotspots of anammox and nitrification in oligotrophic karstic limestone aquifers**

M. Herrmann<sup>1,2</sup>, C. E. Wegner<sup>1</sup>, S. Kumar<sup>1</sup>, P. Geesink<sup>1</sup>,  
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<sup>3</sup>University of Southern Denmark, Department of Biology, Odense, Denmark

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Despite the high relevance of aquifers as drinking water reservoirs, sources and sinks of nitrate in aquifers and the interconnecting biogeochemical processes are still not fully understood. We assessed nitrogen transformation processes and key microbial players across an oligotrophic limestone aquifer system in the Hainich Critical Zone Exploratory (Germany) sampled at 5 to 88 m depth. Metagenomics- and metatranscriptomics-based analyses along with functional gene targeted quantitative approaches and activity measurements revealed a high spatial heterogeneity of nitrogen transformation reactions across the two aquifer assemblages. Under suboxic conditions with low availability of organic carbon and long groundwater travelling times, anaerobic ammonium oxidation (anammox) contributed an estimated 84% to total nitrogen loss. Under oxic conditions, high abundances of *amoA* genes including those related to *Nitrospira sp.* capable of complete oxidation of ammonia (comammox) suggested a contribution of both canonical nitrification and comammox to an overall nitrification activity of  $14.4 \text{ nmol NO}_x \text{ L}^{-1} \text{ d}^{-1}$ . Potential vertical transfer of soil-borne N cycling microbiota was assessed by comparison of nitrifier communities between groundwater and surface soils and seepage water from the respective recharge areas, revealing strong shifts towards groups with high substrate affinities in the groundwater. The observed high spatial heterogeneity in nitrogen transformation processes and their microbial drivers across different aquifer assemblages in an oligotrophic karstic limestone setting is likely driven by heterogeneity of surface conditions at groundwater recharge areas as well as differences in groundwater residence times and oxygen supply.

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#### EMV12

##### **Filamentous *Desulfobulbaceae* as mediators of cryptic sulfur cycling in upland soils and aquifer sediments**

C. Sachs<sup>\*1</sup>, D. Kanaparthi<sup>1</sup>, T. Lüders<sup>1</sup>

<sup>1</sup>Helmholtz Zentrum München, Institute for Groundwater Ecology, Munich, Germany

Upon water saturation of soils, electron acceptors are sequentially reduced. Recycling is assumed to happen only at oxic-anoxic interphases. However, recently discovered *Desulfobulbaceae* can form cm-long filaments, spanning different redox zones and being able to recycle electron acceptors. As such rapid recycling of sulfur compounds might have an impact, here, we aim to provide evidence for a possible role of filamentous *Desulfobulbaceae* in contaminant and organic matter degradation in flooded soils. Soil and aquifer samples from different sites were incubated as static flooded laboratory columns. Depletion of electron acceptors and pH changes over depth were monitored at

mm-scale, using microsensors. Changes in community composition were examined by PacBio SMRT amplicon sequencing and fluorescence *in situ* hybridization, done at regular times and depths. We devised a novel method of enriching filamentous *Desulfobulbaceae*, using a vertical agar pillar embedded within the soil cores. Our results show oxygen consumption and formation of hypoxic zones over several mm of depth within days after flooding. The sulfidic zone gradually shifted deeper into the core, accompanied by appearance of a characteristic pH peak at the oxic-anoxic gradient, indicating long-distance electron transfer (LDET). Microscopy of this zone showed formation of long *Desulfobulbaceae* filaments. This suggests that LDET by filamentous *Desulfobulbaceae* is possible in upland soils, and could be important after occasional flooding events due to intense precipitation. Our attempts to enrich and isolate these bacteria by agar pillar technique are ongoing. To our knowledge, we are the first to report LDET by filamentous bacteria for upland soils, along with a new possible route to enrich these microbes.

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### EMV13

#### Wind-driven emission of *Enterococcus faecium* from agricultural soil fertilized with poultry manure

N. Thiel<sup>\*1</sup>, V. Junker<sup>1</sup>, S. Münch<sup>2</sup>, P. Siller<sup>3</sup>, O. Biniash<sup>4</sup>, M. Faust<sup>5</sup>, K. Schepanski<sup>5</sup>, T. Amon<sup>4</sup>, U. Rösler<sup>3</sup>, R. Funk<sup>2</sup>, U. Nübel<sup>1</sup>

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The application of livestock manure as fertilizer on agricultural fields results in environmental pollution with fecal bacteria. We investigated the persistence of *Enterococcus faecium* from chicken manure in fertilized agricultural soil on an experimental field site, and measured wind-driven microbiological emissions during the fertilizer application process, during subsequent tillage operations incorporating the manure into soil, and in the weeks after fertilization. Enterococcal isolates from manure and the various samples were matched by genome sequencing.

Dust formation was measured and air samples collected for microbial quantification at varying distances from the dust sources. We found that the emission of *E. faecium* was highest during fertilizer application, followed by tillage operations, even though dust development was stronger during the latter process. Viable *E. faecium* strains genomically indistinguishable from those in the manure were detectable in air samples collected at least 100 meters from the dust source. Regular sampling of the field site revealed that the same *E. faecium* strains survived in the soil for up to seven weeks following fertilization. In addition, we measured the wind-driven release of Enterococci from fertilized soil in controlled wind-tunnel experiments, to determine the parameters critical for bacterial emission and long-distance transport.

In conclusion, we demonstrate the persistence of pathogenic fecal bacteria in manure-fertilized agricultural soil for several weeks and we have assessed their potential for wind-driven emission. We use our data to model bacterial emission fluxes from agricultural soil and their dispersal, to assess the risk of dust-associated transmission, and to evaluate strategies for mitigation.

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### EMV14

#### Unravelling the identity, metabolic potential, and global biogeography of the atmospheric methane-oxidising Upland Soil Cluster $\alpha$

J. Pratscher<sup>1</sup>, J. Vollmers<sup>2</sup>, S. Wiegand<sup>3</sup>, M. Dumont<sup>4</sup>, A. K. Kaster<sup>\*2</sup>

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<sup>2</sup>Karlsruhe Institute of Technology, Institut für Biologische Interfaces (IBG5), Eggenstein-Leopoldshafen, Germany

<sup>3</sup>Radboud University, Nijmegen, Netherlands

<sup>4</sup>University of Southampton, Southampton, United Kingdom

Understanding of global methane sources and sinks is a prerequisite for the design of strategies to counteract global warming. Microbial methane oxidation in soils represents the largest biological sink for atmospheric methane. However, still very little is known about the identity, metabolic properties, and distribution of the microbial group proposed to be responsible for most of this uptake, the upland soil cluster  $\alpha$  (USC $\alpha$ ). This microorganism has evaded cultivation and identification attempts for the past decades. Here, we were able to reconstruct an almost complete draft genome of USC $\alpha$  from a combination of targeted cell sorting via FACS and metagenomes from forest soil, providing the first insights into its phylogeny, metabolic potential and environmental adaptation strategies. The 16S rRNA gene sequence recovered was distinctive and suggests this crucial group as a new genus within the Beijerinckiaceae, close to *Methylocapsa*. Application of a fluorescently labelled suicide substrate for the particulate methane monooxygenase enzyme (pMMO) coupled to 16S rRNA fluorescence *in situ* hybridisation (FISH) allowed for the first time a direct link of the high-affinity activity of methane oxidation to USC $\alpha$  cells *in situ*. Analysis of the global biogeography of this group further revealed its presence in previously unrecognized habitats, such as subterranean and volcanic biofilm environments, indicating a potential role of these environments in the biological sink for atmospheric methane. In addition, a *XoxF*-type methanol dehydrogenase was found in the genome, suggesting the dependence of USC $\alpha$  on lanthanides for growth and activity and making a more targeted cultivation approach possible in the future. These findings were recently (January 2018) published in *Env. Microbiol.*

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### EMV15

#### *Burkholderiaceae* are primary acetate assimilating denitrifiers in peat circles of the arctic tundra capable of complete denitrification at pH 4

S. Hetz<sup>\*1</sup>

<sup>1</sup>Leibniz University Hannover, Institut für Mikrobiologie (Bodenmikrobiologie), Hanover, Germany

Acidic peat circles (pH 4) in the Eastern European Tundra harbor up to 2 mM nitrate and emit the greenhouse gas nitrous oxide (N<sub>2</sub>O) like heavily fertilized agricultural soils in temperate regions. The sequential reduction of nitrate via nitrite, nitric oxide, and N<sub>2</sub>O to dinitrogen gas (N<sub>2</sub>), called denitrification, is the main process yielding N<sub>2</sub>O under anoxic conditions. Crucial factors altering denitrification and impacting the N<sub>2</sub>O/N<sub>2</sub> ratio are organic carbon to nitrate ratios (OC/N) and pH. Low pH (<6) blocks the assembly of a functional N<sub>2</sub>O-reductase of classical neutrophilic model denitrifiers and like a low OC/N results in increased N<sub>2</sub>O/N<sub>2</sub> ratios. Active key denitrifiers of peat circles are important but unknown to date. Thus, it is hypothesized that peat circle denitrifiers are (i) new, (ii) adapted to low pH and capable of complete denitrification and (iii) operate under substrate limitation. The effect of [<sup>13</sup>C]- and [<sup>12</sup>C]-acetate on denitrification was tested in anoxic microcosms +/- supplemental nitrate and +/- acetylene at *in situ* near pH 4. Acetate with nitrate stimulated denitrification by 150% and nitrate stimulated acetate consumption and CO<sub>2</sub> production

rates by 200% relative to unsupplemented controls. In the absence of acetylene, N<sub>2</sub>O was not detectable, suggesting complete denitrification at pH 4. 16S rRNA SIP coupled to Illumina MiSeq v3 amplicon sequencing suggested *Burkholderiaceae*, and other Proteo-, Actinobacteria as well as Verrucomicrobia as key acetate assimilating denitrifiers in peat circles. The combined data indicate that peat circle denitrifiers are operating under substrate limiting conditions due to recalcitrant old peat material and produce large amounts of nitrate derived N<sub>2</sub>O despite their capacity for complete denitrification at pH 4.

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## EMV16

### Biogeography of methanogenic archaea in natural environments

X. Wen<sup>\*1,2</sup>, S. Yang<sup>1,3</sup>, F. Horn<sup>1</sup>, M. Winkel<sup>1</sup>, D. Wagner<sup>1</sup>, S. Liebner<sup>1</sup>

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Methanogenic archaea are major methane producers under anaerobic conditions in numerous natural environments. Whether and how environmental change will propagate into methanogenic community structure and diversity remains largely unknown owing to a poor understanding of global distribution patterns and environmental drivers of this specific group. This study investigated the methanogenic biogeography, diversity and their environmental controls in natural environments. We conducted a meta-analysis (Wen *et al.* 2017, *Front. Microbiol.*) on 94 globally distributed *mcrA* gene sequence datasets from NCBI database. The geographical coordinates and environmental variables were retrieved from corresponding publications. Raw sequences were processed with mothur platform. Ecological and statistical analyses were performed using R packages. Our results reveal a global pattern of methanogenic archaea that is more linked with habitat filtering than with geographical dispersal. At global scale, salinity substantially shapes the methanogenic community composition. In nonsaline soils and lakes, the methanogenic community structure is largely controlled by the combination of temperature and pH. Further analysis on 152 described methanogenic isolates also highlights the control of habitat filtering on their physiochemical features. In addition, estuaries were identified as the most diverse methanogenic habitats but with minor contribution to the global methane budget. In conclusion, our study identifies salinity, temperature and pH as major environmental drivers of methanogenic community composition on a large spatial scale, and large changes of these environmental settings will consequently alter in methanogenic community composition.

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## Short Lecture

### Gene Regulation 1

17 April 2018 • 08:00–10:00

#### GRV01

##### Uncovering the regulatory circuits of the glycine betaine synthesizing pathway in *Bacillus subtilis*

B. Warmbold<sup>\*1</sup>, S. Ronzheimer<sup>1</sup>, M. Fischer<sup>1</sup>, E. Bremer<sup>1</sup>

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Confronted with hyperosmotic stress, the soil bacterium *Bacillus subtilis* accumulates compatible solutes to maintain

cell turgor. Glycine betaine is such a compatible solute that can either be taken up from the environment by the Opu-transporters or can be synthesized from the precursor choline by the dehydrogenases GbsB and GbsA. Upstream of the *gbsAB* gene cluster, the *gbsR* gene is located which encodes a MarR-type choline-responsive repressor regulating the *gbsAB* operon as well as the *opuB* operon, encoding a choline-specific ABC transporter (Nau-Wagner *et al.*, (2012) *J. Bacteriol.* 194:2703-2714).

Bioinformatic analysis and mutational studies showed that GbsR binds a palindromic repeat downstream of the transcriptional start site of the *gbsAB* and the *opuB* operons, and therefore acts through a road-block mechanism. Additionally, we conducted a comprehensive targeted mutagenesis study based on a homology model of the GbsR protein. This approach, provided evidence that choline is bound by an aromatic cage present in GbsR, which matches that of substrate binding proteins acting in conjunction with ABC transporters.

Since GbsR acts as a repressor of the *opuB* but not of the closely related *opuC* operon, we studied the networks involved in the regulation of both gene clusters at the transcriptional level using reporter gene fusions. This showed a strikingly different expression pattern between the two gene clusters in response to extracellular salinities. However, they share the regulation through the MarR-type repressor OpuCR. We uncovered the physiological role of OpuCR, by demonstrating that this regulator is involved in the re-establishment of *opuC* repression under high salt concentrations, a function in agreement with the salt-induced expression of the *opuCR* gene itself.

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#### GRV02

##### The RirA transcriptional regulator of *Dinoroseobacter shibae* senses iron via a [3Fe-4S]<sup>1+</sup> cluster as cofactor

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**Introduction:** Next to genes encoding iron regulators Fur and Irr, the *Dinoroseobacter shibae* genome exhibits a gene encoding a protein homolog to the rhizobial iron regulator RirA.

**Objectives:** Role of RirA from *D. shibae* in iron-dependent gene regulation.

**Materials & Methods:** Anaerobic RirA protein was recombinantly produced and purified. UV/Vis, electron paramagnetic resonance (EPR) spectroscopy as well as *in vivo* whole cell Mössbauer analyses were used to determine the nature of the Fe-S cluster. Four cysteine residues of the RirA protein were changed to alanine *via* site directed mutagenesis. DNA binding was analyzed and a specific binding site was defined using Footprint analyses and EMSA.

**Results:** UV/Vis spectroscopy revealed an absorption shoulder at 420 nm, typical for Fe-S cluster containing proteins. Using EPR and Mössbauer spectroscopy, a [3Fe-4S]<sup>1+</sup> cluster was identified. Three of the four cysteine mutants of RirA showed a reduced absorption in UV/Vis measurements and may serve as ligands for the [3Fe-4S]<sup>1+</sup> cluster. Using Footprint and EMSA analyses, binding of RirA under anaerobic conditions at the specific binding site 5'-TTAA-N<sub>10</sub>-AATT-3' of *hemB2* was shown. After exposure to

oxygen, the cluster degraded and RirA is no longer able to form a DNA-protein complex.

**Conclusion:** RirA of *D. shibae* is a novel iron sensor that is different from other members of the Rrf2 family in coordinating a [3Fe-4S]<sup>1+</sup> cluster to respond to changing iron concentrations in the cell. RirA binds to a newly identified DNA binding motif and represses transcriptional activation. Iron limitation leads to the conversion of RirA into its apo form and subsequently to de-repression of *hemB2* gene transcription.

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## GRV03

### The components of the unique Zur regulon of *Cupriavidus metallidurans*

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Zinc is an essential trace element but toxic at high concentrations. The heavy metal resistant bacterium *C. metallidurans* accumulates in mineral salts medium 63.000 zinc atoms/cell (1). To maintain a zinc homeostasis the organism needs a stringent regulation of uptake and efflux processes. A highly efficient removal of surplus zinc from the periplasm by the CzcCBA-system is responsible for the outstanding metal resistance of the organism (2). Rather than having a typical Zur-dependent, high-affinity ZnuABC for zinc uptake at low concentrations, *C. metallidurans* has the secondary zinc importer ZupT of the ZRT/IRT-like protein family. It is important to understand, how this zinc-resistant bacterium manages exposure to low zinc concentrations. Members of the Zur regulon in *C. metallidurans* were identified by comparing transcriptomes of a  $\Delta zur$  mutant and its parent strain, with the prediction for Zur-binding boxes in this organism (3,4). The binding of Zur to these boxes was confirmed by EMSA. Two Zur boxes upstream of the *cobW1*, encoding a putative zinc chaperone, were required for complete repression of *cobW1* and its downstream genes in cells cultivated in mineral salts medium. Zur boxes upstream of *zur*, *cobW3* and *zupT* permitted upregulation under zinc starvation situations. This demonstrates a compartmentalization of zinc homeostasis, where the periplasm is responsible for the removal of surplus zinc and cytoplasmic components are responsible for the management of zinc as an essential cofactor. Both compartments seem to be connected by ZupT.

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## GRV04

### Characterization of LftR, a PadR-like transcription factor in *Listeria monocytogenes*

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**Introduction:** Transcriptional regulators of the PadR family contribute to resistance against antibiotics and small toxic compounds in many bacteria. LftR, a PadR-homologue of the important human pathogen *Listeria monocytogenes* was previously shown to affect ethidium bromide resistance and virulence.

**Objectives:** To better understand the role of LftR in physiology of *L. monocytogenes*, we studied the phenotype of a  $\Delta lftR$  mutant using various genetic and physiological approaches.

**Materials & methods:** Antibiotic resistance of  $\Delta lftR$  and wildtype bacteria was determined. The influence of LftR on global gene expression was studied using RNA sequencing. LftR-dependent genes were confirmed using promoter-*lacZ* fusions, Northern blotting as well as enzyme activity assays. Agents inducing the LftR response were identified using a screening strain expressing an LftR-dependent  $\beta$ -galactosidase gene.

**Results:** RNA-Seq showed that LftR tightly represses its own operon and that of the *lieAB* drug efflux pump. Measurements of LacZ activity revealed that both operons are induced in the presence of rhodamine. Furthermore, the *lftR* deletion had a strong effect on genes regulated by the alternative sigma factor  $\sigma^B$ . Phenotypic changes include altered resistance to antibiotics and aromatic dyes, as well as chitinase inactivity and invasion deficits into HeLa cells.

**Conclusion:** LftR controls the transcription of the LieAB efflux pump and the *lftRS* operon in *L. monocytogenes* EGD-e. This repression is relieved by rhodamine dyes. However, LftR also interferes with activity of the alternative sigma factor  $\sigma^B$  and thus has a broad effect on  $\sigma^B$  dependent phenotypes.

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## GRV05

### Synthetic countersilencing as an approach to study evolutionary network expansion

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**Introduction:** Horizontal gene transfer is a major driver of microbial evolution. However, the acquisition of foreign genes requires stringent regulation of gene expression. Previously, we described CgpS as a xenogeneic silencer of *Corynebacterium glutamicum* prophages (1). Interference by the binding of a specific TF may oppose silencer activity and relieve gene repression. However, the underlying rules of this disruptive countersilencing mechanism are poorly understood so far.

**Objectives:** In this study, a synthetic approach was used to provide insights in the evolution of TF-mediated xenogeneic countersilencing.

**Methods:** Synthetic CgpS target promoters with and without additional TF operator sites were fused to a reporter gene (*venus*) and analyzed by fluorescence microscopy and a microtiter cultivation system.

**Results:** The insertion of different TF operator sites into CgpS target promoters revealed that sequence composition as well as the position have a significant impact on countersilencing efficiency. Insertion at the center of the CgpS peak showed a very low basal expression level and an effector dependent induction mediated by the binding of the specific TF. The binding of TFs and CgpS to synthetic

promoter constructs was confirmed *in vitro* by EMSAs and surface plasmon resonance. Based on the synthetic countersilencing principle, a metabolic toggle switch under control of the gluconate-dependent TF GntR was constructed.

**Conclusion:** We propose that the presented synthetic countersilencing approach will enhance our understanding of evolutionary network expansion in bacteria and that the mechanism of xenogeneic countersilencing bears a great potential for the modular design of synthetic regulatory circuits.

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#### GRV06

##### Cell cycle regulator DnaA modulates the switch between solo and social lifestyles of *Sinorhizobium meliloti* via multiple regulatory feed-forward loops

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**Introduction:** Bacteria typically exist in populations of socially interactive team players. However, knowledge of how and when bacteria "decide" to cooperate or to go solo is limited. In *Sinorhizobium meliloti*, a soil-dwelling model bacterium for studying plant-microbe symbiosis, we found that social behavior is primarily subject to nutrient availability. This was unexpected, since most bacteria use quorum sensing as the primary trigger of the social lifestyle.

**Objectives:** In this study, we were primarily interested in two questions: How important is nutrient sensing for determining the onset of social activity, and how is nutrient sensing integrated mechanistically with quorum sensing in this process?

**Methods:** We generated genetic knockouts, and used a novel triple fluorescent reporter cassette to follow the expression of multiple genes and capture the onset of social activity in micro-colonies grown under controlled nutritional conditions using time-lapse microscopy. Furthermore, we used microarray and ChIP-Seq supported by reporter assays to uncover the molecular mechanisms.

**Results:** We found a novel regulatory network consisting of several interacting genes that set social activity. These are controlled by DnaA, the master regulator of the cell cycle, meaning that DnaA also regulates social activity. Curiously, DnaA both activates and represses social activity via multiple mechanisms.

**Conclusions:** Transcription control by DnaA creates tension between opposing mechanisms within the regulatory network controlling social behavior. Nutrient limitation rapidly inactivates DnaA, whereupon social activity begins. Thus DnaA functions as a switch between growth – essentially a competitive behavior – and social behavior.

#### GRV07

##### Unconventional biofilm formation of *E. coli* Nissle 1917

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**Question:** The probiotic, commensal strain *Escherichia coli* Nissle 1917 (EcN) is a good biofilm former at both ambient and human body temperature 37°C. The extracellular matrix produced by EcN biofilms can consist of both proteinaceous curli fimbriae and the exopolysaccharide cellulose. Biofilm formation is promoted by high levels of the second messenger c-di-GMP, which is synthesized by diguanylate cyclases (DGC) and degraded by phosphodiesterases (PDE). Surprisingly, EcN cellulose expression is independent of the regulator CsgD that is essential for biofilm formation in other *E. coli*, and of the DGCs AdrA or YedQ, which deliver the c-di-GMP critical for allosteric activation of the cellulose synthase BcsA. As biofilms might contribute to the probiotic characteristics of EcN, the regulation of cellulose expression at 37°C will be studied.

**Methods:** Genome sequencing was combined with motility tests and biofilm assays in liquid culture and on agar plate.

**Results:** Overexpression of PDEs in EcN indicated the importance of c-di-GMP for production of the extracellular matrix. To identify the responsible c-di-GMP turnover protein(s), strain IMI699, a spontaneous non-biofilm forming laboratory EcN mutant was studied. Analysis of the IMI699 genome sequence revealed a non-sense mutation in *yegE*, encoding a DGC, leading to loss of function. Overexpression of full-length *yegE* cloned from EcN wild type could restore biofilm formation in IMI699.

**Conclusions:** YegE was previously shown to promote c-di-GMP-dependent *csgD* expression, explaining the absence of curli gene expression in IMI699. Presumably, YegE-derived c-di-GMP activates the cellulose synthase BcsA by binding to its PilZ domain at both 30°C and 37°C and is thus critical for cellulose expression in EcN.

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#### GRV08

##### Differential view on the bile acids stress response of *Clostridioides difficile*

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*C. difficile* is an intestinal human pathogen that uses the opportunity of a depleted microbiota to cause an infection. It is known, that composition of the individual bile acids cocktail has a great impact on the susceptibility towards *C. difficile* infections, and that especially secondary bile acids produced by intestinal bacteria hamper *C. difficile* proliferation.

We aim to elucidate the molecular mechanisms of the differential effects single bile acids have on vegetative cells of *C. difficile*.

Sub-lethal concentrations of the main bile acids (cholic-, chenodeoxycholic-, deoxycholic- and lithocholic acid) were determined in shock experiments and for long-term challenge. An LC-MS-based proteomics approach was used to record stress signatures for all four mentioned bile acids and a mixture of them. Comprehensive datasets were

visualized via PCA, Voronoi treemaps and volcano plots. Motility assays were performed on swimming agar plates, and morphological alterations tracked by negative contrast electron microscopy.

Inhibitory concentrations for the single bile acids vary significantly. Due to the common steroid structure, a general overlapping stress response could be determined for all tested bile acids. However, several proteins showed an altered abundance in the presence of only a single or a few of the bile acids indicating the existence of specific stress responses. Proteomics revealed a loss of flagella in bile acids-stressed *C. difficile* cells, which was supported by electron microscopy images and a reduced motility of the bacterium.

Proteins involved in the general and specific stress adaptation to bile acids were identified in this study and will be in the focus of our future work.

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## Short Lecture

### Microbial Diversity and Microbiota

17 April 2018 • 08:00–10:00

#### MDMV01

##### Dead or Alive? Molecular life-dead distinction in human stool samples reveals significantly different composition of the microbial community

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**Question:** The gut microbiome is strongly interwoven with human health. To analyse the composition of the gut microbiome, stool samples are subjected to 16S rRNA gene targeting next generation sequencing (NGS) and results are correlated with clinical parameters. However, some microorganisms might not be alive at the time point of sampling, and thus might have less impact on the human. As conventional next generation sequencing methods lack the opportunity to differentiate between the viable and dead microbial components, retrieved results give only limited information. Propidium monoazide (PMA) is used in food safety monitoring to discriminate living from dead cells. It binds to free DNA and masks it for subsequent procedures, such as PCR reaction and sequencing. It was our goal to test the impact of PMA on the results of 16S rRNA gene-targeting NGS from human stool samples and to determine the optimal concentration and protocol.

**Methods:** We treated fresh stool samples with a concentration series (0-300  $\mu$ M PMA) and subjected the samples to NGS. Data were analysed and compared.

**Results:** A substantial part of the human microbial community is dead at the timepoint of sampling and PMA treatment significantly reduces the diversity and richness of the sample analyzed. Concentrations of 100  $\mu$ M PMA were sufficient to discriminate signals from disrupted microbial cells.

**Conclusion:** The application of PMA in complex microbiome samples is possible, if the PMA concentration is adapted accordingly. We prove, that the classical NGS approach, compared to our PMA-NGS, reveals significant differences in microbial composition and the abundance of certain important microorganisms (i.e. *Akkermansia*, *Bacteroides*).

Our findings challenge a number of suggested microbiome-disease correlations.

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#### MDMV02

##### Metatranscriptome analysis of the vaginal microbiota reveals potential mechanisms for recurrence and protection against metronidazole in bacterial vaginosis

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Bacterial vaginosis (BV) is a prevalent multifactorial disease of women in their reproductive years characterized by a shift from the healthy *Lactobacillus sp.* dominated microbial community towards a highly diverse anaerobic community. BV can initially be cured by antibiotic therapy in most women, but the high recurrence frequency represents a serious challenge. Moreover, for unknown reasons, a small number of women does not respond to therapy. In a clinical study, out of 37 women diagnosed with BV, 31 were successfully treated with a single peroral dose of metronidazole, while 6 still had BV after treatment. Here, we performed a metatranscriptome analysis of the vaginal microbiota of the subgroup of those non-responding patients (N = 6), comparing them to patients (N = 8) who were successfully treated. Moreover, we followed the changes in the metatranscriptome composition over three months in two patients with recurrence and two that were cured. In health, *Lactobacillus iners* and *L. crispatus* contributed more than 90% of all bacterial transcripts, but they did not co-occur. Their functional profiles *in vivo* confirmed the pathogenic role of *L. iners*. In BV, *Gardnerella vaginalis* contributed on average 37% of all transcripts. In some patients, transcripts from *Gardnerella* species isolated from the bladder were abundant. In non-responding patients, *cas* genes of *G. vaginalis* were highly up-regulated. We hypothesize that colonization by *L. iners* and re-infection through the bladder contribute to recurrence of BV. *Cas* genes of *G. vaginalis*, in addition to protecting against phages, might be involved in DNA repair thus mitigating the bactericidal effect of DNA damaging agents like metronidazole. The clinical relevance of these findings needs to be confirmed.

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#### MDMV03

##### Tight associations: insights on the microbiome of barley seeds

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Endophytes are microorganisms colonizing plant internal tissues. They are ubiquitously associated with plants and play a major role in plant growth and health. Among all endophytes, those found inside seeds are of particular interest, as they are the first to colonize the tissue of the seedlings. Although significant efforts have been made to determine the composition of the endophytic bacterial communities, less is known about their activity. Therefore, we investigated the active bacteria communities residing in seeds and their relevance for composition of root tissues of barley in different growth stages, using an RNA-based approach. Moreover, metagenomic analyses of seed-borne root endophytes were carried out to obtain information about the functional traits provided by the seed microbiome. We found out that members

of the genera *Phyllobacterium*, *Paenibacillus* and *Trabusiella* were the most abundant active bacteria inside germinating seeds. The presence of the later families was also confirmed by cultivation (e.g. *Panaenibacillus hordei* and *Pantoea agglomerans*). Metagenomic analysis revealed that these microbes harbor genes related to functions assumed to be essential for plants to grow in different environments, such as antioxidative stress enzymes or osmoprotectants. We also detected genes coding for plant signaling molecules, e.g. indole acetic acid and ACC deaminase. Though some of these populations were also found in the roots, we found a greater influence of soil microbes on the composition of the community, particularly at later growth stages. We hypothesize that the seed endophytes play a role during germination, but becomes less relevant as the plant develops, where endophytic bacterial populations most probably originates from the soil.

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#### MDMV04

##### **High-resolution metaproteomics of biogas plants revealed two major patterns of methanogenesis and highlighted possible impact of Bacteriophages on microbial communities**

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In biogas plants (BGP), microbial communities convert biomass to methane and carbon dioxide. However, the majority of microorganisms and their metabolic functions are unknown. Furthermore, efficiency and performance of biomass conversion depends on interactions, e.g. syntrophy or competition, between microorganisms.

Metaproteomics detected approx. 17,000 protein groups covering the majority of the expected metabolic networks of 11 analysed BGPs. Regarding the methanogenic *Archaea* two metabolic clusters of BGPs were observed among: (i) acetoclastic and hydrogenotrophic methanogenesis and (ii) hydrogenotrophic methanogenesis, exclusively. In the latter case, the presence of enzymes of the Wood-Ljungdahl pathway in *Bacteria* implicated syntrophic acetate oxidation.

Investigation of microbial interactions showed that besides syntrophic interactions and competition for substrates host-phage interactions might play a crucial role regarding the taxonomic and functional composition of the microbial communities. Considering the small size of phages in comparison to bacterial cells the average abundance of phage proteins (2% of total protein) related to about five phages per cell. Main targets of phages are presumably *Bacteria*, in particular *Bacillaceae*, *Clostridiaceae*, whereas *Archaea* are most likely infected, rarely. A higher abundance of CRISPR proteins found for *Archaea* could contribute to a lower infection risk by phages. Regarding the impact of phages on metabolic functions, the presence of phages infecting hydrolytic and fermenting bacteria, e.g. *Clostridiales*, could disturb anaerobic digestion. But the release of nutrients including essential growth factors from lysed cells could support the growth of auxotrophic microbes depending on complex media.

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#### MDMV05

##### **The evolutionary diversity of the "prokaryote" cell membrane from the perspective of lipid components**

B. J. Tindall<sup>\*1</sup>

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The cell membrane is the single most complex part of the cell, with up to half of the genome encoding components that are integral parts of it or are directly associated with it. The cell membrane defines a boundary between the cytoplasm and the external environment and is an important aspect of our concept of the cell. All components entering or leaving the cell must cross the cell membrane. An essential class of compounds that make up the cell membrane and are indispensable for its proper functioning are lipids. Despite the wide diversity of lipids documented in prokaryotes the "text book" view of lipids is severely limited. An appreciation of the diversity of lipids is fundamental to understanding the evolutionary origin(s) of the cell membrane as well as the origin and diversification of cellular life. Current knowledge indicates that lipids are divided into two fundamentally different classes, those derived from fatty acids and those derived from isoprenoids. While both classes may be found in all cellular forms of life specific structures may be limited to defined evolutionary groups. A small number of enzymes may be responsible for the synthesis of compounds that point to a polyphyletic origin of compounds that make up the cell membrane. Furthermore, there appears to be no evidence for the presence of even one polar lipid that is universally distributed in all members of the Bacteria and Archaea. Combining chemical analysis with enzymological and genomic information allows one to begin to document the diversity at all three levels and to begin to appreciate evolutionary and functional diversity imparted by the lipid components of the cell membrane to the evolution and functioning of modern membranes and the resulting cellular systems.

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#### MDMV06

##### **EDGAR: a versatile tool for phylogenomics**

J. Blom<sup>\*1</sup>, M. Dieckmann<sup>1</sup>, T. Juhre<sup>1</sup>, J. G. Schrader<sup>1</sup>, S. P. Gläser<sup>2</sup>, A. Goesmann<sup>1</sup>

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The deployment of next generation sequencing approaches has caused a rapid increase in the number of completely sequenced genomes. As one result of this development, it is feasible to analyze not only single genomes, but large groups of related genomes in a comparative approach. In the last 8 years, the EDGAR platform has become one of the most established software tools in the field of comparative genomics. Since, the software has been continuously improved and a large number of new analysis features have been added. In recent years, the use of EDGAR for core genome based phylogenomic/taxonomic analysis has become a main application field of the software.

The web-based user interface offers Venn diagrams, synteny plots, and a comparative view of the genomic neighborhood of orthologous genes. Recently, the software was extended with various new features like statistical and phylogenetic analyses, replicon grouping options and second level analyses of meta-genesets. Furthermore, EDGAR calculates core genome based phylogenetic trees as well as amino acid identity (AAI) and average nucleotide identity (ANI) matrices. Thus the software supports a quick survey of evolutionary relationships and simplifies the process of obtaining new

biological insights into the differential gene content of kindred genomes.

EDGAR provides 322 genus based public databases with 8079 genomes. In addition, there are ~500 private projects with more than 18,000 genomes that are used by scientists from all over the world to analyze unpublished data. EDGAR is a free-for-academic-use service, funded as a service of the 'German Network for Bioinformatics Infrastructure – de.NBI'. EDGAR is available via the public web server <http://edgar.computational.bio>.

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#### MDMV07

##### Genome-scale data resolve phylogeny and classification of various bacterial phyla

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Due to the staggering advances in sequencing technology it should now be possible, in principle, to base phylogeny and classification of microorganisms on information from genome-scale data. The lack of genome sequences for type strains of major lineages of prokaryotes is, of course, a real obstacle for a genome-based classification of microorganisms, but phylogeny-driven microbial genome sequencing projects such as the Genomic Encyclopedia of Archaea and Bacteria (GEBA) and the One Thousand Microbial Genomes (KMG) project, which are briefly introduced, are currently solving this issue. However, genome sequencing alone will not yield an adequate taxonomic classification of *Archaea* and *Bacteria*. Adequate methods are needed to infer phylogenies from genome-scale data and taxonomic classifications from these phylogenies. For this reason, I will recapitulate the goal of phylogenetic classification and its historic origin and then contrast it with contemporary approaches in microbial systematics. The relationship between phenotypic and genomic data with respect to phylogenetic classification will also be discussed. Phylogenies and classifications should be derived from genome-scale data despite the prevalence of horizontal gene transfer, which had led some authors to instead conclude that both the idea of a microbial tree of life and the hierarchical classification of prokaryotes should be dismissed. Examples from distinct phyla of prokaryotes illustrate genomic approaches to obtain a phylogenetic classification of microorganisms.

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#### MDMV08

##### A phylogenetic framework for the kingdom Fungi based on 18S rRNA gene sequences

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The usage of molecular phylogenetic approaches is critical to advance the understanding of systematics and community processes in the kingdom Fungi. Among the possible phylogenetic markers (or combinations of them), the 18S rRNA gene appears currently as the most prominent candidate due to its large availability in public databases and informative content. The purpose of this work was the creation of a reference phylogenetic framework that can serve as ready-to-use package for its application on fungal classification and community analysis. The current database contains 9,329 representative 18S rRNA gene sequences covering the whole fungal kingdom, a manually curated alignment, an annotated and revised phylogenetic tree with all the sequence entries, updated information on current taxonomy, and

recommendations of use. Out of 201 total fungal taxa with more than two sequences in the dataset, 179 were monophyletic. From another perspective, 66% of the entries had a tree-derived classification identical to that obtained from the NCBI taxonomy, whereas 34% differed in one or the other rank. Most of the differences were associated to missing taxonomic assignments in NCBI taxonomy, or the unexpected position of sequences that positioned out of their theoretically corresponding clades. The strong correlation observed with current fungal taxonomy evidences that 18S rRNA gene sequence-based phylogenies are adequate to reflect genealogy of Fungi at the levels of order and above, and justify their further usage and exploration.

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#### Short Lecture

##### Fungal Biology 2

17 April 2018 • 08:00–10:00

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#### FBV09

##### Functional characterization of a candidate core effector protein with endonuclease features in the endophytic fungi *Serendipita indica*

H. Widmer\*<sup>1</sup>, S. Wawra<sup>1</sup>, A. Zuccaro<sup>1</sup>

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The root endophyte *Serendipita indica* is able to colonize a broad range of plants but can also live saprotrophically. Among the experimental hosts are *Arabidopsis thaliana*, a non-mycorrhizal plant, and barley, an agriculturally important crop. The long-lasting symbiosis between *S. indica* and the plants leads to growth promotion and also to protection against biotic and abiotic stress. After an initial biotrophic phase *S. indica* triggers cell death in different plant hosts during compatible root colonization. The fungal elicitors/effectors that initiate this process and the host biochemical pathway that underpins the execution of this cellular program remain largely unknown. We functionally characterized a fungal secreted endonuclease which was found in the apoplastic fluid of colonized barley roots. Using fluorescence microscopy, biochemical assays and overexpression/ gene deletion we studied the role of this endonuclease in interaction with the plant host and its involvement in fungal triggered root cell death. We propose that the type of induced cell death is decisive to the outcome of fungus-root interactions.

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#### FBV10

##### *Exophiala dermatitidis*: stress resistance and pathogenicity

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*Exophiala dermatitidis*, belongs to the order *Chaetothyriales* (Ascomycetes) and to the group of so called "black yeasts". The fungus is able to thrive in extreme environments such as deserts, glaciers or salterns but also in man-made extreme niches such as sauna facilities or dishwashers. In warm blooded animals *E. dermatitidis* can cause subcutaneous and cutaneous phaeohyphomycosis but also systemic infections especially in immunocompromised patients. Knowledge about the different courses of disease which differ between etiologies, virulence factors and host response are scarce while the number of cases affecting

cystic fibrosis patients is increasing. One known virulence factor is melanin, a black pigment in the cell wall, which is thought to be an agent to withstand harsh and extreme conditions. To understand more about the infection mechanism of the fungus we set up an artificial ex-vivo skin model infection and did a transcriptome analysis. Different up- and downregulated pathways and genes during infection and a complex genome organization were revealed. To overcome the small genome size, the fungus uses multi-functional transcripts, either working as coding or/and ncRNAs. The melanin pathway used by the fungus during infection could be determined. Based on this results, a melanin deficient mutant was produced using the Crispr/Cas9 technology for the first time in black yeast. To understand the contribution of melanin for pathogenicity we repeated the artificial skin infection and compared the mutant with the wildtype on a transcriptome level.

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## FBV11

### Fungus-feeding amoeba *Protostelium aurantium* identifies the survival strategies of *Candida parapsilosis* during predatory-prey interactions

S. Novohradská<sup>1</sup>, J. Sprague<sup>1</sup>, M. Sprenger<sup>2</sup>, R. Tóth<sup>3</sup>, J. Linde<sup>4</sup>, T. Wolf<sup>4</sup>, S. Brunke<sup>2</sup>, A. Gáscher<sup>3</sup>, F. Hillmann<sup>1</sup>

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<sup>3</sup>University of Szeged, Department of Microbiology, Szeged, Hungary

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Amoeba predation as an evolutionary driver towards the generation and maintenance of microbial virulence traits represents a prominent hypothesis for many bacteria, but has rarely been considered for fungal pathogens. Here, we have studied an exclusively mycophagous amoeba, *Protostelium aurantium*, which we have isolated and completely sequenced its genome and transcriptome. Large-scale screenings revealed a broad prey spectrum across the fungal kingdom with an extremely rapid recognition followed by a highly efficient intracellular killing. This process is mediated by the disruption of the fungal heavy metal homeostasis, which subsequently leads to an impaired oxidative stress response. Overexpression of copper and iron exporters during amoeba predation, together with the simultaneous down-regulation of respective importers, might induce the loss of internal heavy metal resources indispensable for the anti-oxidative function of superoxide dismutases. In order to stabilize the redox and pH balance, *Candida parapsilosis* upregulates thioredoxin-linked peroxidase, which reduces both hydrogen peroxide and tert-butyl hydroperoxide. A deletion mutant for this enzyme showed diminished growth in the presence of ROS and absence of methionine, residues of which are oxidized in order to protect proteins from oxidative damage. As a new natural model to study phagocytic interactions between amoeba and its fungal prey, we further conducted a dual-transcriptome approach using three *Candida* species: *C. parapsilosis*, *C. albicans*, and *C. glabrata*. Interestingly, we observed similar responses of the two edible yeasts towards the amoeba predation in comparison to the non-edible *C. glabrata*, which did not show significant changes in gene expression despite the massive uptake of this yeast.

## FBV12

### *Randia spinosa* L: A Potential Drug Candidate Targets Cell Membrane Of *Candida Albicans* And Its Associated Virulence Factors

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Resistance to present antimicrobial drugs and the incidence of life-threatening opportunistic fungal infections are extensively growing across in worldwide. In this study, we have investigated the anticandidal potentials of partially purified aqueous fraction (AF10) obtained from *Randia spinosa* fruits. We evaluated the active fraction AF10 MIC, MFC and mechanism of action against *Candida albicans* virulence factors include secretory aspartyl protease (SAPs), phospholipases (PLAs), biofilm formation, yeast to hyphal transition and cell membrane damage using *in vitro* assays. The AF10 significantly inhibited the growth of *C. albicans* at a MIC value of 0.1 mg ml<sup>-1</sup>. Further, we observed a significant inhibition of the secretion of virulence enzymes - SAPs and PLAs and also germ tube formation when used AF10 at 50 and 25µg ml<sup>-1</sup>. Interestingly, confocal, scanning electron microscope and UV spectrum studies revealed that the AF10 is capable to damage yeast cell membrane flux through ergosterol biosynthesis and specifically inhibited lanosterol-14-alpha demethylase (ERG11). In addition, the active fraction AF10 promises significant inhibition of *C. albicans* biofilm formation. Further, the active fraction AF10 was subjected to analyze for GC-MS, FT-IR and the resulted spectral data revealed one possible active molecule as randinoside. In conclusion, this active molecule randinoside mechanistically acts through targeting cell membrane and thereby it promotes intracellular leak followed by inhibition of virulence factors. This present investigation assures and leading to identify and develop novel anticandidal molecules with the potential mechanism of action against *C. albicans* from *R. spinosa*.

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## FBV13

### Ability of Extracellular proteases of micromycetes *Aspergillus flavipes*, *Aspergillus ochraceus* and *Aspergillus sydowii* to affect proteins of the human hemostatic system

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Micromycetes produce proteases capable to promote proteolysis of haemostasis proteins or, along with hydrolytic activity, to show the ability to activate proenzymes of this system transforming them into an active form. In this regard, it is actual to reveal the spectrum of proteolytic action of extracellular proteases of micromycetes towards the haemostasis proteins and allows us to consider them perspective substitutes of components in corresponding diagnostic sets and therapeutic remedies.

Study the influence of *A. flavipes* A17, *A. ochraceus* L-1 and *A. sydowii* 1 proteinases on the human haemostasis proteins.

Micromycetes were cultivated in submerged conditions, protease activity detection was made using chromogenic

peptide substrates specific for haemostasis proteases, with (direct amidolytic activity) and without the addition of blood plasma (activator activity).

It was shown, that all three strains, to a different extent, possess proteolytic activity and their proteolytic enzymes are capable to cleave some of used substrates, mainly to thrombin and plasmin substrates. Moreover, proteases of *A. flavipes* A17 and *A. ochraceus* L-1 showed activator activity against protein C and factor X, while *A. sydowii* 1 proteases did not show any activator activity. For proteases of *A. flavipes* A17, the activator activity towards protein C and factor X was shown for the first time, but their activity was lower than activity of proteases of *A. ochraceus* L-1.

The effect of extracellular proteases of *A. flavipes* A17, *A. ochraceus* L-1, and *A. sydowii* 1 on proteins of the human haemostasis system was studied. *A. ochraceus* L-1 as a producer of potent activators of protein C and factor X for diagnostic medicine was selected for further study.

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## FBV14

### SCF Ubiquitin Ligase F-box Protein Fbx15 Controls Nuclear Co-repressor Localization, Stress Response and Virulence of the Human Pathogen *Aspergillus fumigatus*

B. Jöhnk<sup>\*1</sup>, Z. Bayram<sup>1,2</sup>, A. Abelmann<sup>\*1</sup>, T. Heinekamp<sup>3</sup>, D. J. Mattern<sup>3</sup>, A. A. Brakhage<sup>3</sup>, I. D. Jacobsen<sup>4</sup>, O. Valerius<sup>1</sup>, G. H. Braus<sup>1</sup>

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E3 ubiquitin ligases ubiquitinate specific target proteins, eventually triggering their proteasomal degradation. F-box proteins share the F-box domain to connect substrates of E3 SCF ubiquitin RING ligases through the adaptor Skp1/A to Cul1/A scaffolds. Fbx15 is a fungal specific F-box protein, which is required for the general stress response in the human pathogen *Aspergillus fumigatus*. Oxidative stress induces *fbx15* gene expression leading to 3x elevated Fbx15 protein levels. In addition it triggers the dephosphorylation of Fbx15, which is phosphorylated during non-stress conditions. Fbx15 binds to SsnF(Ssn6), a part of the RcoA(Tup1)-SsnF(Ssn6) transcriptional co-repressor complex and is essential for its nuclear localization. Dephosphorylation of Fbx15 prevents the nuclear localization of SsnF and results in derepressed gliotoxin gene expression. Fbx15 exhibits a second function dependent on its F-box domain, which acts as SCF interaction site and is required for complete stress tolerance and gliotoxin biosynthesis repression. The absence of Fbx15 in *A. fumigatus* leads to an avirulent phenotype *in vivo* in immunosuppressed CD-1 mice. Fbx15 has a novel dual function by controlling transcriptional repression and being part of SCF E3 ubiquitin ligases, which is essential for stress response, gliotoxin production and virulence in the opportunistic human pathogen *A. fumigatus*.

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## FBV15

### A functional homolog of Hippo kinase is required for fungal development

D. Radchenko<sup>\*1</sup>, U. Kück<sup>1</sup>

<sup>1</sup>Ruhr University Bochum, Allgemeine und Molekulare Botanik, Bochum, Germany

**Introduction:** Hippo pathway is a large eukaryotic signaling network with a core composed of a germinal center (GC) kinase and NDR kinase two-step cascade. Its malfunction in animals leads to tissue overgrowth and tumor formation. The signaling architecture of the Hippo core resembles the fungal septation initiation network (SIN), which regulates cytokinesis in fission yeast as well as septation in filamentous fungi. The conserved striatin interacting phosphatases and kinases (STRIPAK) complex acts as a negative regulator of Hippo in animal cells and SIN in fission yeast. Recently, STRIPAK in filamentous fungi was characterized extensively at the molecular level, but its connection with SIN remains vague.

**Objectives:** Genetic and functional analysis of a STRIPAK-associated kinase KIN3 in *Sordaria macrospora*, an ascomycetous model organism. KIN3 is believed to be the link between the SIN and the STRIPAK complex.

**Materials & Methods:** Generation of deletion mutants, site-directed mutagenesis, phenotypic and bioinformatics analysis.

**Results:** BLAST analysis confirms KIN3 as a homolog of fungal SIN GC kinases.  $\Delta$ kin3 deletion strain shows an arrest in early fruiting body formation stage, hyphal fusion defect and septation deficiency with a few septa prevalently localized at the base of hyphal branches. ATP-binding mutant KIN3<sup>K39R</sup>, as well as a double deletion strain, lacking KIN3 and a core STRIPAK subunit PRO11, demonstrated more severe developmental defects.

**Conclusion:** KIN3 emerges as a dual function kinase in *S. macrospora* SIN and STRIPAK complexes. Both of them regulate multiple cellular processes, including septation, essential for vegetative growth and sexual development.

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## FBV16

### About how *Colletotrichum graminicola* shapes its lifestyle

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<sup>2</sup>University of Halle-Wittenberg, Institute for Agricultural and Nutritional Sciences, Halle a. d. S., Germany

Since the late 1980s the formation of two different, dimorphic conidia by the hemibiotrophic corn pathogen *Colletotrichum graminicola* is known. Nevertheless, research regarding maize infection is focusing exclusively on the investigation of deep colored, falcate conidia. Our current work suggests that falcate and the smaller ovoid conidia have specific functions in *C. graminicola* lifestyle and infection, depending on environmental factors like nutrients and light.

Developmental programs of falcate conidia depend mostly on nutrients, as high starch contents induce conidia formation on surface cultures, whereas free sugar triggers germination and appressoria formation. Life style of ovoid conidia is more complex: Being generated in liquid cultures in the absence of light, the germination of this spore type is independent of available nutrient sources, taking place in both in liquid and surface cultures. Starving conditions further

allow the generation of conidial anastomosis tube (CAT) formation by ovoid conidia, a so far unknown feature of *C. graminicola*, which is strictly absent in cultures of falcate spores. Most interestingly, formation of penetration structures on hydrophobic surfaces is regulated inversely in both conidial types: a high nutrient content leads to the formation of appressoria from falcate conidia, whereas exact the same process is induced under starving conditions in ovoid conidia. Taken together, our results support a new model for *C. graminicola* life style, in which different conidial types undergo specific developmental and pathogenic programs allowing successful invading of plant tissues, propagation, and distribution depending on natural condition faced.

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## Mini Symposium

### Microbial Pathogenicity (FG MPV)

17 April 2018 • 08:00 – 10:00

#### MPV-FG01

##### Organization and biological significance of lipid rafts in bacteria

D. Lopez\*<sup>1</sup>

<sup>1</sup>Centro Nacional de Biotecnología CNB, Universidad Autónoma de Madrid, Madrid, Spain

No abstract has been submitted.

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#### MPV-FG02

##### Polyphosphate – an important bacterial posttranslational stress defense system against inflammatory oxidants

J. U. Dahl\*<sup>1</sup>, B. Groitl<sup>1</sup>, M. J. Gray<sup>1</sup>, U. Jakob<sup>1</sup>

<sup>1</sup>University of Michigan, Molecular, Cellular, and Developmental Biology, Ann Arbor, MI, United States

Innate immune cells use a powerful strategy to kill invading pathogens: they produce high levels of oxidants, including hypochlorous acid (HOCl), the active ingredient of household bleach, and hypothiocyanous acid (HOSCN). Little is known about the physiological consequences of HOCl/HOSCN-mediated oxidative stress, the major targets that are affected, and the strategies that bacteria employ to reduce cellular damage. Understanding the molecular mechanisms behind bacterial responses to HOCl/HOSCN will open up new treatment possibilities by targeting the crucial players in these processes and sensitizing pathogens to the oxidative burst.

We directly compared the antimicrobial efficacy of the two oxidants on the *P. aeruginosa* strain PA14. Both oxidants caused extensive protein aggregation. We identified common and oxidant-specific response systems by RNAseq analysis and found that both oxidants elicit the production of high levels of polyphosphate (polyP), an universally conserved chaperone that confers resistance against the stressors by preventing the formation of toxic protein aggregates. We then discovered that mesalamine, an FDA-approved drug that serves as gold standard in treating ulcerative colitis, substantially depletes polyP in a wide variety of pathogens as well as in human gut microbiota. Equivalent to strains lacking the ability to produce polyP, we found that treatment of various pathogens with mesalamine significantly reduced their ability to form biofilms and persisters, increased their sensitivity towards HOCl/HOSCN, and resulted in impaired colonization of the pathogen in the host. Our data suggest that inhibition of bacterial polyP production has the potential to increase the efficacy of the host to kill colonizing bacteria.

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#### MPV-FG03

##### Assembly, structure, and function of the core export apparatus components of bacterial type III secretion systems

S. Wagner\*<sup>1</sup>, C. Robinson<sup>1,2</sup>, S. Lea<sup>2</sup>

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<sup>2</sup>University of Oxford, Oxford, United Kingdom

Bacterial type III protein secretion systems inject effector proteins into eukaryotic host cells in order to promote survival and colonization of Gram-negative pathogens and symbionts. Secretion across the bacterial cell envelope and injection into host cells is facilitated by a so-called injectisome that is evolutionary related to the bacterial flagellum. Its small hydrophobic export apparatus components SpaP, SpaQ, and SpaR were shown to nucleate assembly of the needle complex and to form the central "cup" substructure of a *Salmonella* Typhimurium secretion system. However, the *in vivo* placement of these components in the needle complex and their function during the secretion process remained poorly defined. We have recently obtained a high resolution structure of the flagellar homologs FliP, FliQ, and FliR, forming a unique supra-membrane helical assembly that gates the export channel of type III secretion systems. I will present this structure and discuss its implications for our understanding of membrane protein complex assembly and type III secretion function.

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#### MPV-FG04

##### The impact of redox regulation on the stress response of *Aspergillus fumigatus* and its role in host-pathogen interaction

L. Ivanova\*<sup>1</sup>, E. Shekhova<sup>1</sup>, T. Krüger<sup>1</sup>, O. Kniemeyer<sup>1</sup>, A. A. Brakhage<sup>1</sup>

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Recent studies have shown that reactive oxygen species (ROS) are not only involved in protein and cell damage, but also act as regulators for important cellular functions, mainly through reversible cysteine oxidation. Previously, our group has shown (manuscript in preparation), that *A. fumigatus* experiences high levels of intracellular oxidative stress during adaptation to hypoxia or antifungal agents. However, it still remains unclear how ROS are distributed within the cell during these stress conditions and the physiological processes influenced by cysteine oxidation remain to be elucidated.

The project aims to establish genetically encoded fluorescent redox sensors, on the basis of molecules like HyPer-2 and roGFP2/Grx1-roGFP2, to allow observation of the flow and quantification of intracellular ROS under different stress conditions. Additionally, a quantitative redox proteomics method is being established in order to improve the identification and characterization of oxidative post translational modifications (PTMs). Combining the established iodoTMT and oxICAT methods with the iTRAQ approach would also allow measuring changes in overall protein turnover.

Ultimately, the main goal will be to observe intracellular redox changes in *A. fumigatus* during *in vitro* interaction with immune cells, allowing us to draw conclusions about the role of redox regulation in the host-pathogen interactions.

## MPV-FG05

### C-type lectin receptor (CLR)-Fc fusion proteins as tools to screen for novel CLR/bacteria interactions: an exemplary study on *Campylobacter jejuni* isolates

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**Introduction and Aims:** C-type lectin receptors (CLRs) are eukaryotic carbohydrate-binding receptors that recognize their glycan ligands often in a Ca<sup>2+</sup>-dependent manner. Upon ligand binding, myeloid CLRs in innate immunity are able to trigger or inhibit a variety of signaling pathways, thus modulating effector functions such as cytokine production, phagocytosis and antigen presentation. CLRs are known to bind to various pathogens, including viruses, fungi, parasites and bacteria. The bacterium *Campylobacter jejuni* (*C. jejuni*) is a very frequent Gram-negative zoonotic pathogen of humans, causing severe acute intestinal symptoms. Interestingly, *C. jejuni* has the enzymatic capacity for both O- and N- glycosylation. It expresses multiple glycosylated surface structures, for example the capsular polysaccharide (CPS), lipooligosaccharide (LOS) and envelope proteins.

**Methods and Results:** The present work describes innovative methods and applications based on CLR-Fc fusion proteins to screen for yet unknown CLR/bacteria interactions using *C. jejuni* as a proof-of-principle example. ELISA-based detection of CLR/bacteria interactions offers the possibility for a first pre-screening that can be confirmed using flow-cytometric-based binding analyses and visualized using confocal microscopy. By applying these methods, we identified mDectin-1 as a novel CLR recognizing two selected *C. jejuni* isolates with different LOS and CPS genotypes.

**Summary:** In conclusion, the here-described methods and applications of CLR-Fc fusion proteins represent useful methods to screen for and identify novel CLR/bacteria interactions.

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## MPV-FG06

### Trimeric Autotransporter Adhesins - Understanding adhesive function and screening for inhibitors

A. Saragliadis<sup>1</sup>, D. Linke<sup>\*1</sup>

<sup>1</sup>University of Oslo, Department of Biosciences, Oslo, Norway

Trimeric Autotransporter Adhesins (TAAs) are key virulence factors in various Gram-negative pathogenic species, including *Yersinia*, *Bartonella*, *Neisseria*, and others. Their main function is to adhere to host cells, enabling persistence, tissue invasion and inflammatory processes. Some of the TAAs in addition can bind and inactivate components of the complement system, rendering the bacterial cell partially invisible to the immune system. In recent work, we have studied details of the adhesive process using different systems, from ELISA-like assays and x-ray crystallography to electron microscopy. We have developed assays suitable for high-throughput screening of inhibitors of adhesion, that can potentially be adapted to other adhesins and adhesin classes, using live bacterial cells. In a first round of screening with a library of 28.000 compounds, we have found 17 promising candidate hits for future projects. We hope to develop anti-adhesive substances that can be used to treat different diseases, and that are suitable for coating implants

and other medical devices where bacterial adhesion is problematic.

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## MPV-FG07

### Auto-regulatory control and phenotypic heterogeneity in expression of the flagellar master operon *flhDC* in *Salmonella enterica*

C. Kühne<sup>\*1</sup>, M. Erhardt<sup>1</sup>

<sup>1</sup>Humboldt-Universität zu Berlin, Bacterial Physiology, Berlin, Germany

**Question:** Flagellar motility plays a crucial role for *Salmonella* pathogenicity by enabling directed movement. The flagellar master regulator FlhD<sub>4</sub>C<sub>2</sub> initiates transcription of genes required for assembly of the bacterial flagellum. Expression of *flhDC* is under extensive regulatory control, but the details are poorly understood.

**Methods:** We used bacterial genetics and biochemical approaches to analyze the autogenous regulation of *flhDC*. Bistable expression of *flhDC* was analyzed using flow cytometry and single-cell fluorescence microscopy.

**Results:** We investigated the molecular mechanism of the auto-regulatory control of *flhDC*. We show that autogenous regulation of *flhDC* involves the global Rcs phosphorelay system and a FlhD<sub>4</sub>C<sub>2</sub>-specific repressor, RfIM. RfIM and RcsB form a heterodimeric complex that represses *flhDC* transcription from the primary P<sub>1*flhDC*</sub> promoter with enhanced affinity compared to RcsB alone [1]. Additionally, we show that the autogenous repression of *flhDC* through the RcsB-RfIM complex results in phenotypic heterogeneity in expression of the flagellar master operon. Furthermore, we demonstrate that RfIM-dependent repression of *flhDC* exhibited hysteresis, suggesting that the system is bistable.

**Conclusion:** The bistable expression of the master regulator *flhDC* reveals another level in the regulation of flagella synthesis. We propose that RfIM acts as novel RcsB co-regulator that mediates target specificity of RcsB in repression of *flhDC* transcription. The bistable regulation of *flhDC* might allow *Salmonella* to fine-tune initiation of flagellar synthesis and to rapidly adapt to changing environments during infection by maintaining stable flagellated and non-flagellated subpopulations.

#### References:

[1] Kühne C et al. (2016) Mol Microbiol 101:841-855

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## Short Lecture

### Systems Biology and Metabolomics

17 April 2018 • 08:00–10:00

#### STMV01

##### Engineering an artificial bypass for glucose degradation in *Escherichia coli* K12 by expression of a mutant gene of fructose 6-phosphate aldolase (*fsaA*)

E. Guitart Font<sup>\*1</sup>, G. A. Sprenger<sup>1</sup>

<sup>1</sup>University of Stuttgart, Institute of Microbiology, Stuttgart, Germany

Deletions of genes that code for key enzymes of glycolysis and the pentose phosphate pathway have a major impact on growth behavior and survival of cells on different carbon (C) sources. Although there are genes (*fsaA*, *fsaB*) present in the *E. coli* chromosome that code for fructose 6-phosphate aldolase (FSA), *E. coli* mutants lacking phosphofructokinase

(PfkA, PfkB) activity are impaired in the use of C-sources entering the cell at or above the level of fructose 6-phosphate (F6P) [1]. FSA cleaves F6P to dihydroxyacetone and glyceraldehyde 6-phosphate [2]; thus, an artificial bypass for carbon degradation might be established. We investigated this by expression of the recombinant genes *fsaA* and *fsaA*<sup>A129S</sup> [3] in different *E. coli* K-12 mutant strains (LJ110-derivates) growing in minimal media with glucose as sole C-source.

GL3 lacks glucose 6-phosphate dehydrogenase (*zwf*) and PfkA, PfkB; and GL4 is a GL3-derivate deficient in dihydroxyacetone kinase (*dhaKLM*). Both strains only grow on glucose when they carry pJF119*fsaA*<sup>A129S</sup>. GL35 (GL3-derivate) and GL5 (GL4-derivate) have *P*<sub>lac</sub>-*fsaA*<sup>A129S</sup> integrated in the *lac* operon and have regained the ability to use glucose (generation time of 14h and 40h respectively compared to 1h for wild type). GL5-derivates that grow 3 times faster on glucose than GL5 were also selected. In all media tested, wild type cells had the highest growth rate.

Restored growth of all strains on glucose by expression of *fsaA*<sup>A129S</sup>, which enables cleavage of F6P, strongly suggests that a glycolytic bypass was functional. Physiological and biochemical data will be supplied.

#### References:

- [1]Roehl & Vinopal (1976) *J Bacteriol* 126:852-860
- [2]Schürmann & Sprenger (2001) *J Biol Chem* 276:11055-11061
- [3]Castillo et al. (2010) *Adv Synth Catal* 352:1039-1046

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#### STMV02

##### Making and breaking the wall – a systems approach to cell wall homeostasis in *Bacillus subtilis*

H. Piepenbreier<sup>1</sup>, G. Fritz<sup>\*1</sup>

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Growth and survival of gram-positive bacteria critically hinges on the faithful synthesis of the cell wall. Accordingly, in the fierce competition for limited resources many microbial species produce antimicrobial peptides (AMPs) that interfere with the lipid II cycle of cell wall biosynthesis, such as nisin or vancomycin. Although the molecular targets of most of these AMPs are well characterized, the *in vitro* binding constants for the AMP-target interactions are often orders of magnitude lower than the MIC values required to kill bacteria *in vivo*. While for some AMPs this is due to the presence of highly efficient resistance determinants, for other AMPs we still found a remarkable discrepancy between *Kd* and MIC also in the absence of resistance mechanisms. In order to rationalize this counterintuitive behaviour, we developed a mathematical model integrating measured pool levels of lipid II cycle intermediates as well as enzyme kinetic parameters from previous biochemical studies. Strikingly, without any parameter fitting the model correctly predicted the MICs for 5 different AMPs from their *Kd* values, suggesting that the experimentally determined ratios between MIC and *Kd* are not the result of spurious AMP inactivation *in vivo*, but rather a systems level property of the lipid II cycle. Indeed, the model shows that low-abundance intermediates of the cycle, such as lipid II itself, are much harder to block than high abundance intermediates, such as undecaprenyl pyrophosphate. Finally, our analysis reveals that cooperative drug-target interactions can greatly boost the potency of

AMPs, as predicted, e.g., for the dimeric binding of vancomycin to lipid II.

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#### STMV03

##### ADM1 + FBA: Bringing anaerobic digestion modeling to the next level

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Anaerobic digestion, the microbial conversion of organic material to methane, has been modeled since more than 15 years by approaches that do not resolve intracellular microbial activity. Modeling approaches capable of doing this have been developed for monocultures and allow for precise predictions which can be compared to OMICS data.

We here augment the Anaerobic Digestion Model No. 1 (ADM1) as the standard kinetic model of anaerobic digestion by coupling it to Flux-Balance-Analysis models of methanogenic species capable of either acetotrophic and hydrogenotrophic methanogenesis (*Methanosarcina barkeri*) or only the latter pathway (*Methanococcus maripaludis*). We replace one or both methanogenesis pathways in ADM1 by minimal or genome-scale metabolic models.

Steady state results of the coupled models are comparable to classic ADM1 simulations if the energy demand for non-growth associated maintenance is chosen adequately. In comparison to ADM1, the coupled model delivers predictions of intracellular fluxes (up to 1,000 per species), providing a more detailed representation of metabolic pathway activity within the community.

Our study proves the feasibility of augmenting ADM1, an ordinary differential equation based model, by Flux-Balance-Analysis models implementing individual steps of anaerobic digestion. The coupling approach reduces the number of model parameters to be estimated (yield coefficients) and provides detailed predictions on intracellular activity of microbial species in a community which can be compared to experimental meta-OMICS data. This advances the simulation of microbial community driven processes to the next level by establishing a direct link to experimental validation.

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#### STMV04

##### Adaptation of *Dinoroseobacter shibae* to peroxide stress

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The photoheterotrophic bacterium *Dinoroseobacter shibae* occurs in the photic zone of marine ecosystems where oxygen is ubiquitous, and therefore reactive oxygen species are prevalent. Hence, an effective antioxidant defense mechanism is required to prevent oxidative damage. In the present study we focused on the adaptation of *D. shibae* to peroxide stress and the regulation of the antioxidative response utilizing a GeLC-MS/MS approach. Exposure of exponentially growing *D. shibae* cells to 10 mM hydrogen

peroxide resulted in significant changes (at least 1.5 fold) in amount of 122 cytosolic and surface-associated proteins. Besides proteins involved in detoxification of hydrogen peroxide and protection and repair of oxidative damage in cellular structures, iron transport associated proteins have been observed to accumulate under these conditions. Notably, the amount of the rhizobial iron regulator RirA in *D. shibae* was significantly reduced at the same time. A *rirA* deficient strain showed an improved adaptation to peroxide stress indicating that RirA dependent proteins may be important for oxidative stress resistance. The most interesting candidates that might mediate the observed phenotype are peroxiredoxin, thioredoxin, Hsp20, ClpB, DnaJ and RecA. To gain further insights into the regulation of the peroxide stress response in *D. shibae*, a mutant strain lacking the extrachromosomal encoded OxyR was generated. Preliminary results revealed that inactivation of *oxyR* led to an increased resistance to hydrogen peroxide possibly provoked by an increased production of catalase.

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### STMV05

#### Metabolome analysis of xylose and xylitol degradation in *Phaeobacter inhibens*

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*Phaeobacter inhibens* is a gram-negative marine bacterium which is able to utilise a wide range of substrates. In comparison to the well investigated reference strain *P. inhibens* DSM 17395, the *P. inhibens* isolate P88 differed in its growth behaviour on xylose and xylitol. The aim of this study was to reveal the utilisation and degradation pathways of these substrates.

Both *P. inhibens* strains were grown on xylose, xylitol, glucose and a mixture of those. The intracellular metabolites were analysed by GC-MS. To elucidate the degradation pathways and their impact on the metabolism, isotope labeling experiments were performed with <sup>13</sup>C-labeled substrates.

While the reference strain showed good growth on xylose and glucose but no growth on xylitol, the isolate was able to grow on xylitol whereas the growth on xylose was noticeably reduced compared to glucose or xylitol. However, it was not possible to explain the reduced growth behaviour on xylose based on genomic data. Metabolome analysis confirmed that xylitol is catabolized via xylulose and xylulose-5-phosphate and subsequently in the pentose phosphate pathway. During the cultivation on xylose several yet unidentified metabolites were strongly accumulated in the isolate compared to the reference strain. Two of them could be identified as a heptulose and a heptitol. It is known that these metabolites can act as storage compounds or compatible solutes in plants and fungi. We showed for the first time that a bacterium such as a *Phaeobacter* strain is also able to produce these carbohydrate compounds as storage compounds or compatible solutes.

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### STMV06

#### Metabolic and regulatory adaptation of *Staphylococcus aureus* to different carbon sources

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*Staphylococcus aureus* generally resides as a harmless commensal on the mucous membranes of the nares and on the skin of humans. However, in the immunocompromised host or following penetration of mucosal and skin barriers, *S. aureus* can switch to a more aggressive lifestyle often causing severe or even life-threatening infections. We hypothesize, that this switch is also modulated by the nutrients available to *S. aureus*, which in addition to providing energy and carbon may also act as a habitat identification signal.

For example, during invasive disease glucose is the major carbon source in the blood stream, whereas pyruvate, present in mucous secretions of the nose, may correlate with a colonizing lifestyle. Lactate is produced in high amounts by proliferating T-cells at the site of infection and may therefore function as a signal of the hosts' state of immune defense. Finally, many intracellular pathogens utilize glycerol as a major carbon source during host cell invasion.

For a better understanding of the *S. aureus* adaption processes during life as a harmless commensal or as a pathogen during invasive disease, we analysed carbon source utilization of *S. aureus* strain COL using a combined metabolomic and proteomic approach. *S. aureus* was grown in a synthetic medium with glucose, pyruvate, lactate or glycerol as sole carbon source. To also investigate the impact of carbon catabolite repression in this process, we included a catabolite control protein A mutant in our analyses and exposed *S. aureus* to combinations of glucose with either pyruvate, lactate or glycerol. Quantitative data will be presented describing the intra- and extracellular protein repertoire and metabolic profile of *S. aureus* when grown with different carbon sources.

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### STMV07

#### Arginine deiminase pathway in *Acetobacterium woodii*

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**Question:** A major interest with acetogenic bacteria is to improve energy conservation, for example with new substrates that benefit growth. By the arginine deiminase pathway (ADI pathway) arginine is metabolized to the products ornithine, ammonia, and ATP. *A. woodii* harbors all genes necessary for the ADI pathway on its genome, but genes are not clustered as this is the case for other anaerobes such as *Clostridium autoethanogenum*. The aim of the study was to boost growth of recombinant *A. woodii* by providing cells with additional ATP generated by the ADI pathway.

**Methods:** The recombinant strains *A. woodii* [pMTL83151\_P<sub>tet</sub>\_boost] and *A. woodii* [pMTL83151\_P<sub>pta-ack</sub>\_boost] were constructed, which both harbor the genes for the ADI pathway under the control of an anhydrotetracycline-inducible *tet* or a constitutive *pta-ack* promoter. Mixotrophic growth experiments were performed with fructose and CO<sub>2</sub> as carbon and arginine or yeast extract as nitrogen source.

**Results:** *A. woodii* control strains not harboring plasmids with the ADI pathway genes did not consume arginine or produce ornithine. *A. woodii* [pMTL83151\_P<sub>tet</sub>\_boost] consumed a third of the initial arginine and produced ornithine after induction of the ADI pathway gene expression. Compared to the control strains, *A. woodii* [pMTL83151\_P<sub>tet</sub>\_boost] reached the highest optical density. *A. woodii* [pMTL83151\_P<sub>pta-ack</sub>\_boost] consumed all of the provided arginine and produced ornithine. Furthermore, citrulline accumulation was detected, which is an intermediate of the ADI pathway.

**Conclusions:** The growth of recombinant *A. woodii* strains may be improved by arginine, which provides the cells with additional energy while fructose and CO<sub>2</sub> is used for biomass production.

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#### STMV08

##### Characterization of nitrogen metabolism of *Streptococcus pneumoniae* by isotopologue profiling

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**Introduction:** *Streptococcus pneumoniae* is an opportunistic bacterium residing often symptomless in the human respiratory tract. During infection pneumococci have to adapt to provided carbon and nitrogen sources. To assess the nitrogen metabolism we used <sup>15</sup>N-isotopologue profiling and because of the different regulation of the arginine metabolism in strain D39 and strain TIGR4, we compared these two strains.

**Methods:** We studied nitrogen metabolism by <sup>15</sup>N-isotopologue profiling by culturing pneumococci under microaerophilic conditions in chemically defined medium supplemented with labeled <sup>15</sup>N<sub>2</sub>-glutamine, <sup>15</sup>N<sub>4</sub>-arginine or a mixture of <sup>15</sup>N-labeled amino acids. We detected the amounts of resulting labeled amino acids by GC-MS analysis.

**Results and conclusion:** Growth of strain D39 with <sup>15</sup>N-labeled glutamine revealed a detection of twelve labeled amino acid indicating that glutamine can provide NH<sub>4</sub><sup>+</sup> for biosynthesis of other amino acids. We measured no labeled serine and only traces of labeled glycine. When we used strain TIGR4 in the labeling experiments, Gly and Ser significantly acquired the <sup>15</sup>N-label demonstrating different biosynthesis pathways in pneumococci for these amino acids. Arginine is catabolized by arginine deiminase (AD) providing NH<sub>4</sub><sup>+</sup> and generated ATP in strain TIGR4 whereas no functional AD is present in strain D39. Our experiments with <sup>15</sup>N<sub>4</sub>-labeled arginine revealed fifteen labeled amino acids for TIGR4. Due to the nonfunctional arginine deiminase in D39 arginine was not used as N-source. Taken together, our <sup>15</sup>N-isotopologue profiling revealed the use of glutamine

as major nitrogen source and arginine only in strain with a functional AD.

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## Plenary Lecture

### Infection Biology and Evolution

17 April 2018 • 10:30–11:30

#### IL08

##### Suppression of adaptive immunity by *Salmonella*

D. Holden<sup>\*1</sup>

<sup>1</sup>*Imperial College London, MRC Centre for Molecular Bacteriology and Infection, London, United Kingdom*

*Salmonella enterica* can cause persistent infections such as typhoid fever. Following bacterial entry into host cells, the pathogen replicates in a membrane-bound compartment called the *Salmonella*-containing vacuole (SCV). Bacteria respond to nutritional deprivation and the acidic pH in the vacuole lumen by activating the expression of the SPI-2 type III secretion system (T3SS). After assembly of a T3SS-linked pore in the vacuole membrane, bacteria sense the near-neutral pH of the host cell cytoplasm. This triggers the translocation of bacterial virulence proteins (effectors) into the host cell.

Approximately 30 different effectors are translocated by the SPI-2 T3SS. These have been implicated in several physiological activities, including the control of SCV positioning within the host cell, maintenance of vacuole membrane integrity, bacterial replication, interference with lysosome function and innate immune signaling. I will discuss our recent progress on an effector that suppresses the development of adaptive immunity, which is likely to contribute to the ability of *Salmonella* to persist in host tissues.

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#### IL09

##### Analysis of 100,000 prokaryotic (meta-)genomes shows clear species boundaries

K. Konstantinidis<sup>\*1</sup>

<sup>1</sup>*School of Civil & Environmental Engineering and School of Biological Sciences, Georgia Institute of Technology, Atlanta, GA, United States*

No abstract has been submitted.

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## Plenary Lecture

### Natural Products

17 April 2018 • 13:00–14:30

#### IL10

##### Cryptic Mediators of Microbial Interactions

C. Hertweck<sup>\*1</sup>

<sup>1</sup>*Leibniz Institute for Natural Product Research and Infection Biology (HKI), Jena, Germany*

The vast structural diversity of secondary metabolites has evolved over millions of years to address specific needs of the producing microorganisms in their niches. Thus, microbial natural products are not only highly specific mediators of microbial interactions, but also a valuable source of molecular tools and therapeutics that have been pre-optimized for particular biological targets. Recent research has led to a massive body of knowledge on biosynthetic mechanisms, structures and functions. Yet, we know only very little about how structural diversity evolved. We are also perplexed by the hugely underestimated

biosynthetic potential that remains invisible outside of the specific ecological context. This talk will present some compounds and pathways from various less explored bacteria, highlight the importance of considering the ecological interactions in the search for biologically active molecules, and shed light on some evolutionary aspects of natural product biosynthesis.

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## IL11

### Environmental dimensions of antibiotic resistance

J. Larsson<sup>\*1,2</sup>

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<sup>2</sup>University of Gothenburg, Department of Infectious Diseases, Institute for Biomedicine, Sahlgrenska Academy, Goeteborg, Sweden

The environment plays critical roles in the development and spread of antibiotic resistance; Water and food polluted with fecal material serves as important transmission routes for several enteric bacteria, particularly in low-resource settings. The enormous diversity of environmental bacteria also provides a vast source for resistance factors that over time and under the selection pressure from antibiotics are transferred to pathogens. Knowing where and under what circumstances selection of resistance takes place in the environment is therefore critical to assess and manage risks. Several approaches have been adopted, including both sensitive lab-based assays to more realistic field studies. Most if not all of these studies, however, have important limitations. Hence, it is currently difficult to judge the role of low levels of antibiotics in the environmental selection and evolution of resistance. Having said that, there are also environments polluted with exceptionally high concentrations of antibiotics where resistant bacteria, without doubt, are strongly selected for – environment polluted directly from the manufacturing of drugs. In this lecture, I will attempt to provide an overview of the environmental dimensions of antibiotic resistance, with some emphasis on evolution, selection and possible mitigations. I will also provide examples on how studying antibiotic resistance in the environment could be used to extract information on the current, regional clinical resistance situation - information that is sorely lacking in many low income countries, thereby hampering effective empirical treatment of infections.

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## IL12

### Mining the dark: Metabolic novelty from elusive bacteria

J. Piel<sup>\*1</sup>

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Most areas of the bacterial tree of life are functionally uncharacterized. These regions include numerous deep-branching taxa that lack cultivated representatives and live in diverse habitats. Our lab uses metagenomic and single-cell-based mining strategies to investigate whether this remarkable taxonomic and ecological diversity is a resource for metabolic novelty. We have previously reported uncultured 'Entotheonella' symbionts of the sponge *Theonella swinhoei* as rich source of bioactive and biosynthetically unusual compounds [1,2] These bacteria were assigned to a newly proposed candidate phylum named 'Tectomicrobia' and contain large genomes with dozens of biosynthetic gene clusters. This talk presents new data obtained from broader studies on the metabolism of sponge-associated bacteria. They confirmed 'Entotheonella' as talented producer taxon with a rich and diverse chemistry comparable to that of streptomycetes, but they also revealed the existence of further, unrelated producers. These data

suggest fundamental contributions of sponge microbiomes to the natural product repertoire of their hosts and validate microbial dark matter as a resource of distinct chemistry. While most of the gene clusters had no counterparts in known cultured bacteria, we also identified unprecedented, mechanistically surprising enzymes that are widespread in conventional prokaryotes, indicating the existence of large natural product families with as-yet unknown ecological and pharmacological functions [3].

### References:

[1] *Science* (2012) **338**, 387.

[2] *Nature* (2014) **506**, 58.

[3] *Science* (2018), in print.

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## Poster Session 2

### Archaea (ARP)

17 April 2018 • 14:30–17:00

#### ARP001

#### ***Halobacterium salinarum* under heavy metal stress: A proteomic comparison between planktonic cells and biofilms**

S. Völkel<sup>\*1</sup>, C. Lenz<sup>2,3</sup>, N. Benker<sup>4</sup>, S. Hein<sup>5</sup>, G. Losensky<sup>1</sup>, F. Pfeifer<sup>1</sup>

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**Introduction & Aim:** *Halobacterium salinarum* R1 is an extreme halophilic archaeon that is able to attach to the surface and form tower-like biofilm structures. In a previous study, we investigated the effect of heavy metal ions on planktonic cells and mature biofilms by CLSM analysis, indicating metal-specific differences of the biofilm architecture. This study focuses on scanning- and transmission electron microscopic (SEM, TEM) investigations of the biofilms and analysis of the proteome of planktonic cells and mature biofilms exposed to copper and nickel ions.

**Materials & Methods:** *Hbt. salinarum* R1 planktonic cells and mature biofilms were exposed to copper and nickel ions for 24 h. The proteome was analyzed by label-free mass spectrometric SWATH-LC/MS/MS. Differences in biofilm structure and cell shape were investigated by SEM and TEM analysis.

**Results:** Microscopic analysis of metal-treated biofilms showed a decreased number of adherent cells and the formation of small cell aggregates when treated with copper, whereas nickel exposure resulted in a massive increase in adherent cells forming dense cell aggregates. A quantitative proteome analysis comparing metal-treated and non-treated planktonic cells and biofilms yielded 269 significantly altered proteins, i.e. 23% of the proteins quantified. In planktonic cultures, we identified 112 similar proteins in copper and nickel treated samples of which 36% are increased and 64% decreased. In contrast, only 6 similar proteins were found in biofilm samples with an increase of 67% and a decrease of 33% of these proteins.

**Conclusion:** Copper and nickel ions have a strong and similar effect on planktonic cells, whereas biofilms are less affected and show a metal-specific stress response as seen by proteome and microscopic analysis.

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#### ARP002

##### Deletions of putative pilin genes in *Halobacterium salinarum* R1

G. Losensky<sup>\*1</sup>, F. Pfeifer<sup>1</sup>

<sup>1</sup>Technical University Darmstadt, Microbiology and Archaea, Darmstadt, Germany

**Introduction:** *Halobacterium salinarum* R1 is able to form biofilms, *i.e.* multicellular microbial communities. Adhesion of the cells is the first step in biofilm formation and facilitated by specialized type IV pili. The *pil-1* locus is crucial for the formation of the adhesion pili, but lacks pilin genes.

**Objectives:** We focused on the identification of the *Hbt. salinarum* R1 adhesion pilins.

**Materials & methods:** Bioinformatic analyses of the *Hbt. salinarum* R1 proteome were conducted to search for type IV-like pilins. qRT-PCR was used to test the transcription of the pilin gene candidates in planktonic and adherent cells. Gene deletion mutants were generated based on a double-homologous recombination strategy. Gene deletions were verified by PCR, sequencing and Southern analyses. The deletion mutants were characterized by microscopy, fluorescence-based adhesion assays and swimming assays.

**Results:** More than 30 proteins containing N-terminal type IV pilin signatures were identified. The three putative pilin genes *pilA5*, *pilA6* and *pilA7* showed increased transcript amounts in adherent cells. Single deletions of these genes were obtained in a previously generated *Hbt. salinarum*  $\Delta fla$  strain, that is devoid of archaea. However, the deletions did not affect the adhesion of the cells, but the deletion of *pilA5* resulted in a significantly higher motility. Double and triple deletions of various pilin genes are in progress.

**Conclusion:** The present work joins previous studies on type IV pili, that are abundant in archaea. *Hbt.* contains a large number of putative pilin genes, demanding further examinations, especially with regard to the constituents of the adhesion pili and the interplay between motility and adhesion of the cells.

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#### ARP003

##### An ultramicrobial *Vampirococcus*-like epibiont on *Archaea* and *Bacteria*

J. Kizina<sup>1</sup>, S. Jordan<sup>1</sup>, A. Resteu<sup>1</sup>, G. Martens<sup>1</sup>, C. Probian<sup>1</sup>, R. Mellwig<sup>2</sup>, S. Littmann<sup>3</sup>, E. Rhiel<sup>4</sup>, S. Markert<sup>5</sup>, M. Richter<sup>6</sup>, K. Stüber<sup>7</sup>, T. Schweder<sup>5</sup>, R. Reinhardt<sup>7</sup>, J. Harder<sup>\*1,7</sup>

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Electron micrographs provided 1986 a view on *Vampirococcus*, an anaerobic epibiont predating freshwater *Chromatium* spp. (Guerrero et al., *Proc. Natl. Acad. Sci. U.S.A.* **83**: 2138–42). *Vampirococcus* was informally described as genus of unknown phylogeny and is currently without standing in nomenclature. We have identified an epibiotic bacterium showing structures in prey-predator interactions which resemble the micrographs of *Vampirococcus*' attack on *Chromatium*.

A stable hydrocarbon-degrading methanogenic enrichment culture was established in a dilution-to-extinction experiment in 1999. A culture inoculated with one microliter grew and then the enrichment culture was annually transferred. 16S rRNA gene clone libraries and *in situ* hybridization experiments revealed an abundant phylotype affiliating with an uncultivated candidate phylum. After physical separation, a fraction highly enriched with cells of the phylotype was used for a metagenome. PCR reactions with DNA of the enrichment culture allowed us to resolve repetitive elements in the population genome and to assemble the first single-contig (closed) genome for the candidate phylum. Additional metagenomes confirmed the high-quality genome and characterized the archaeal and bacterial populations in the nearly clonal enrichment culture. Metatranscriptomes and metaproteomes gave insight into the biology of the epibiont. Cells of the enrichment culture were characterized by a variety of electron microscopic techniques and *in situ* hybridisation experiments focusing on *Methanosaeta* and the epibiont.

We present evidence that the epibiotic bacterium makes a living on *Archaea* and *Bacteria* and discuss some far-reaching implication of the ecology of ultramicrobacteria in living systems.

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#### ARP004

##### C-di-AMP as second messenger in the Euryarchaeon *Haloferax volcanii*

F. Braun<sup>\*1</sup>, S. V. Albers<sup>1</sup>

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Nucleotide-based second messengers are involved in the signal transduction of various extra- and intracellular stimuli and thereby crucial for the regulation of cellular adaptation processes. One quite recently discovered signaling nucleotide in Bacteria is cyclic di-adenosine monophosphate (c-di-AMP), which was shown to have various essential functions in diverse species (Corrigan & Gründling, 2013). Despite the fact that bioinformatical studies already predicted the presence of c-di-AMP producing enzymes, so called Di-Adenylatcyclases (DACs), also in Archaea in 2008 (Römling, 2008), yet, no information, neither about c-di-AMPs' presence nor its possible functions, is available for the third domain of life.

Therefore, the objective of this study is to examine the presence of c-di-AMP and reveal its function in the euryarchaeal model organism *Haloferax volcanii*, whose genome encodes for one putative DAC.

Analyzing whole cell pellet extracts with LC-MS/MS it could be shown for the first time that c-di-AMP is present in an archaeal species. Several genetic approaches are used to address the questions whether this second messenger is also essential in Archaea and what possible function c-di-AMP could serve. To this end, pulldown assays using c-di-AMP coupled agarose-beads are used to identify putative c-

di-AMP binding (and thereby putatively regulated) proteins. Furthermore, the only DAC present in *H. volcanii*, DacZ, is heterologously expressed and *in vitro* DAC-activity is examined.

In summary, this is the first study addressing the occurrence and possible functions of c-di-AMP in an archaeal model organism.

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#### ARP005

##### Interaction of accessory gas vesicle proteins determined by split-GFP in *Haloflex volcanii*

K. Winter<sup>\*1</sup>, F. Pfeifer<sup>1</sup>

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**Introduction:** The use of split-GFP to study protein-protein interaction (PPI) is already established, but the method is not adapted to the high salt in haloarchaea. The principle is based on the fragmentation of the GFP into 2 fragments that are fused to the interacting proteins or peptides under investigation. Only then, GFP is able to reassemble a fluorescent protein. We used split-GFP to analyze PPI of the accessory gas vesicle proteins in *Haloflex volcanii* to gain a better understanding of the initial stages in gas vesicle (GV) formation.

**Objectives:** We were seeking for a method to visualize PPI in *Hfx. volcanii* cells *in vivo*. Also, we determined the individual interaction sites in the proteins tested.

**Material & Methods:** The salt-adapted and highly fluorescent JB-GFP was split between residues 157 and 158 and the resulting fragments NGFP and CGFP were fused to the proteins tested at the N- or C-terminus. The respective *Hfx. volcanii* transformants were examined for fluorescence.

**Results:** We verified the interaction of GvpL and GvpM in *Hfx. volcanii*. Only transformants carrying a C-terminally tagged GvpM were fluorescent, indicating that the interaction site might be near the N-terminus of GvpM. Thus, GvpM variants with deletions in the N- or C-terminal portion and also the peptides M(25N), consisting of the first 25 aa, or M(25C), with the last 25 aa of GvpM were analyzed. The results supported that GvpM interacts with GvpL *via* its N-terminal part.

**Conclusion:** The use of split-GFP is a powerful method for the detection of *in vivo* PPI under high salt conditions. We are now able to examine individual interaction sites in the putative nucleation complex formed by the interacting accessory Gvp FGHIJKLM at the onset of GV formation.

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#### ARP006

##### Communication between the chemotaxis and motility machinery in archaea

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Archaea use their motility structure, the archaeellum, to colonize new habitats. This structure assembles in a similar fashion as type IV pili and has no structural homology with the bacterial flagellum. The archaeellum consists of 7-15

different subunits of which most were shown to be essential for archaeellum assembly and motility. Several archaea have received the chemotaxis system via horizontal gene transfer. In bacteria, the chemotaxis system enables directional movement along chemical gradients. Environmental signals are transferred via the central chemotaxis protein, CheY, to the switch complex at the base of the bacterial flagellum resulting in a directional change. Homologs of the bacterial switch complex are lacking in archaea. We aim to identify the archaeal switch complex required for communication with the chemotaxis system. We hypothesize that the switch complex might be formed by FlaC,D and E, which are proteins of unknown function encoded in the archaeellum operon. Previous interaction studies indicated that they bind to proteins of the chemotaxis cascade. FlaC,D and E are not present in crenarchaea, which correspondingly lack the chemotaxis system. Using the halophilic euryarchaeon *Haloflex volcanii* as a model, we showed that FlaD and FlaCE (fused in *H.volcanii*) are essential for directed movement. The cellular localization of these proteins was studied with fluorescent microscopy, revealing their presence at the cell poles. Interestingly, FlaD is required for correct localization of FlaCE at the archaeellum motor complex. These results give the first insights into the role of the uncharacterized *flaCDE* genes, suggesting that they are an essential part of the archaeellum motor complex that might be involved in communication with the chemotaxis system.

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#### ARP007

##### Insights into substrate specificity of archaeal Entner-Doudoroff aldolases: the structure of *Picrophilus torridus* 2-keto-3-deoxygluconate aldolase.

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The euryarchaeon *Picrophilus torridus* catabolises glucose via a non-phosphorylative Entner-Doudoroff pathway involving 2-keto-3-deoxygluconate aldolase (Pt-KDGA) which is highly specific for the non-phosphorylated substrate, 2-keto-3-deoxygluconate (KDG) (1). In contrast, the crenarchaeon *Sulfolobus solfataricus* degrades glucose via a branched Entner-Doudoroff pathway (2,3) using a different aldolase, 2-keto-3-deoxy-6-phosphogluconate aldolase (Ss-KD(P)GA), that catalyses the cleavage of both KDG and 2-keto-3-deoxy-6-phosphogluconate (KDPG) (2,3), with a preference for KDPG. So far, the structural basis for the high specificity of KDGA from *P. torridus* for KDG as compared to the promiscuous KD(P)GA from *S. solfataricus* has not been analysed.

Here we report the elucidation of the structure of KDGA from *P. torridus*. KDPG was modelled into the active sites of both KDGA from *P. torridus* and KD(P)GA from *S. solfataricus*. By superimposition of the active sites of the two enzymes, and by subsequent site-directed mutagenesis studies, a network of four amino acids, namely Arg106, Tyr132, Arg237 and Ser241, was identified in KD(P)GA from *S. solfataricus* as likely to interact with the negatively-charged phosphate group of KDPG. The KDPG-binding network is absent in KDGA from *P. torridus*, which may explain the low catalytic efficiency of KDPG cleavage.

#### References:

[1] Reher M *et al.* (2010) J Bacteriol 192: 964-974

[2] Lamble HJ *et al.* (2005) FEBS Lett 579: 6865-6869

[3] Ahmed H *et al.* (2005) Biochem J 390: 529-540

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#### ARP008

##### Identification of a potential transcriptional activator of D-glucose-catabolism in *Haloferax volcanii*

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The halophilic archaeon *Haloferax volcanii* degrades D-glucose to pyruvate via the semiphosphorylative Entner-Doudoroff pathway (1). This pathway is initiated by a glucose-dehydrogenase (GDH) and gluconate-dehydratase (GAD) which are both induced by D-glucose. So far, the transcriptional regulation of these enzymes is unknown. Here we report that HVO\_1082 encodes a transcriptional activator of GDH and GAD: (I) HVO\_1082 is located in close vicinity to the encoding gene of GDH and its amino acid sequence is homologous to the uncommon transcriptional regulators of the PRT (phosphoribosyltransferase) protein family, which are involved in purine- and pyrimidine-metabolism of few gram-positive bacteria (2,3). (II) A knockout mutant of HVO\_1082 exhibited reduced growth on D-glucose rather than on casamino acids; the wild type phenotype could be recovered by a complementation *in trans*. (III) Promotor-activities of GDH and GAD, analysed with  $\beta$ -galactosidase as a reporter gene, showed a strong D-glucose specific induction. The deletion of HVO\_1082 resulted in a significant decrease in promotor-activity of GDH and GAD compared to the wild type, indicating that HVO\_1082 functions as a transcriptional activator. This is the first report of a transcriptional regulator in D-glucose metabolism of archaea that belongs to the PRT protein family.

#### References:

[1] Sutter JM *et al.* (2016) J Bacteriol 198: 2251-2262

[2] Sinha SC and Smith JL (2001) Curr Opin Struct Biol 11:733-739

[3] Kilstrup M and Martinussen J (1998) J Bacteriol 180:3907-3916

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#### ARP009

##### Different growth behavior of *Sulfolobus acidocaldarius* and *Sulfolobus solfataricus* on L-glutamate or L-pyroglutamate as carbon source

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Growth of microorganisms in glutamate-containing media can be influenced by pyroglutamate, a lactam formed by spontaneously cyclization of the internal amide of glutamate in the supernatant. Under aerobic conditions, the highest tendency of glutamate for cyclization is observed under thermoacidophilic conditions. Some thermoacidophilic microorganisms such as *Sulfolobus solfataricus* show limited or no growth at high pyroglutamate concentrations which is attributed to an acidification of the cytosol. However, the extent to which this limitation applies to microorganisms of the same genus has not been analysed so far.

During cultivation of the thermoacidophilic archaea, *Sulfolobus acidocaldarius* and *S. solfataricus*, on glutamate, we followed the formation of pyroglutamate in the culture supernatant. Interestingly, *S. acidocaldarius* consumed glutamate and pyroglutamate simultaneously, showing no restriction of growth. Consequently we cultivated *S. acidocaldarius* on pyroglutamate and observed the ability to grow on pyroglutamate as sole carbon source with similar growth parameters as on glutamate. In contrast, *S. solfataricus* first consumed glutamate while pyroglutamate was effectively taken up in the stationary phase. *S. solfataricus* was not able to grow on pyroglutamate as sole carbon source. Apparently *S. acidocaldarius* – though considered to be metabolically less versatile than *S. solfataricus* – exhibits a higher tolerance against pyroglutamate and allows the use of pyroglutamate as sole carbon source.

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#### ARP010

##### Title: Investigation of Extracellular Polymeric Substances (EPS) from *Halobacterium salinarum* R1 Biofilms Johannes Born, Robin Wloch and Felicitas Pfeifer Technische Universität Darmstadt, Microbiology and Archaea, Darmstadt

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**Introduction:** Biofilms provide several advantages for the microorganisms. The EPS formed by bacteria consists of different substances such as carbohydrates, proteins, lipids, or extracellular DNA (eDNA). Extracellular proteins in particular play an important role within biofilms like cell attachment, stabilization or dissolving of the biofilm. In contrast, the formation of archaeal biofilms as well as the composition of their EPS are not very well investigated. One method is to visualize biofilms and EPS by confocal laser scanning microscopy (CLSM) by tagging the cells with *gfp* and staining different EPS compounds with specific fluorescent dyes. The aim of this study was to investigate biofilm formation with regard to the EPS development and the identification of extracellular proteins of *gfp*-expressing *Hbt. salinarum*.

**Methods:** *Hbt. salinarum* cells expressing *gfp* were grown for 10 days. Individual EPS components were stained with different fluorescent dyes and visualized by CLSM. Integral proteins of the EPS were isolated and identified by mass spectroscopy.

**Results:** CLSM analysis showed bulky tower-like microcolonies containing glycoconjugates, eDNA and proteins. The presence of extracellular proteases was determined by zymography. Classification of the identified proteins by mass spectrometry yielded 5 proteins in the category information storage and processing, 10 proteins in the category cellular processes and signalling, 9 proteins in the category metabolism and 4 hypothetical proteins.

**Conclusion:** A multi-faceted approach including the visualization, isolation and identification of EPS-components of sessile *gfp*-expressing *Hbt. salinarum* cells was found to be a good tool for investigating the development of halophilic biofilms.

## ARP011

### Analysis of two heme-based sensor kinases and their role in putative two-component systems from

#### *Methanosarcina acetivorans*

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The multidomain protein MsmS from *Methanosarcina acetivorans* is one of the first examples for the biochemical characterization of an archaeal sensor kinase with autophosphorylation activity. MsmS consists of two alternating PAS and GAF domains and a C-terminal H<sub>2</sub>ATPase domain [1]. A homolog to MsmS is the sensor kinase MA0863, which shares 68% identity and 84% similarity with MsmS and contains an additional PAS domain at the N-terminus. MA0863 also contains an amber codon for pyrrolysine in the second PAS domain. The second GAF domain of both proteins covalently binds a heme cofactor via a cysteine residue. MsmS and MA0863 are proposed to form two-component systems with closely encoded transcriptional regulator proteins belonging to the dimethylsulfide (DMS) metabolism. To investigate the function of these archaeal signal transduction systems and their redox sensory function kinase assays, protein-protein interaction studies and UV-vis spectroscopy were used. For both kinases, the redox state of the heme cofactor was shown to influence the autophosphorylation activity of the adjacent kinase domain. The kinase activity was independent on bound ligands to the heme cofactor, like CO or DMS, but inhibited by the presence of the coenzyme CoM. Interaction studies of MsmS with the corresponding regulator protein MsrG resulted in a kinase-regulator interaction dependent on the redox state of the heme cofactor of the kinase. Furthermore, the redox potential of wild type MsmS-sGAF2 and a protein variant lacking the cysteine residue for heme binding were determined to investigate the redox sensory function and the influence of the covalent linkage on the oxidation state of MsmS-sGAF2.

#### References:

[1] Molitor *et al.* (2013), *J. Biol. Chem.* **288**, 18458-18472

## ARP012

### Translational coupling via termination/reinitiation in *Haloferax volcanii* and *Escherichia coli*

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Many genes in archaea and bacteria are organized in operons and are transcribed into polycistronic mRNAs. This enables translational coupling, i.e. translation of a downstream gene depends on translation of an upstream gene. One mechanism of translational coupling, "Termination-Reinitiation" (TeRe), is known from eukaryotic viruses and can operate on closely spaced or overlapping genes. In this case ribosomes that terminate translation of an upstream gene remain on the transcript (at least the small subunit) and reinitiate translation of a downstream gene. The genome of the haloarchaeon *H. volcanii* contains 4128 genes, 886 of which form 443 overlapping gene pairs. The genome of the bacteria *E. coli* contains 4288 genes, 820 of which form 410 overlapping gene pairs. These gene pairs contain 4 nt (ATGA) or 1 nt (TG/AATG) overlaps. Translational coupling was analyzed for 9 native gene overlaps from *H. volcanii* and 5 from *E. coli*. The gene

overlaps where fused to reporter genes (*dhfr* for *H. volcanii*, *glpD* + *gusA* for *E. coli*), and variants were compared that did or did not, respectively, allow the ribosome to reach the overlap. Quantification of translational efficiencies revealed that strict translational coupling via TeRe operates in the archaeal as well as in the bacterial species. Typically, overlapping gene pairs contain Shine Dalgarno (SD) motifs in the 3'-region of the upstream gene. In 12 cases the SD motif was replaced, and comparison of wildtypes and mutants revealed that the SD motif is of gene-specific varying importance for coupling efficiency

## ARP013

### Translation initiation factor network in *Haloferax volcanii*

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In all three domains of life initiation is the rate-limiting step of translation and thus determines the rate of protein synthesis. The efficiency of translation initiation and its differential regulation depends on translation initiation factors (IFs). Bacteria contain only three IFs, whereas the number is significantly higher for Archaea (aIFs) and Eukaryotes (eIFs). For example, *H. volcanii* contains 14 genes that encode (subunits of) aIFs. To elucidate their role, single gene deletion mutants were generated. This was successful in nine cases, but aIF1, aIF2 $\gamma$ , aIF5A, aIF5B and aIF6 turned out to be essential. Phenotypic analyses of all single gene deletion mutants revealed fitness defects for all mutants under at least one environmental condition.<sup>1</sup>

Further work concentrated on IF2 and IF1A, based on their central importance for preinitiation complex formation in all analyzed archaea and eukaryotes. His-tagged versions of the three subunits of the heterotrimeric aIF2 and of aIF1A were produced in the respective deletion mutants (except for the essential aIF2 $\gamma$ , which was produced in the wild-type). Co-affinity purification was used to isolate the aIFs and their binding partners, which were identified by peptide mass fingerprinting. Currently we are generating a protein interaction network, which contains various unexpected proteins, in addition to the expected aIFs and other translation-related proteins.

#### References:

[1] K. Gäbel *et al.*: A comprehensive analysis of the importance of translation initiation factors for *Haloferax volcanii* applying deletion and conditional depletion mutants. *PLoS One*. 2013;8(11):e77188. doi:10.1371/journal.pone.0077188.

## ARP014

### Single-domain zinc finger $\mu$ -proteins in *Haloferax volcanii*

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Very small proteins ( $\mu$ -proteins) have hardly been studied for a long time, but recently became an emerging field of research. Characterization of the "low molecular weight proteome" of *Halobacterium salinarum* led to the identification of 380 proteins of less than 20 kDa<sup>1</sup>. Most of them had no assigned function, and 20 contained two CPXCG motifs that were postulated to form a zinc finger. Currently, *Haloferax volcanii* is annotated to encode 282  $\mu$ -proteins with a length of less than 70 amino acids, only 24 of which have an assigned function. 43 of these  $\mu$ -proteins

contain at least two CPXCG motifs and are, therefore, most probably single-domain zinc finger  $\mu$ -proteins.

Until now 18 of the respective genes have been deleted, and 11 deletion mutants differed from the wildtype under at least one condition, e.g. growth capabilities with different carbon/phosphate/nitrogen sources, resistance against UV-/pH-/oxidative or osmotic stress, biofilm formation, or motility/chemotaxis.

The deletion of *HVO\_2753* coding for the only zinc finger  $\mu$ -protein containing four CPXCG motifs led to a complete loss of swarming activity and considerably decreased biofilm formation. The expression of the native *HVO\_2753* gene completely restored swarming. In contrast, production of single amino acid substitution mutants of the first cysteine in each CPXCG motif did not enhance swarming, showing that all four CPXCG motifs are essential for function. Further characterization of *HVO\_2753* is currently under way, including the identification of binding partners and structure determination via NMR.

#### References:

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#### ARP015

##### **PhyMet2: complex database containing records on methanogens with unique feature (MethanoGram) allowing prediction of culture conditions based on 16S rRNA**

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**Introduction:** Methanogens are methane-producing anaerobic archaea, that can be found in many anaerobic habitats. They are recognized as the largest biogenic source of methane, which is a potent greenhouse gas, and consequently as an important factor in the global carbon cycle. They also show growing potential for biotechnological uses. Our rudimentary knowledge about them results from difficulties with their isolation and culturing in laboratory conditions, which are necessary to describe their phenotype. Innovations in DNA sequencing technologies allowed for rapid development of metagenomics. DNA sequencing of environmental samples resulted in identifying a plethora of new uncultivated methanogens. Therefore, we created PhyMet2, the first database that combines description of methanogens and their culturing conditions with genetic information.

**Results:** The database, with a user-friendly interface design, contains a set of utilities for interactive data browsing and comparing as well as exploring phylogeny and searching for sequence homologues. Pnique feature of PhyMet2 is MethanoGram, a web server that can be used to significantly reduce the time and cost of searching for optimal culturing conditions of methanogens by predicting them based on given 16S rRNA sequences. The database will facilitate many researchers to explore the world of methanogens and their application in biotechnological processes. Moreover, we hope to apply our algorithm to prediction of culturing conditions of other microorganisms.

**Availability:** PhyMet2 with MethanoGram predictor is available at <http://metanogen.biotech.uni.wroc.pl>

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#### ARP016

##### **Trehalose metabolism in the hyperthermophilic Crenarchaeon *Sulfolobus acidocaldarius*: Enzymatic and functional analyses of the newly identified trehalose-6-phosphate synthase**

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The role of trehalose as compatible solute has been reported in various representatives from all three domains of life. In the Crenarchaeon *Sulfolobus acidocaldarius* trehalose represents the major compatible solute and the trehalose synthesis from glycogen via the two enzymes TreY (maltooligosyltrehalose synthase) and TreZ (maltooligosyltrehalose trehalohydrolase) is well established. The most common trehalose synthesis pathway via trehalose-6-phosphate synthase (TPS) (glucose 6-phosphate + UDP-glucose trehalose 6-phosphate + UDP) and trehalose-6-phosphate phosphatase (TPP) (trehalose 6-phosphate + UDP) trehalose + Pi) has so far been assumed to be absent from *Sulfolobus*. However, first analyses indicated that a *S. acidocaldarius* mutant lacking the TreY/TreZ pathway could still produce trehalose suggesting an additional trehalose synthesis pathway in this organism. In this work, a novel TPS from *S. acidocaldarius* was identified using bioinformatic analyses. The protein was recombinantly produced and enzymatically characterized. Additionally, a *tps* knock out strain of *S. acidocaldarius* was constructed and intracellular trehalose concentrations were comparatively analyzed to the wt and the treY/treZ mutant. The recombinant protein was characterized as TPS and showed a  $V_{max}$  of 5.8 U/mg, a  $K_m$ -value of 3.2 mM (G6P) and accepted UDPG and ADP-glucose as glycosyl donors. The enzyme exhibited a pH optimum of 7 and high thermal stability. Deletion of the *tps* gene in *S. acidocaldarius* resulted in a lower intracellular trehalose concentration in comparison to the wild type and to the treY/treZ mutant. These results indicate that in *S. acidocaldarius* a complete TPS/TPP pathway is operative in addition to the TreY/TreZ synthesis route.

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#### ARP017

##### **Development of a MurB based biosensor system for application in *Sulfolobus acidocaldarius***

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**Introduction:** The thermoacidophilic Archaeon *Sulfolobus acidocaldarius* grows optimally at 75-80°C and pH 2-3. These conditions match those of diluted acid/heat pretreatment required for lignocellulosic biomass conversion which makes *S. acidocaldarius* a high potential platform organism for such biotechnological applications. However, under such conditions toxic byproducts like furfural and 5-hydroxymethylfurfural (5-HMF) are formed inhibiting cellular processes. Thus, a reliable sensor system for these compounds is desirable to enable robust process control. The FAD containing, fluorescent UDP-N-acetylenolpyruvoylglucosamine reductase MurB which is involved in peptidoglycan synthesis in bacteria was recently introduced as reporter protein in mesophilic hosts.

**Objectives:** The aim is to develop a *murB* based biosensor system for application in *S. acidocaldarius*.

**Material & Methods:** Thermophilic *murB* homologs were identified, cloned and expressed in *E. coli* and the fluorescence was checked under different temperatures. For

promoter identification furfural and 5-HMF responsive genes were discovered in *S. acidocaldarius* grown in the presence of these compounds by transcriptomics.

**Results:** A MurB homolog from *Thermoanaerobacter italicus* was identified which showed sufficient thermostable fluorescence under the desired conditions. Furthermore, transcriptomic analyses of *S. acidocaldarius* revealed several upregulated genes in the presence of furfural/5-HMF some of which putatively involved in carotenoid biosynthesis.

**Conclusion:** These findings allow for plasmid design combining the identified promoters of the responsive genes with the *T. italicus murB* as well as the subsequent evaluation of the biosensor strain using fluorescence measurements upon induction furfural/5-HMF.

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#### ARP018

##### Investigation of the central carbohydrate metabolism in *S. solfataricus* P2 with a special focus on the upper Embden-Meyerhof-Parnas pathway

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*Sulfolobus solfataricus* P2 uses the branched Entner-Doudoroff (ED) pathway for D-glucose degradation. Additionally, it possesses a nearly complete set of proteins involved in the Embden-Meyerhof-Parnas (EMP) pathway, but due to a missing phosphofructokinase (PFK), *S. solfataricus* uses the EMP only in the anabolic direction. The reactions from the ED and lower EMP pathway are already well understood, but detailed informations on the upper EMP pathway and its role for biosynthetic processes in *S. solfataricus* are still scarce. In this work, the genes encoding hexokinase (HK) and phosphoglucose isomerase (PGI) from the upper EMP as well as phosphoglucomutase (PGM), the first enzyme of glycogen synthesis, were cloned and expressed in *E. coli* and the enzymes were investigated in respect to their kinetic and regulatory properties. HK showed the highest activity with D-glucose and ATP yielding D-glucose 6-phosphate (G6P) and ADP. To a lesser extend also mannose and GTP were accepted as substrates. PGI catalyzed the reversible conversion of G6P and D-mannose 6-phosphate (M6P) to D-fructose 6-phosphate (F6P) identifying the protein as PGI/PMI family enzyme also indicated by sequence analyses. The enzyme was inhibited by known effectors of PGI/PMI enzymes like erythrose 4-phosphate (E4P) and 6-phosphogluconate (6PG). The PGM converted G6P and M6P to the corresponding C1 phosphorylated derivatives G1P and M1P and was dependent on glucose 1,6-bisphosphate (G1,6BP). Fructose 1,6-bisphosphate (F1,6BP) acted as inhibitor which has already been described as competitive inhibition in other organisms. The results will contribute to a comprehensive understanding of essential biosynthetic processes e.g. the formation of glycogen as energy storage and trehalose as compatible solute.

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#### ARP019

##### Regulation of pentose transport and metabolism in *S. acidocaldarius*

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The thermophilic archaeon *S. acidocaldarius* can utilize different carbon sources, including the pentoses D-xylose

and L-arabinose. For the uptake of sugars membrane transport proteins are essential, since sugars cannot cross the cell membrane by diffusion. However, the corresponding transporter was still unknown. Here, we present the ABC transporter involved in the uptake of the pentoses from which the sugar binding protein (SBP) is novel to Archaea. In addition, we identified the possible regulator (Saci\_2116), which will be referred to as *xylR*. *XylR* induces transcription of the respective ABC-transporter.

The transport, regulation and utilization of sugars in *S. acidocaldarius* is poorly understood. In the study we aimed to identify and characterize the regulator responsible for the transcription of the pentose transporter.

RNA sequencing data revealed three highly upregulated genes encoding a so far unknown and uncharacterized ABC transport system. To confirm the role of the transport system as well as its regulator, deletion mutants were grown on different carbon sources. A quantitative RT-PCR was used to examine transcriptional levels of the SBP. The transcriptional levels of the transporter operon were induced upon the presence of L-arabinose and D-xylose. However, after the deletion of *xylR* this induction is no longer detected. The binding of *XylR* to the Ara-box was tested with an ONPG assay. When the regulator or the Ara-box was deleted the growth on the pentose substrate was strongly reduced. Also, deletion of either the regulator or the Ara-box caused a significant decrease in the  $\beta$ -galactosidase activity.

Taken together our results deepen the knowledge about this unique transport system for L-arabinose and D-xylose in *Sulfolobus acidocaldarius*.

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#### ARP020

##### Small proteins in *Methanosarcina mazei* strain Gö1

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**Introduction:** For the methanoarchaeon *Methanosarcina mazei* strain Gö1 new research topics revealed small RNA (sRNA) encoding putative open reading frames (sORF) putatively translated in small proteins (sP).

These sPs were found in all three domains of life and regulate several different cell mechanisms for example gene expression or the activity of other proteins. For *M. mazei* Gö1 several sPs have been identified by analyzing protein extracts of differently grown cells using LC-MS/MS and a combination of Bottom-up 2D-LC-MS and Semi-top-down GelFree-LC-MS, but the functions of these sPs are still unknown.

**Objective:** Our aim is the characterization of sPs and the identification of their interaction partners in *M. mazei* Gö1 to elucidate the biological functions.

**Materials and Methods:** The syntheses of the sPs are studied using Western Blot analysis under different stress conditions. Further pull-down experiments and LC-MS/MS analysis are performed to reveal the interaction partners. Interactions will be verified by microscale thermophoresis to biochemically characterize the sPs and their partners.

**Results:** In *M. mazei* Gö1 we found three promising small proteins: sP26 and sP36 are assumed to interact with the

nitrogen metabolism, due to their upregulation under nitrogen limiting conditions. sP44 is expressed on a stable level independently from stress conditions or growth phase.

**Conclusion:** Strong conservation of these sPs within the genus *Methanosarcina*, argue for an important role(s). Further research is required to unravel the whole coverage of the regulating and influencing network of the sPs in archaea using biochemical and genetic studies.

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#### ARP021

##### Improving protocols for the detection and quantification archaeal signatures in human tissue samples

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**Background and question:** The human body is the host to a huge number of microorganisms, the human microbiome. The microbiome is composed of bacteria, eukaryotes and archaea. However, the detection of archaea is often hindered due to methodological problems, so that their presence, abundance and importance are largely underestimated. The goal of our work is a) to improve the methods for archaeal detection and b) to reveal the archaeal contribution to the human microbiome in terms of: abundance, diversity and function.

**Methods:** Numerous different primer combinations were analysed for their capacity to comprehensively target the human archaeome for NGS sequencing and quantitative PCR in stool, appendix, nose, oral and skin samples, whereas the stool samples served as natural mock communities.

**Results:** Different primer combinations revealed a different pattern of the human archaeome in the various samples, emphasizing the importance of selecting the appropriate methodology. *In silico* analyses were proven to not properly reflect the primer specificity and efficiency.

**Conclusions:** Here, we propose novel protocols for the detection and quantification of archaeal 16S rRNA genes in human tissue samples. Our protocols will help to reveal the important roles of Archaea in the body-microbiome interplay and their clinical relevance.

#### References:

[1] Moissl-Eichinger, C et al.: Human age and skin physiology shape diversity and abundance of Archaea on skin. *Sci Rep.* 2017; 7(1): 4039-4039.

[2] Koskinen, K; Pausan, MR; Perras, AK et al.: First Insights into the Diverse Human Archaeome: Specific Detection of Archaea in the Gastrointestinal Tract, Lung, and Nose and on Skin. *MBio.* 2017; 8(6)

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#### ARP022

##### Enzymes of the 3-hydroxypropionate/4-hydroxybutyrate cycle in *Nitrosopumilus maritimus*

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Ammonia-oxidizing archaea (*Thaumarchaeota*) are among the most abundant microorganisms on Earth, contributing significantly to the global N and C cycles. All known representatives of this group are autotrophs. Being capable to grow at extremely low concentrations of ammonia, they need special metabolic adaptations to constantly low energy supply. Indeed, autotrophic CO<sub>2</sub> fixation proceeds in *Thaumarchaeota* via a modified 3-hydroxypropionate/4-hydroxybutyrate (HP/HB) cycle representing the most energy efficient aerobic autotrophic pathway [1]. Interestingly, most of the enzymes of this pathway are not homologous to the enzymes of the HP/HB cycle functioning in thermophilic *Crenarchaeota*, and the crenarchaeal HP/HB cycle is much less energy efficient than the thaumarchaeal variant, suggesting independent emergence of the HP/HB cycle in *Cren-* and *Thaumarchaeota*. Here we studied conversions of 3-hydroxypropionyl-CoA into acryloyl-CoA and of crotonyl-CoA into acetoacetyl-CoA in the HP/HB cycle in a thaumarchaeon *Nitrosopumilus maritimus*. A specific 3-hydroxypropionyl-CoA hydratase and a bifunctional crotonyl-CoA hydratase/3-hydroxybutyryl-CoA dehydrogenase catalyze these reactions in a crenarchaeon *Metallosphaera sedula*, whereas *N. maritimus* has different set of enzymes for these conversions, a promiscuous 3-hydroxypropionyl-CoA/3-hydroxybutyryl-CoA dehydratase and a monofunctional 3-hydroxybutyryl-CoA dehydrogenase. The analysis of biochemical properties of the corresponding enzymes in *N. maritimus* and *M. sedula* could not identify any advantage of one variant of the conversion over another and suggested their independent emergence, thus supporting the idea of a convergent evolution of autotrophic pathways in these two archaeal groups.

#### References:

[1] Könneke et al., *PNAS*, 111:8239 (2014)

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#### ARP023

##### Analysis of transcription factor 3 in *Sulfolobus acidocaldarius* using RNA-Seq

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In comparison to bacteria, the knowledge about enzymes, metabolic pathways or transcriptional networks in archaea is rather poor. Since the search for industrially relevant thermo- and pH-stable enzymes in these organisms has been intensified, a deeper understanding of the basic functions and the transcriptional architecture gets more and more important. Both identification of new enzymes, as well as the elucidation of transcriptional networks can be addressed by the analysis of transcriptomes via RNA-Seq. The alternative general transcription factor 3 (Tfb3) in *S. acidocaldarius* MW001 is involved in UV stress response. The *tfb3*-disruption mutant *tfb3::pyrEF* and *S. acidocaldarius* MW001<sup>pyrEF+</sup> as a reference were treated with UV light and cells were harvested before and 45 /90 minutes after the treatment. RNA was isolated for all samples and used for RNA-Seq. The analysis of the transcriptomes allowed to differentiate between a Tfb3-dependent and Tfb3-independent UV-response. The direct comparison of the transcriptomes of the mutant and the reference revealed seven Tfb3-dependent genes with a strongly reduced transcriptional level in the mutant at all timepoints. Since the promoters had already been identified in a different approach using a modified protocol for the preparation of the sequencing libraries to enable a genome-wide detection of transcription start sites(TSS), the promoters of the seven

genes were compared. The search for a conserved motif revealed the sequence ACTTCA, located 15-17nt upstream of the TSS. It can be assumed, that Tfb3 specifically binds to this sequence and thus increases transcription starting at these promoters.

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## Poster Session 2

### Environmental Microbiology and Ecology (EMP)

17 April 2018 • 14:30–17:00

#### EMP128

##### Sulfite respiration in *Wolinella succinogenes*: the role of MccC and MccD

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Reduction of sulfite (SO<sub>3</sub><sup>2-</sup>) to hydrogen sulfide (H<sub>2</sub>S) is an essential step in the sulfur cycle. The Epsilonproteobacterium *Wolinella succinogenes* uses the heme c-copper sulfite reductase MccA for sulfite respiration [1,2]. MccA is encoded by the first gene of the *mcc* gene cluster, whose transcription is presumably induced by the two-component regulatory system MccRS in response to sulfite and/or sulfur dioxide. It has been proposed that the iron-sulfur protein MccC, the putative quinol dehydrogenase MccD as well as menaquinone (MK) and/or 8-methylmenaquinone (8-MMK) were involved in the electron transport chain of *W. succinogenes* sulfite respiration [1]. Here, mutants of *W. succinogenes* were constructed that lacked *mccC*, *mccD* or *mqnK*, whose gene product is responsible for MK methylation to yield 8-MMK [3]. In contrast to previous experiments [1] all mutants possessed a frameshift-corrected *mccR* gene that enabled *mcc* induction during cell growth in the presence of a mixture of fumarate and sulfite as terminal electron acceptors. Under these conditions, cells lacking *mccC*, *mccD* or 8-MMK were severely hampered in sulfite turnover despite the presence of mature MccA. The results indicate that MccC, MccD and 8-MMK are essential components for efficient sulfite respiration in *W. succinogenes*.

#### References:

[1] Kern *et al.* (2011) *Mol. Microbiol.* 82, 1515-1530

[2] Hermann *et al.* (2015) *Nature* 520, 706-709

[3] Hein *et al.* (2017) *Mol. Microbiol.* 104, 449-462

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#### EMP129

##### Making the stress-protectant Ectoine: Structural and Biochemical Insights into the Acetyltransferase EctA

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**Introduction:** Ectoine is a compatible solute widely synthesized by microorganisms as an osmoprotectant. Due to its stabilizing effect on macromolecules, it is described as a chemical chaperone and used for medical and biotechnological applications.<sup>[1]</sup> The 2,4-diaminobutyrate acetyltransferase (EctA) catalyzes the second step of the ectoine synthesis pathway, the regiospecific acetylation of 2,4-diaminobutyric acid (DAB) to N-γ-acetyl-2,4-diaminobutyric acid (ADABA).<sup>[2]</sup>

**Objectives:** Here we focus on the biochemical and structural characteristics of EctA from the thermo-tolerant bacterium *Paenibacillus lautus*. To understand the biocatalytic mechanism of EctA, crystal structures in complex with DAB and the co-substrate acetyl-CoA are desired. Furthermore the biochemical characteristics, including enzyme kinetics, are of interest.

**Materials & Methods:** A codon optimized *ectA* of *P. lautus* was cloned into expression vectors for heterologous expression in *E. coli*. The produced protein was purified and crystallized or used for enzymatic assays. The activity of EctA was assessed by HPLC-based quantification of ADABA.

**Results:** Crystal structures of the native EctA, EctA co-crystallized with CoA and EctA with DAB and CoA were obtained and revealed the architecture of the active site. Preliminary biochemical characterization showed the optimal working conditions at 45 °C, pH 7.5 and the presence of 150 mM NaCl.

**Conclusion:** The crystal structures reveal a functional dimer. Relevant amino acids of the active site will be tested regarding their influence on the enzyme activity by site-directed mutagenesis studies. The further biochemical characterization is in progress.

#### References:

[1] J. M. Pastor, *et al.* (2010), *Biotechnol. Adv.* 28, 782–801.

[2] H. Ono, *et al.* (1999), *J Bacteriol.* 181, 91-9.

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#### EMP130

##### The effect of genetic modification of maize on soil fungi, bacteria, and bacterial denitrifiers at different field sites across Europe

M. Szoboszlay\*<sup>1</sup>, A. Näther<sup>1</sup>, C. Tebbe<sup>1</sup>

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The cultivation of transgenic maize expressing the insecticidal toxin of *Bacillus thuringiensis* (BT maize) is under debate in the European Union. Considering that maize is cultivated in diverse biogeographical regions in Europe, the environmental impact of BT maize should be evaluated at different growing areas. To assess the effects of BT maize on the soil bacterial and fungal community, four field sites located in Denmark, Slovakia, Spain, and Sweden representing four main biogeographical regions of Europe (atlantic, continental, mediterranean, boreal) were sampled in 2012, 2013, and 2014 within the AMIGA (Assessing and Monitoring the Impacts of Genetically modified plants on Agro-ecosystems) initiative of the European Commission. At each site, rhizosphere soil samples were collected from ten plots of BT and ten plots of near-isogenic non-BT maize at flowering stage. Metagenomic DNA extracted from the samples was subjected to qPCR to measure the abundance, and MiSeq amplicon sequencing targeting the 16S, ITS and *nirK* genes to determine the community structure of *Bacteria*, *Archaea*, *Fungi*, and bacterial denitrifiers. There were

significant differences in the composition of both the prokaryotic and the fungal communities in the maize rhizosphere between the four field sites which approximated their geographical distances. Variation between sampling years was also detectable. However, the genetic modification of maize did not have a significant effect on the rhizosphere prokaryotic and fungal community structure. Bacterial and fungal taxa that were detected independent of the field site and sampling year, and thus form the core community of the maize rhizosphere, were identified.

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#### EMP131

##### **Climate change and the rhizosphere microbiome of wheat: the effects of elevated atmospheric CO<sub>2</sub>, temperature, drought, and different N-fertilization regimes in a 2-year FACE experiment**

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Abstract has been withdrawn.

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#### EMP132

##### **Vibrio cholerae Combines Individual and Collective Sensing to Trigger Biofilm Dispersal**

P. K. Singh<sup>\*1</sup>, S. Bartalomej<sup>1</sup>, R. Hartmann<sup>1</sup>, C. D. Nadell<sup>2</sup>, K. Drescher<sup>1</sup>

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Bacteria can generate benefits for themselves and their kin by living in multicellular, matrix-enclosed communities, termed biofilms. The advantages of the biofilm mode of life include increased stress resistance and access to concentrated nutrient sources. However, there are also costs associated with biofilm growth, including the metabolic burden of biofilm matrix production, increased resource competition, and limited mobility inside the community. The decision-making strategies used by bacteria to weigh the costs between remaining in a biofilm or actively dispersing are largely unclear, even though the dispersal transition is a central aspect of the biofilm life cycle and critical for infection transmission. Using a combination of genetic and novel single-cell imaging approaches, we show that *Vibrio cholerae* integrates dual sensory inputs to control the dispersal response: cells use the general stress response, which can be induced *via* starvation, and they also integrate information about the local cell density and molecular transport conditions in the environment *via* the quorum sensing apparatus. By combining information from individual (stress response) and collective (quorum sensing) avenues of sensory input, biofilm-dwelling bacteria can make robust decisions to disperse from large biofilms under distress, while preventing premature dispersal when biofilm populations are small. These insights into triggers and regulators of biofilm dispersal are a key step towards actively inducing biofilm dispersal for technological and medical applications, and for environmental control of biofilm populations.

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#### EMP133

##### **Myxobacterial diversity: we only see the tip of the iceberg**

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Many bacteria are able to generate resistances against antibiotics leading to an uncontrolled spreading of pathogenic strains. The majority of medically important antibiotic drugs are derived from secondary metabolites, which are produced by bacteria and fungi. Myxobacteria, for example, are a rich source for new antibiotics. To date more than 100 new metabolites and more than 500 derivatives were described from these soil dwelling, predatory microorganisms. In particular new families, genera and species of myxobacteria are reliable sources for new bioactive compounds. Therefore the isolation of hitherto undescribed myxobacteria is of high importance. However, approximately only 1-10 % of all bacteria are cultivable under standard laboratory conditions and it was shown for many bacterial groups that there is a large discrepancy between cultivable species and those which are only detectable by cultivation-independent approaches. In order to enable the effect of common isolation techniques, we compared the diversity of cultivable myxobacteria from different habitats, i.e. Kiritimati beach, German compost, peat bog and fen to those which are detectable by cultivation independent, clone-bank analyses. 16S rRNA sequencing revealed that the majority of myxobacteria is exclusively represented by clone sequences and could not be acquired by cultivation. This means that there is a strong need to develop new and innovative isolation techniques to reach the numerous groups of myxobacteria which resist cultivation approaches. Especially uncommon habitats like moors, but also classical habitats like compost consist of numerous unknown myxobacterial species, genera and even families which in turn harbour a great potential of urgently needed, new antibiotics.

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#### EMP134

##### **Bacterial-algal interactions mediated by siderophores**

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Iron is essential for virtually all organisms, but its bioavailability is limited. In order to solubilize iron from the environment, many microorganisms produce siderophores, which are low molecular weight iron chelators. Upon their secretion, siderophores complex ferric iron and deliver it to the cell. The freshwater bacterium *Cupriavidus necator* H16 produces the siderophore cupriachelin [1]. This siderophore is special in that it is photoreactive. Once it has complexed ferric iron, cupriachelin rapidly undergoes a light-induced cleavage reaction, thereby releasing ferrous iron into the environment. The released iron can thus be easily taken up by the surrounding planktic community. In this study, we investigated the cupriachelin-based interaction between *C. necator* H16 and the diatom *Navicula pelliculosa*. The proposed exchange of iron and organic carbon between the two organisms is supported by a positive growth effect of the bacterium on the alga. We analyzed the effect of algal exudates on bacterial siderophore transcription levels using a  $\beta$ -galactosidase assay. Furthermore, we investigated the transcription factors regulating cupriachelin biosynthesis under different conditions using a DNA protein pulldown assay.

#### References:

[1] Kreutzer, M. F., Kage, H., & Nett, M. (2012). Structure

and biosynthetic assembly of cupriachelin, a photoreactive siderophore from the bioplastic producer *Cupriavidus necator* H16. *Journal of the American Chemical Society*, 134(11), 5415-5422.

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### EMP135

#### Microbial diversity, abundance and activity in a dry CO<sub>2</sub> degassing mofette in Hartoušov, NW Bohemia

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The Cheb Basin (CZ) is a shallow neogene intracontinental basin filled with fluvial and lacustrine sediments. The central and northern parts of the seismic active rift are characterized through diffuse degassing of mantle-derived CO<sub>2</sub> in form of so-called mofettes. The Hartoušov mofette field shows the highest CO<sub>2</sub> flux (> 99%) and soil gas concentration among those mofettes. The present study aimed to characterize the influence of elevated CO<sub>2</sub> concentrations on the geochemistry and microbial community. Two 3 m drillings were performed, one located in the centre of the degassing structure and the other 8 m away served as an undisturbed reference. The sites were compared in terms of their geochemical features, microbial abundances, community structures and methanogenic activity. The results highlight the strong impact of elevated CO<sub>2</sub> concentrations and associated side effects on microbial processes. Illumina MiSeq sequencing of the 16S rRNA genes and multivariate statistics revealed that the pH strongly influenced species distribution and explained around 35.3 % of the variance between mofette and the undisturbed site. Accordingly, acidophilic microorganisms (e.g. *Acidobacteriaceae* and *Acidithiobacillus*) showed a much higher relative abundance in the mofette. Additionally, genera potentially linked to carbon fixation were found in deep sediments of the mofette. Activity tests performed with sediments from both sites showed that the potential microbial methane production rate is significantly higher in the mofette. We demonstrated that an increased availability of hydrogen (occurring during seismic events) can trigger methanogenic activity. The present study provides insight into microbial life and bio-geo interactions in CO<sub>2</sub>-dominated habitats such as mofettes.

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### EMP136

#### Actinobacterium that can produce ibuprofen via a novel styrene degradation pathway

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**Introduction:** The actinobacterium *Gordonia rubripertincta* CWB2 was isolated on styrene as sole source of carbon and energy. While offering a fast degradation of styrene, strain

CWB2 is also able to withstand high concentrations of this toxic compound compared to other actinobacteria. Styrene degradation is usually initiated by epoxidation to styrene oxide and leads to phenylacetic acid as central metabolite. This is done by a styrene monooxygenase (SMO), an isomerase (SOI) and an aldehyde dehydrogenase (PAD).

**Objectives:** Metabolomic analysis of strain CWB2 indicated for an anomaly in the styrene degradation pathway as a key enzyme (SOI) could not be detected. As it was also possible to produce ibuprofen via that pathway, it was consecutive to clarify the strategy of that high-GC Gram-positive actinobacterium.

**Materials & methods:** Metabolites, enzyme activities, genome, transcriptome and proteome of *G. rubripertincta* CWB2 were analyzed.

**Results:** Omic investigations allowed identifying a gene cluster of about 32 kbp, which is upregulated under styrene exposure. It contains SMO and PAD, which were recombinant expressed and approved to be active. Connection of both steps is supposed to be done by a glutathione dependent transferase. Glutathione dependent degradation of styrene oxide was confirmed in crude extract. This is remarkable as glutathione utilization is unusual in Gram-positive bacteria.

**Conclusion:** *G. rubripertincta* CWB2 assembled gene clusters from foreign origin to enable metabolization of styrene and analogous compounds. Therewith, strain CWB2 is able to produce ibuprofen.

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### EMP137

#### Metaproteomic analyses of antibiotic resistances and antibiotic resistant bacteria in wastewater treatment plants

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**Introduction:** The massive use of antibiotics (AB) in human medicine as well as animal breeding leads to a growing input of antibiotics and antibiotic resistant bacteria (ARB) into the environment. Urban wastewater treatment plants (UWTP) have been considered as hot spots for the emergence and dissemination of ARB. The high bacterial density in these facilities promotes horizontal gene transfer and therefor the exchange of antibiotic-resistance determinants between pathogenic and non-pathogenic bacteria. There is a growing interest in investigating the role of UWTP in the spread of ARB and the dissemination of resistant determinants.

**Objective:** Using a metaproteomics approach, we aim at the identification and quantification of metabolically active and antibiotic-resistant bacteria as well as resistance determinants at different sites of the UWTP.

**Material & methods:** Samples from different stages of sewage treatment were analyzed by GeLC-MS/MS. For protein identification sequence databases based on metagenome data were created. Antibiotic resistance proteins were predicted using online tools (ARDB, Resfams). In addition, culture-dependent methods were applied to determine the proportion of ARB.

**Results:** Bacterial community changes during sewage treatment. Members of human gut microbiome and bacteria related to infections of the gastro-intestinal/urinary tract that are known to carry antibiotic resistances dominate the inlet. Activated sludge processes add bacteria that remove nutrients. Treated wastewater contains significantly decreased bacterial numbers but still includes ARB and pathogens.

**Conclusion:** Sewage treatment leads to reduction but not clearance of ARB and potential pathogens. Their release into the environment could promote the spread of AB resistances.

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### EMP138

#### A glyphosate pulse to a brackish long-term microcosm leads to a prolonged shift in the stoichiometric composition of the *phn* operon within the bacterial metagenome

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The herbicide glyphosate has been detected in aquatic coastal zones of the southern Baltic Sea with yet unknown consequences for this brackish ecosystem. We investigated the impact of glyphosate on the succession of bacterial community assemblages and functions in brackish and phosphorus-limited microcosms. These were incubated for 69 days prior adding of glyphosate to obtain stable community dynamics; eventually, the microcosms were incubated for further 72 days. The system was sampled up to twice a week for the whole incubation period and analyzed concerning glyphosate degradation and bacterial succession. Succession of bacterial assemblages and their functions was determined by next generation 16S rRNA (gene) amplicon as well as shotgun metagenomic sequencing, respectively. As result, glyphosate was degraded without detectable amounts of aminomethylphosphonic acid. Glyphosate addition revealed shifts in the bacterial community compositions, as well as increased cell counts, the abundance of specific operational taxonomic units, microbial diversity, and species richness. Metagenomic analyses revealed a shift in the stoichiometric composition of the *phn* operon, resulting in increased numbers of the *phnM* gene. This shift was detected till the end of the experiment whereas the other described responses ended with glyphosate concentrations below 1 mg·L<sup>-1</sup>. Thus, glyphosate impacted bacterial assemblages in a brackish system on different levels, but especially their functional gene compositions for a prolonged period of time.

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### EMP139

#### The distribution of active iron-cycling bacteria in marine and freshwater sediments is decoupled from geochemical gradients

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Microorganisms can oxidize Fe(II) with oxygen, with nitrate or with light during CO<sub>2</sub> reduction. Recently, it was shown that these three physiological types of neutrophilic Fe(II)-oxidizers (FeOx) co-exist in marine (Aarhus Bay, Denmark) and freshwater (Lake Constance, Germany) surface sediments. However, the *in-situ* abundance, depth distribution, and diversity of metabolically active Fe(II)-

oxidizers and Fe(III)-reducers (FeRed) remained largely unexplored in typical low-iron aquatic environments.

Here, we characterized the microbial community composition at the oxic-anoxic interface of marine and freshwater sediments using DNA-/RNA-based next generation 16S rRNA (gene) amplicon sequencing. All three physiological groups of neutrophilic FeOx were found to be active in marine and freshwater sediments, revealing up to 0.2% anoxygenic photoferrotrophs (e.g. *Rhodobacter*, *Chlorobium*), 0.1% microaerophilic FeOx (e.g. *Mariprofundus*, *Gallionella*), and 0.3% nitrate-reducing FeOx (e.g. *Thiobacillus*, *Pseudomonas*, *Hoeflea*). Active FeRed (e.g. *Shewanella*, *Geobacter*) were most abundant (up to 2.8%) in marine sediments and co-occurred with FeOx and cable bacteria (up to 2.5%).

Geochemical profiles of Fe(III), Fe(II), O<sub>2</sub>, light, nitrate, and total organic carbon revealed a redox stratification of the sediments and explained 75-85% of the vertical depth distribution of most microbial groups, while active Fe-cycling bacteria were found to be decoupled from geochemical gradients within the upper 3 cm of the sediments. We suggest that metabolic flexibility, micro-niches in the sediments, and particularly interrelationships of Fe-cycling bacteria with filamentous cable bacteria might explain the unexpected depth distribution patterns of active Fe-cycling bacteria.

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### EMP140

#### Establishment of a metaproteomics pipeline to unravel the mechanisms of polysaccharide degradation of particle-associated microbial communities

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**Introduction:** Marine snow plays a central role in carbon cycling. It consists of organic particles and particle-associated microbial communities. The potential for distinct compositionality and functionality among planktonic marine bacteria has been recently demonstrated by integrated omics-approaches. In contrast, structure and function of particle-associated bacterial communities are largely unexplored.

**Objective:** Our working hypothesis is that abundant particle-associated bacteria differ from their planktonic counterparts. Using a metaproteomics approach combined with FISH-analyses, metagenomics and -transcriptomics, we want to unravel the taxonomic groups and metabolic key-players present on marine aggregates, the key-functions involved in polysaccharide degradation, and how the taxonomy and expressed functions respond to spring algae blooms in the North Sea.

**Material & Methods:** For the metaproteomic approach bacterioplankton samples (3 and 10 µm filter fractions) collected during spring phytoplankton bloom in 2009, were analysed by GeLC-MS/MS. As a starting point an efficient and reliable metaproteomics protocol had to be established.

**Results:** A combination of SDS-buffer extraction and bead beating led to highest protein identification rates for both fractions. First results indicate that the most abundant taxonomic groups are *Flavobacteria*,  $\alpha$ - and  $\gamma$ -*Proteobacteria*. Different types of transporter proteins (TonB-

dependent transporter) and hydrolytic enzymes (galactosidases, sulfatases) have been identified.

**Conclusion:** The established metaproteomics pipeline allowed for the identification of proteins that have been assigned to taxonomic groups and functions that were also found in planktonic samples collected in parallel to the marine particle (Teeling 2012).

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#### EMP141

##### **Molecular detection of denitrifying bacteria and N-transformation processes in groundwater and surface water**

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Worldwide, agriculture is the most extensive anthropogenic source of nitrate to surface water and groundwater systems. Due to the broad application of N fertilizers, the nitrate threshold value of 50 mg/L (German TrinkwV) is already exceeded in many wells across Germany. Microbial denitrification is the key process involved in natural attenuation of nitrate and thus able to improve water quality. This study focuses on the assessment of heterotrophic denitrification as ecosystem service to naturally attenuate nitrate. Molecular methods are developed to monitor the extent of microbial nitrate degradation in an ecological system. Therefore, pure cultures isolated from contaminated water samples were cultivated and grown in batch experiments. Ion chromatography was used to analyse the degradation of nitrate to nitrite as the first step of denitrification. To analyse the denitrification process on a molecular level, standards were developed and extraction methods adjusted. The transcription levels of mRNA (*narG*, *nirK*, *nirS*, *nosZ*) encoding enzymes involved in denitrification were quantified by quantitative polymerase chain reaction (qPCR). First results with laboratory cultures show that the analytical results correlate with the transcription levels and therefore indicate this technique as a successful monitoring method of active denitrification. As a next step, these methods will be tested with environmental samples which require a lower detection limit regarding DNA and mRNA concentrations. The study will help understanding natural attenuation in contaminated sites and will provide a quick screening method for potential denitrification. The research is funded by the BMBF within the projects "GroundCare" and "SIGN" (grant numbers 033W037B and 02WCL1336A).

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#### EMP142

##### **Organic soil amendments impact on bacterial community structure and co-occurrence patterns in a long-term field trial**

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In this study, the consequences of long-term application of organic amendments to soil for bacterial co-occurrence have been investigated. Previous research focussed mostly on species diversities and abundance changes of single species, neglecting effects on bacterial co-occurrence. In our study we systematically compared two frequently used soil amendments, manure and straw, and their impacts on bacterial co-occurrence in a long-term field trial in Speyer,

Germany. Using 16S rRNA gene amplicon sequencing combined with bacterial network analysis, we compared the different management regimes evaluating each factor's impact on the bacterial community. We observed an increase in bacterial diversity as well as an accumulation of bacteria of the order Bacillales, when plots were amended with horse manure as compared to a control treatment. Straw-amended plots, however, did neither show changes in diversity nor indicative species could be identified. Network analyses indicated an increased complexity of the bacterial co-occurrence structure in plots amended with either manure and straw. Furthermore, we could demonstrate the clear consequences of mineral fertiliser addition for the connectivity of these networks. Differences in the effects of manure and straw might be attributed to their differences in their nutritional / chemical contents. We concluded that bacterial interactions are a crucial parameter for the assessment of amendment effects with respect to their roles in soil health and sustainability.

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#### EMP143

##### **Nitrogen metabolism in aquaponic systems**

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**Introduction:** In the last 20 years aquaponic systems have become increasingly popular as innovative and sustainable technology for fish and vegetable production. These systems combine aquacultures (fish cultivation) and hydroponics (plant cultivation) in one recirculating system. The ammonium enriched effluent from the aquaculture is pumped into the hydroponic part serving as fertilizer for the vegetables and fruits. So far, the nitrification process has only been investigated by measuring the concentrations of ammonium, nitrite and nitrate.

**Objectives:** In this study, we investigated the bacterial communities in the sump of a back yard aquaponic system to understand the metabolic processes involved in nitrogen cycling and how steering of the nitrogen metabolism can be improved.

**Methods:** Curriers filled with sand were incubated in the aquaponic sump and regularly sampled over two years. The DNA was extracted from the sand grains, amplified using general primers for bacterial and archaeal 16S rRNA gene sequences, and the amplicons were sequenced.

**Results:** The sequencing results showed a very heterogenous community composition changing over time into a highly specified community performing nitrification. The most abundant genus with the potential of nitrification is *Nitrospira*. Interestingly, the commonly named nitrifying genera *Nitrosomonas* and *Nitrobacter* could not be found. Additionally, some very abundant genera are capable of denitrification and nitrogen fixation.

**Conclusion:** The bacterial community in an aquaponic is highly diverse and the genetic potential for all parts of the microbial nitrogen cycle is present in an aquaponic system.

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#### EMP144

##### **Droplet Digital PCR for quantification of *Macrococcus* spp. in bioaerosols**

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Strains of *Macrococcus caseolyticus* and *M. canis* have been shown to be potential sources of conferring methicillin resistance to *Staphylococcus aureus*. Additionally, *M. canis* is described as a possible opportunistic animal pathogen. Thus, detection of both organisms is important for occupational and environmental research. Here, a specific Droplet Digital PCR (ddPCR) system was established for detection and quantification of both species in bioaerosols.

The established probe-based ddPCR assay targets species specific 16S rRNA gene sections. Bioaerosol samples were collected in chicken poultry houses by personal air sampling. DNA from droplets was extracted and subsequently sequenced in order to verify specificity of the assay in bioaerosol samples.

Specificity of the assay was positively tested against other members of the genus *Macrococcus*. The ddPCR assay was analysed over two orders of magnitude with different target genome amounts. Correlation analyses for both target species were above 0.99. The limit of quantification is at 50 genes per reaction. Cloning and sequencing of DNA from droplets revealed highly specific amplification of target organisms in the bioaerosol samples. Taken together these findings support the applicability of this assay for target organism detection and quantification in complex bioaerosol samples. Quantitative analysis of bioaerosol samples from chicken poultry houses showed concentrations of *M. caseolyticus* and/or *M. canis* increasing up to 2,000 cells/ m<sup>3</sup> air.

A quantitative ddPCR assay was established for *M. caseolyticus* and *M. canis*. Application of this assay in poultry houses revealed a ubiquitous exposure of workers to these organisms and their emission into the environment.

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#### EMP145

##### **The distribution of benthic *Roseobacter* group members in the Pacific is shaped by nutrient availability at the seafloor and productivity in the water column**

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The *Roseobacter* group (short: roseobacters) represents a significant part of pelagic microbial communities, but information on their biogeography in deep-sea sediments is sparse. Hence, Pacific seafloor sediments along the 180° meridian from New Zealand to Alaska were analyzed for the abundance and diversity of roseobacters. We hypothesized a distinct distribution of the *Roseobacter* group linked to environmental conditions within different oceanic provinces. Cell counts were lowest in both nutrient depleted mid-ocean gyres and increased to the North showing maximum values in the highly productive Bering Sea. Specific quantification of roseobacters by CARD-FISH and qPCR revealed on average a relative abundance of 1.7% and 6.3%, respectively. Sequencing of 16S rRNA genes and transcripts showed different compositions containing on average 0.7% and 0.9% *Roseobacter*-affiliated OTUs of the present and active communities. Most OTUs were assigned to uncultured roseobacters, but also *Sedimentitalea*- and *Sulfitobacter*-affiliated OTUs made up substantial proportions. The different oceanic provinces with low nutrient content such as both ocean gyres were characterized by specific *Roseobacter*-communities, distinct from those of the more productive Pacific subarctic region and the Bering Sea.

The chlorophyll content of the water column influenced the diversity of active roseobacters at the seafloor, while iron(III) oxide and silicic acid concentrations had an impact on the present *Roseobacter*-communities. However, linking their community structure to specific metabolic processes in sediments was hampered by the dominance of so-far uncultured members of the *Roseobacter* group, indicating a diversity that has yet to be explored.

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#### EMP146

##### **Acetone degradation in sulfate-reducing bacteria**

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Acetone degradation is well understood in aerobic and nitrate-reducing bacteria. The latter activate acetone via carboxylation to acetoacetate consuming at least two ATP equivalents. Due to their limited energy budget such an expensive activation is hardly possible for sulfate-reducing bacteria (SRB). Acetoacetate was ruled out as an intermediate as no acetone carboxylases were found in the genome of the acetone-degrading sulfate reducer *Desulfococcus biacutus*.

Studies on *D. biacutus* indicated an involvement of ATP-, TDP (thiamine diphosphate)- and B12-dependent enzymes, leading finally to acetoacetyl-CoA. Comparative 2D-PAGE of *D. biacutus* allowed the identification of several induced proteins during growth with acetone, which are potentially involved in acetone degradation.

Some candidate enzymes (two dehydrogenases, a TDP-dependent enzyme and a B12-dependent mutase) were successfully cloned and overexpressed in *Escherichia coli*. Purified recombinant enzymes were further characterized. One of these enzymes exhibited aldehyde/ketone oxidoreductase activity, the mutase performed the isomerization of 2-hydroxyisobutyryl-CoA to 3-hydroxybutyryl-CoA and a dehydrogenase oxidized 3-hydroxybutyryl-CoA to acetoacetyl-CoA. However, the initial activation step (possibly involving an activated formyl residue) of acetone degradation by *D. biacutus* is still under research. Analysis for homologous genes in other organisms revealed only three other strains that contain comparable genes: *Desulfosarcina cetonica* (the only other described acetone-degrading SRB), *Desulfotomaculum arcticum*, and *Desulfotomaculum geothermicum*. These bacteria are of special interest to finally solve the biochemistry behind this new pathway of acetone degradation.

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#### EMP147

##### **Microbial mineral precipitation - a smart tool in fossil preservation**

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Due to the long time periods, not all stages of fossil formation are well understood. Though it should be expected that formation pathways for fossils did not change throughout earth history, it is obvious that the results of some fossilization processes are just observable in specific strata. One peculiar example is the formation of carbonate concretions. Frequently, inside concretions, consisting of e.g. calcite or siderite, exceptionally preserved fossils are found. In the initial steps of fossil formation, microorganisms

hydrolyze the complex organic matter into reduced organic compounds and CO<sub>2</sub> – the latter will be one building block of a carbonate concretion. Sulfate reduction was identified as a key process for concretion formation. In our study we investigated the complex microbial communities involved in formation of the carbonate precipitates. We prepared mesocosms with marine sediments, artificial sea water and various lipid-like substrates. DNA was extracted at certain time points and subjected to analysis of microbial communities based upon next generation sequencing. Mineral precipitates were observed by light and electron microscopy (EM). Mesocosms with Span 80 (sorbitan monolaurate) substrate exhibited more than 50% of retrieved ribosomal RNA sequences related to the sulfate reducer *Desulfofrigus*. EM revealed nanoparticles decorating the cell periphery of the bacterial morphotype. These findings point to the relevance of lipid substrate for initial biomineral formation by sulfate reducers. So, a comparative analysis of carbonate concretions in recent mesocosms and fossils is feasible. We will use model organisms for biomineral formation and will identify the lipid and kerogen biomarkers in concretions.

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#### EMP148

##### **It's the mix: substrate diversity activates soil anaerobes in earthworm gut contents**

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Earthworms feed on soil and litter, and promote soil fertility. Fermentative microbes in the anoxic gut decompose biopolymers derived in part from ingested plant and microbial cells that are subject to rupture by the gizzard. Fermentation products in the gut can be utilized by the earthworm as sources of organic carbon and energy. Given the feeding habits of earthworms, the fermentative microbial partners of their digestive system might be conceptualized to be dominated by ingested "transients" that are activated by the beneficial conditions in the anoxic gut, but syntrophic "residents" might also be a part of the gut microbiome. Soil supplemented with simulated gizzard-ruptured cells to mimic the complex nutrient-richness of the earthworm gut displayed a rapid and robust fermentation profile that was nearly identical to that of gut content. This finding is in marked contrast to the inability of so-called high value compounds (e.g., glucose) to rapidly activate fermentative soil microbes. 16S rRNA and 16S rRNA gene analyses revealed that the same microbial taxa (including *Peptostreptococcaceae*, *Aeromonadaceae*, and *Enterobacteriaceae*) were activated in both soil and gut content microcosms supplemented with simulated gizzard-ruptured cells. These findings (i) indicate that soil-derived transients have the potential to convert complex food sources derived in part from gizzard-ruptured cells to fermentation products that can subsequently be assimilated by the earthworm, trophic interactions that likely contribute to the turnover of matter in the terrestrial biosphere, and (ii) illustrate that it is the combined complexity of the "mix" rather than the availability of one or two high value compounds that might drive the ingested gut microbiome.

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#### EMP149

##### **The first evaluation report of *Nostoc entophyllum* ISC32 cell response to the stress caused by exposure to cadmium at the cellular, molecular and ultrastructural levels**

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The present study is pursuing our previous research, focused on some aspects of *Nostoc entophyllum* ISC32 cell response to the stress caused by exposure to cadmium at the cellular and molecular levels. Variations in the antioxidant system of *N. entophyllum* ISC32 exposed to varying concentrations of Cd (2, and 5 mg/L) resulted in a significant increase in the activity of both catalase and peroxidase. Activity of these enzymes was, however, not significantly changed in the presence of Cd concentrations of 10 and 20 mg/L. Levels of lipid peroxidation, as measured by malondialdehyde (MDA) assay, were observed in response to exposure to Cd (20 mg/L). The content of chlorophyll a and phycobiliproteins of living cells were altered under Cd-induced conditions. TEM images of cyanobacterial cells treated with Cd showed cell surface alteration and modification along with altered cellular microcompartments. Cyanobacterial cells treated with Cd at concentrations below the minimum inhibitory concentration (MIC) remained with no apparent structural changes. However, at a higher concentration of Cd (30 mg/L), a clear detachment effect was observed between the mucilage external layer and cell membrane which may be attributed to cell plasmolysis. Subsequently, the thickness of the ring-shaped mucilage external layer increased likely as a result of the cell defense mechanisms against toxic concentrations of Cd. Energy-dispersive X-ray spectrometry (EDS) analysis suggested that Cd was not present as nanoparticles. The up-regulation of chaperons was confirmed for GroEL and HtpG using real-time PCR and northern blot analyses. Collectively, our findings have led to a better understanding of the events occurred in the cyanobacterial cells in response to Cd stress.

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#### EMP150

##### **Microbial community of the volcanic area at Pantelleria Island, Italy**

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Volcanic ecosystems are characterized by high temperature, low pH and emission of the greenhouse gasses methane and carbon dioxide. Despite these hostile conditions, microbial activity is observed in these ecosystems. Microbes are involved in the elemental cycles of carbon, nitrogen and sulfur. The aim of the project is to obtain a fundamental understanding of the microbial ecology of extremely acid terrestrial volcanic ecosystems. Pantelleria Island, south of Sicily, Italy, is a volcanic area, characterized by a high soil temperature (50-100°C), low pH (2.5-4.5) and methane, hydrogen and carbon dioxide emissions. Hydrogen and methane fractions in the gas are decreasing towards the surface. Oxygen is only measured in the top layer of the soil, up to 20 cm. The measured gas profiles can be described as the combined effect of mixing with air and microbial consumption. In order to link the physicochemical properties of the ecosystem to the microbial community, metagenome studies are performed. Using two different DNA extraction methods and Illumina sequencing, 30 genomes of over 90% completeness could be assembled. Many of the microorganisms in this extreme environment are only distantly related to known, cultivated bacteria. Furthermore,

based on metagenomics analysis, the most abundant microorganism is a methanogen, belonging to the *Methanocella* genus. In addition, aerobic methanotrophs, belonging to the *Verrucomicrobia* and *Gammaproteobacteria*, are found. This research shows that the volcanic area of Pantelleria Island hosts a variety of novel microorganisms and many play an important role in the carbon cycle.

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### EMP151

#### Impact of the viral shunt on microbial community structures and organic matter composition in deep-sea sediments

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Viruses were identified to be responsible for a large part of microbial mortality on the seafloor. The virus-mediated release of cellular components furthermore provides labile organic matter for indigenous prokaryotic communities. Therefore, the so-called viral shunt is expected to have a major impact on the dynamics of microbial populations and biogeochemical cycles. To better understand the role of viruses for shaping the benthic community composition and the organic matter pool, a virus-induction experiment using mitomycin C was performed on deep-sea sediment slurries from the Bering Sea (3,300 m depth). In the mitomycin C treated sediments from 20 cm below seafloor (cmbsf), virus-like particles increased and virus-to-cell ratios nearly doubled from 34 to 76, indicating a successful induction of prophages. Cell lysis led to high releases of amino acids, carbohydrates and dissolved organic carbon. In parallel, Illumina sequencing of 16S rRNA genes and transcripts showed a relatively stable community composition over 2 months of incubation. In contrast, sediments from 0 cmbsf showed no prophage induction. The 16S rRNA libraries exhibited increasing abundances of dominant bacterial taxa (e.g. *Gammaproteobacteria*) at the expense of less abundant phyla. Hence, the induction of prophages functioned as regulating mechanism playing an important role in sustaining microbial diversity in deep-sea sediments. The virus-generated nutrient and carbon release might have resulted in new ecological niches, further supporting bacterial diversity. Ongoing dissolved organic matter (DOM) analysis and correlation with the present and active microbial community structures will lead to a deeper insight in the interactions between the DOM pool and bacterial communities.

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### EMP152

#### Towards the Response Threshold for *p*-Hydroxyacetophenone in the Denitrifying Bacterium *Aromatoleum aromaticum* EbN1

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The denitrifying Betaproteobacterium "*Aromatoleum aromaticum*" EbN1 regulates formation of proteins involved in the sequential *p*-ethylphenol and *p*-hydroxyacetophenone degradation with high substrate-specificity. This process was demonstrated to be mediated by the  $\sigma^{54}$ -dependent transcriptional regulator EtpR that apparently recognizes both aromatic compounds. In this study, the responsiveness of this regulatory system towards the effector *p*-hydroxyacetophenone was studied. The latter is more easily applicable due to its higher water solubility (as compared to *p*-ethylphenol) and well traceable analytically. Cultures of "A.

*aromaticum*" EbN1 were initially cultivated under nitrate-reducing conditions with a growth-limiting supply of benzoate. Upon complete benzoate depletion, *p*-hydroxyacetophenone was added at varying concentrations (500  $\mu$ M to 0.1 nM). Depletion profiles of this substrate and effector, respectively, were determined by highly sensitive reversed phase microHPLC. Irrespective of the added *p*-hydroxyacetophenone concentration of, depletion commenced after approx. 5 min and suggested a response threshold of below 10 nM. Time-resolved transcript profiles (qRT-PCR) of genes located at the start, center and end of the *p*-ethylphenol/*p*-hydroxyacetophenone degradation gene cluster (e.g. *pchF* encoding a subunit of the predicted *p*-ethylphenol methylenehydroxylase) and its related efflux system gene cluster indeed revealed a threshold concentration in a range of 1 – 10 nM. Furthermore, the extend of the transcriptional response was observed to be effector concentration-dependent, with a maximal expression level reached at  $\geq 1 \mu$ M effector and highest expression levels for genes located at the beginning of the two clusters (i.e. *acsA* and *ebA335*).

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### EMP153

#### Amino Acid and Sugar Catabolism in the Marine Bacterium *Phaeobacter inhibens* from an Energetic Viewpoint

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Energetics and efficiency of growth belong to the key determinants of microbial habitat performance and success. The former were studied with *Phaeobacter inhibens* DSM 17395, a member of the alphaproteobacterial marine roseobacters, by combining theoretical considerations on the known catabolic network (9 amino acids, 5 sugars) with quantitative growth experiments (7 substrates e.g. glucose, leucine) using process-controlled bioreactors. Energetic efficiency was studied based on catabolic ATP yields calculated for the 14 degradation pathways and resulted, in combination with the experimental data, in yields for ATP ( $Y_{X/ATP}$ ). Experimentally determined growth rates ( $\mu_{max}$ ) ranged from  $\sim 0.058 \text{ h}^{-1}$  (lysine) to  $\sim 0.179 \text{ h}^{-1}$  (threonine). The bioreactor studies with *N*-acetylglucosamine and phenylalanine (phe) revealed similar yields for carbon consumed in total ( $Y_{X/Ctotal}$ ) and for ATP, while those for dissimilated carbon ( $Y_{X/Cdiss}$ ) and consumed oxygen ( $Y_{X/O2}$ ) were significantly lower for phe translating accordingly into lower shares of assimilated carbon and P:O ratio. This reduction of performance correlates with the markedly higher production of trophodithiic acid (TDA) during growth with phe, a secreted secondary metabolite which has previously been shown to collapse the proton motive force. Additionally these data allowed for interpretation of the assimilation share for each tested substrate in a defined substrate mixture (8 amino acids and 3 sugars) revealing insights of the specific substrate consumption and its impact on the energy flux.

## EMP154

### The influence of bursting bubbles on marine bacterial communities: from transport into the air-water interface to atmospheric export

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Countless bubbles rise through the surface ocean after wave breaking. These bubbles are effective transport vehicles for organic material and microorganisms. When reaching the air-sea interface, the bubbles break and release material into the interface itself or into the atmosphere. While the interaction of bubble bursting, interfacial properties and air-sea exchange processes is of profound importance for biogeochemical cycles, consistent studies addressing these interactions are missing.

We artificially bubbled the upper water column during several field campaigns in the coastal Baltic Sea and studied its effect on the abundance of organic gel particles and microorganisms at the air-water interface. A similar approach was applied using a 1300 L tank filled with North Sea water to better characterize the material aerolised.

We consistently observed an enhanced enrichment of organic gel particles at the air-sea interface after bubbling. While the abundance of small phytoplankton and prokaryotic cells at the air-sea interface increased after bubbling in the tank, this effect was not that clear in the field. Nevertheless, both set-ups revealed positive effects of bubbling on proxies for bacterial activity (e.g. thymidine incorporation, high-nucleic-acid-containing cells). First results of the bacterial community composition analysis based on 16S rRNA-gene amplicon sequencing of aerosol and water samples from the tank indicate an enhanced transport of e.g. *Flavobacteriales* or *Alteromonadales* into the air. These organisms are known to actively inhabit the air-sea interface and suggest that they might also play an important role in atmospheric processes. Overall, our results show the importance of bubbles for transporting material into the air-sea interface and beyond.

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## EMP155

### The impact of groundwater seepage on benthic microbial community structures of a sandy beach

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The discharge of fresh groundwater and recirculated seawater into the beachface is supposed to be a major nutrient-source to the coastal ocean. To study the microbial response and associated biogeochemical processes to variations in salinities and redox conditions, we have performed four interdisciplinary sampling campaigns at a barrier island in the German North Sea. At these islands, rainwater percolates into their sand body and forms a deep freshwater lens. This groundwater contains orders of magnitude more nitrogen, silicate, dissolved organic carbon, phosphate, and iron, than the coastal seawater (Beck et al., 2017). The final composition is influenced by porewater chemistry, aquifer geology, residence time, and microbial

community structures within the subterranean estuary. During the sampling campaigns on Spiekeroog island, groundwater seeps were detected *in situ* via salinity anomalies at different sediment depths within a grid between the high and low water line. Along with biogeochemical measurements, we have determined the microbial abundance and identified horizontal and vertical differences in microbial community structures by 16S rRNA-based analyses. Total cell counts varied by one order of magnitude with depth and two orders of magnitude between sites. Sulfate reduction rates were unexpectedly low (~0.3-88.4 pmol cm<sup>-3</sup> d<sup>-1</sup>), yet seemingly connected to iron reduction rates. Laboratory experiments mimicking tidal cycles and groundwater seepage will elucidate the role of these phenomena on biogeochemical processes as well as microbial community structures. In general, the polyphasic approach will lead to a deeper understanding of biogeochemical processes in the beach subsurface.

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## EMP156

### Molecular biological live/dead differentiation in water treatment

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Drinking water is commonly disinfected by ozone, UV or chlorine. Molecular biological techniques allow a rapid detection of pathogen organisms, but lack in the essential differentiation of infectious and non-infectious viruses.

In this study, bacteriophage detection after disinfection was compared using plaque assay, short and long amplicon qPCR, and PMA-qPCR. The main goal is the development of a rapid detection method for viruses in disinfected samples. PhiX174 bacteriophages spiked in drinking water were treated with different doses of ozone, UV and chlorine. Samples were quantified using conventional and long-amplicon qPCR, PMA-qPCR and the standardized plaque assay (DIN EN ISO 10705-2:2001).

The results show that every disinfection method has different effects on the methods used depending on the caused damage.

UV treatment revealed minimal effect on standard qPCR. Inter-calating dyes had no effect on the result, whereas the use of larger amplicons increased signal reduction. A correlation was found between amplicon size, UV-dose and signal reduction, allowing the calculation of infectivity from qPCR results.

Low chlorine doses did only affect the cultural assay with nearly 6 log-levels signal reduction. No effect was observed in qPCR and PMA-qPCR. Both qPCR and PMA-qPCR were affected with higher doses of chlorine. PMA addition caused an additional signal reduction in the qPCR.

For ozone treatment, loss of infectivity could be detected in all methods including simple qPCR without modification.

The results underline the suitability of PCR for rapid virus monitoring after water treatment. In particular long amplicon PMA-qPCR proved to be promising for monitoring capsid and genomic damage of viruses. The research was funded by BMBF (01DR17014 and 033W010B).

## EMP157

### Fermentative microbes in gut contents of the tropical earthworm *Rhinodrilus alatus*

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The earthworm alimentary canal is an anoxic microzone in aerated soils, constituting an oasis for ingested soil microbes capable of anaerobiosis. Although a considerable amount of information is available on the gut microbiology of model earthworms such as *Lumbricus terrestris*, very little is known about the gut microbiology of tropical earthworms such as the extremely large Brazilian earthworm *Rhinodrilus alatus* that can attain lengths of over 1 meter. The gut of *R. alatus* was found to contain mM concentrations of fatty acids indicative of fermentation, including succinate, formate, and acetate which decreased in concentration along the alimentary canal. Acetate, methylbutyrate, and hydrogen were fermentation products in unsupplemented anoxic microcosms of gut contents, and complex organic matter stimulated the formation of diverse fermentation products (CO<sub>2</sub>, H<sub>2</sub>, acetate, formate, methylbutyrate, propionate, isobutyrate, and succinate). Fermentation was greater in anoxic gut content microcosms compared to anoxic preingested-soil microcosms, suggesting that the gut-associated microbiome was predisposed to conditions that enhanced its ability to react to nutrient input under anoxic conditions. Analyses of 16S rRNA, 16S rRNA genes, and the *in situ* chemistry of the alimentary canal indicated that (i) *Peptostreptococcaceae* and *Enterobacteriaceae*, likely representing ingested obligate anaerobes and facultative aerobes, respectively, are engaged in the collective fermentation profile of the alimentary canal of *R. alatus*, and (ii) nitrate and iron reducers might be engaged in the overall turnover of matter during gut passage.

## EMP158

### You beta believe it: $\beta$ -amino acid degradation by a newly isolated *Paraburkholderia* strain clearly differs from type strain PsJN and we don't know why

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$\beta$ -amino acids ( $\beta$ -aa) are part of natural compounds, degradation products of amine antibiotics and important building blocks for the synthesis of chiral pharmaceuticals; soil and water microorganisms can be expected to be regularly affected with these molecules. However, little is known about the metabolic fate of  $\beta$ -aa.

We are investigating the biodegradation of  $\beta$ -aa using  $\beta$ -phenylalanine ( $\beta$ -Phe) as model compound. While former studies with *Paraburkholderia phytofirmans* PsJN (type strain) revealed strictly (*S*)-selective degradation of *rac*- $\beta$ -Phe by a transaminase reaction, a closely related strain isolated in our lab additionally degrades the (*R*)-enantiomer by mechanisms we were not able to identify so far.

Interestingly, these mechanisms seem to be induced during fermentation with *rac*- $\beta$ -Phe as sole nitrogen source whereas the strain is not capable to grow with enantiopure (*R*)- $\beta$ -Phe. Also puzzling, an extracellular slime capsule is constituted after complete exhaustion of the (*S*)-enantiomer of *rac*- $\beta$ -Phe. Although morphological alterations following substrate changes are not uncommon among bacteria, to the best of

our knowledge this has never been reported when the substrate change is "simply" the usage of different enantiomers of the sole nitrogen source.

We are looking for your input to answer these open questions which we think is crucial in terms of (1) environmental aspects referring to the persistence of  $\beta$ -aa in soil and water (2) defense mechanisms of microorganisms affected with natural compounds containing  $\beta$ -aa and (3) pharmacokinetics of these natural compounds when used as drugs, e.g. cytostatics containing  $\beta$ -Phe derivatives.

## EMP159

### Discovery of new *Pseudomonas* spp. by isolation of siderophore producing bacteria from acidic habitats

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Siderophores are small metal chelating compounds produced by many soil organisms under iron limiting conditions. Due to their ability to complex many metal and metalloid ions, they find medical and environmental application. To find new interesting siderophore producers and extend the knowledge of siderophore production among bacteria living in acidic habitats, three environmental soil samples were collected around Freiberg (Saxony) and suspended in saline. These suspensions showed pH values between 2.7 and 6.3 and were plated on agar plates containing blue Chrome azurol S Fe(III) dye to screen for siderophore production. Orange halos around the colonies indicated siderophore producing strains. Eight very active siderophore producing colonies were isolated and identified as neutrophilic *Pseudomonas* spp. using 16S rDNA sequencing. Quantitative experiments with liquid Chrome azurol S assay showed, that the isolates produce up to 200  $\mu$ M siderophore (DFOB equivalent). Next to Fe, the siderophores of all isolates show metal binding activity towards Al, some of them towards Ga, Cu, and V. Four of the isolates were further investigated regarding their growth on different substrates, culture doubling time, production of biosurfactants and fluorescence of produced siderophores. Most of the isolates showed high similarity to known siderophore producers as *P. fluorescens* and *P. salomonii*, while one isolate seems to be very different from other *Pseudomonas* species. In this experiment we have not only been able to show the presence of siderophore producing species in acidic habitats and show their ability to bind different metal ions, we also discovered a *Pseudomonas* isolate which we could not match with any known *Pseudomonas* species on phylogenetic and biochemical level so far.

## EMP160

### Multiple resistance to toxic compounds of soil microbial community from Ecuador

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Soil microbial communities (MC) are microcosms with high metabolic plasticity. The aim of our work was to determine adaptation regularities of MC to simultaneous presence of 3 toxic stress factors.

**Methods:** MC (high lands cliffs, 4020 m, Papallacta, Ecuador) was cultivated in the simultaneous presence of 3 stress factors during 10 days: KNO<sub>3</sub> 25000-100000 ppm, 1-chloro-4-nitrobenzene (CNB) – 50-200 ppm, CuCl<sub>2</sub> – 2500-10000 ppm Cu<sup>2+</sup>. Microbial metabolic activity controlled parameters: OD was measured by spectrophotometer KFK-2MP; gas phase content (O<sub>2</sub>, N<sub>2</sub>, H<sub>2</sub>, CO<sub>2</sub>) by gas chromatography system LHM; pH, Eh by potentiometric method; CNB concentration by gas chromatography-mass-spectric system.

**Results:** MC was highly adaptive and resistant to 3 stress factors and grew in 50000 ppm of KNO<sub>3</sub>, 5000 ppm Cu<sup>2+</sup>, 100 ppm of CNB – in 2 orders higher than generally accepted bactericidal concentrations. The growth was naturally inhibited. Thus, H<sub>2</sub> synthesis was observed only in control, pH and Eh levels decreased, CO<sub>2</sub> concentration was 5 times lower, OD was in 6 times lower than in control. However MC was highly adaptive and even in these stress conditions was able to decrease CNB concentration in 2 times and overcame the toxicity of stress factors. The presence of stress factors caused succession of microorganisms. Yeasts were more resistant than bacteria. Thus, bacterial forms were dominated in control, yeast forms – in experiment. Colonies size became smaller with increasing of stress.

**Conclusions:** Soil MC of Ecuador were highly resistance and adaptive to 3 toxic stress factors in high concentrations. Microorganisms can survive in conditions that considered incompatible with life. Ecuador soil MC could be used as a source of biotechnologically promising strains isolation.

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#### EMP161

##### The influence of complex substrates on microbial diversity from four Azorean hot springs

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**Introduction:** Heterotrophic thermophilic bacteria were found to be highly abundant in hydrothermal springs of the Azores. To investigate their efficiency on plant biomass degradation, heterotrophic microbial communities were enriched on recalcitrant substrate, spent coffee ground (SCG), as well as on its ingredients cellulose, arabinogalactan, galactomannan and proteins.

**Material and methods:** Hot spring samples were incubated at 60 °C with (i) 1% SCG, (ii) 0.5% carboxymethyl-cellulose + casein and (iii) 0.5% arabinogalactan + galactomannan as substrates. Diversity of the cultures were analyzed by DGGE. DNA from enrichment cultures were pooled and analyzed for metagenomic diversity studies.

**Results:** DGGE analysis revealed differences in community composition and genus presence depending on the substrate utilized. Data indicated that recalcitrant substrate consists of a totally different thermophilic community (dominated by *Dictyoglomus*) and genus presence than enrichments on galactomannan + arabinogalactan (dominated by *Thermodesulfobivrio*) and casein + cellulose (dominated by *Thermus*).

**Discussion:** We used a comparative analysis by DGGE coupled with a metagenomic approach to get insights into heterotrophic enrichment cultures of four Azorean hot springs. This study demonstrates the high potential of thermophilic bacteria capable of degrading recalcitrant

substrate and furthermore shows the role of the substrates on microbial diversity.

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#### EMP162

##### Purification of 2-naphthoate:CoA-Ligase, the second enzyme of the anaerobic naphthalene degradation pathway

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**Introduction:** Anaerobic degradation of naphthalene is initiated by carboxylation to 2-naphthoate followed by a thioesterification by the 2-naphthoate:CoA-Ligase (2-NA:CoA-Ligase). This CoA-Ligase belongs to the superfamily of ATP-dependent adenylation enzymes, similar to the benzoate:CoA-Ligase from *Thauera aromatica*. The ATP-dependent and oxygen insensitive activity of the 2-NA:CoA-Ligase was measured in cell free extracts of the sulfate-reducing cultures N47 and NaphS2.

**Objectives:** Here we wanted to purify the 2-NA:CoA-Ligase from the sulfate-reducing cultures N47 and NaphS2 to homogeneity. The enzyme should be characterized and the substrate specificities of the 2-NA:CoA-Ligase determined.

**Materials & Methods:** Different chromatographic steps were used for the native purification of the 2-NA:CoA-Ligase. These include DEAE-ion exchange, blue sepharose affinity, and gel-filtration chromatography. LC-MS analysis of 2-NA:CoA-Ligase assays was used to identify fractions containing the desired enzyme and determine the substrate specificity.

**Results:** The 2-NA:CoA-Ligase could be further purified using ion exchange and affinity chromatographic methods. The ligases showed a narrow specificity for 2-NA and a few other carboxylic acids. Fluorinated substrate analogues were converted with nearly the same activity as the natural substrate 2-NA. In contrast, substitutes with a hydroxyl group next to the carboxyl group were not converted to CoA thioesters.

**Conclusion:** The identified substrate specificities show similarities to the benzoate:CoA-Ligase, indicating a similar reaction mechanism and conformational change of the 2-NA:CoA-Ligase. However, the enzyme exhibits highest activity with 2-NA and seems to be specific for the anaerobic naphthalene degradation pathway.

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#### EMP163

##### The rhizosphere of methane-emitting fens: an oasis for H<sub>2</sub>-cycling anaerobes

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Fen soils are O<sub>2</sub>-limited and emit large quantities of methane. H<sub>2</sub> is an important substrate for methanogens, and it has been observed that H<sub>2</sub> can be produced from roots of *Carex rostrata*, a typical fen plant, and that the production of H<sub>2</sub> is due to root-associated fermenters that metabolize root-derived material/exudates. Anoxic microcosms of soil-free roots or root-free soil from the rhizosphere of *C. rostrata* were utilized to evaluate where hydrogenotrophic (i.e., H<sub>2</sub>-consuming) methanogens might be located in the rhizosphere and if they might compete with H<sub>2</sub>-consuming acetogens. Almost no methane was produced by roots with or without supplemental H<sub>2</sub>, whereas supplemental H<sub>2</sub>

stimulated acetate production by roots. In contrast, reductant from consumed H<sub>2</sub> was recovered in both methane and acetate in soil microcosms, with methane being more dominant. H<sub>2</sub> accumulated in unsupplemented root microcosms, suggesting that the capacity of root-associated H<sub>2</sub> producers exceeded the capacity of root-associated H<sub>2</sub> consumers. In the case of the rhizosphere of *Carex*, hydrogenotrophic methanogens appear to prefer the rhizosphere soil rather than the root surface where acetogens might be more capable because of high in situ H<sub>2</sub> availability and their greater ability to cope with the oxidative stress associated with the release of O<sub>2</sub> by plant roots. However, results obtained with *Molinia caerulea* (another fen plant) were different to those obtained with *C. rostrata* (e.g., H<sub>2</sub>-dependent production of acetate exceeded that of methane with root-free soil derived from the rhizosphere of *M. caerulea*). These results support the proposal that the rhizosphere of mire plants are a "hotspot" for H<sub>2</sub>-producing and H<sub>2</sub>-consuming processes and indicate that plant species might influence these processes.

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#### EMP164

##### Genetic studies on biofilm formation in *Sphingomonas* sp. strain S2M10 isolated from drinking-water filtration membranes

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Alphaproteobacteria of the family *Sphingomonadaceae* are ubiquitously distributed in natural environments, e.g. in association with plants. However, they are also very often found in nutrient-limited anthropogenic habitats like drinking water distribution systems and water filtration units where they form biofilms. This can lead to hygienic and technic problems. For investigating the mechanisms of biofilm formation in sphingomonads, we isolated several bacterial strains of the genus *Sphingomonas* from a used water filtration membranes. One strain, *Sphingomonas* sp. strain S2M10, was chosen for further analysis. Transposon mutants were generated and screened for altered biofilm formation. Out of about 400 mutants two mutants with an interesting phenotype were found. In mutant strain Tn36, the *spnB* gene necessary for the production of sphingans, a group of typical *Sphingomonas* extracellular polysaccharides, was affected. This mutant was incapable of biofilm formation. In the second mutant strain Tn299, the transposon was inserted in a gene for a FabG homolog encoding a putative 3-oxoacyl-ACP-reductase. This gene is located in a putative cluster with genes for an acyl carrier protein, an acyl ligase, LuxC and LuxE homologs and a sugar transferase. This mutant showed no significant difference in the amount of biofilm quantified by crystal violet staining, but the macroscopic biofilm structure was clearly distinguishable from the wildtype. Strain Tn299 showed strong cell aggregation and lacked the typical macroscopically visible extracellular slime. The gene cluster is also found in few other sphingomonads and some more distantly related bacteria. Its function is currently being characterized for elucidating its role in biofilm formation of these hygienically relevant bacteria

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#### EMP165

##### Effect of salt stress on aerobic methane oxidation and associated methanotrophs

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Salinization by intrusion of seawater is a threat to rice cultivation and rice yields in coastal areas. The impact of salinization extends to belowground microbially-mediated processes, including methane cycling. Rice paddies are an important source of methane, a potent greenhouse gas. Methane emission is regulated by methane production and oxidation rates. While recent studies showed that salinization adversely affects methanogenesis, less is known on the response of methane oxidation and the associated methanotrophs. We determined the response of aerobic methane oxidation and the associated methanotrophs to salt stress in a NaCl gradient ranging from 0 M (un-amended reference) to 0.6 M NaCl (seawater salinity) in a laboratory microcosm study with paddy soil. Methanotrophs were quantified using group-specific quantitative PCR (qPCR) assays, whereas the community composition was monitored using MiSeq sequencing targeting the *pmoA* gene. Methane oxidation potential was not affected at 0.005 M NaCl, but activity was inhibited at > 0.3 M NaCl amendment. Ammonium concentration increased proportionally with NaCl concentration, indicating a release of soil adsorbed ammonium and possibly, an associated competitive inhibition of certain methanotrophs. Within gammaproteobacterial methanotrophs, the type Ia subgroup was selectively stimulated by increasing salinity (< 0.3 M NaCl), while the type Ib subgroup was negatively affected. Although some methanotrophs were compromised by salt stress, methanotrophic activity was remarkably resistant and showed a rather high salinity threshold at more than half seawater strength before activity fully ceased.

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#### EMP166

##### German-Australian Cooperation for Water Microbiology

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Prompt water monitoring and quality control are essential for public health protection and risk regulation. Consequently, the rapid, specific and sensitive detection of microbial indicators and waterborne pathogens represent a key challenge in modern water quality monitoring. Overarching objective of German-Australian collaboration ([www.gabi-cooperation.org](http://www.gabi-cooperation.org)) is to develop fast monitoring methods of microbiological key parameters.

Following research questions are main topics of both partners:

- PCR detection of hygienically relevant microorganisms in wastewater, surface water and ground water
- Microbial Source Tracking with culture-independent methods
- Detection of relevant antibiotic resistance genes (ARGs)
- Improved methodology for the concentration and detection of viruses
- PCR-based live-dead differentiation

- Application of microarray technology in water monitoring

In a first step, different enrichment methods for viruses and bacteria were compared with regard to recovery rates and removal of PCR inhibitors. The use of ultrafiltration columns was found to be the most promising method. Future developments will focus on mobile devices allowing rapid sampling in the field. In addition, the analysis of ARGs showed their ubiquitous dissemination in aquatic isolates and German and Australian surface waters. The investigations contributed to a better understanding of antibiotic resistance and mobility of genes in the aquatic environment.

The methods for molecular biological monitoring are further developed to facilitate their way to routine diagnostics. For quick exchange of data, references and methods, the "Joint PCR Reference Lab" will be established thus strengthening the German-Australian cooperation.

The project is funded by the BMBF (grant number 01DR17014).

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### EMP167

#### Occurrence of Antibiotic Resistant Bacteria and Resistance Genes in Surface and Ground Water

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Due to the widespread use of antibiotics, resistances have spread in the environment. Resistant bacteria are also increasingly found in the population, unrelated to hospitalization, suggesting the transfer of resistance genes from the environment into humans.

Several German joint projects focus on the occurrence of resistances in different environmental compartments (e.g. surface water and soil). In our studies, the presence of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) in water used for drinking water production is examined.

ARGs were detected using PCR based methods. Culture based methods (selective CHROM agar plates containing antibiotics) were used to prove the presence of vancomycin-resistant enterococci (VRE), methicillin resistant *Staphylococcus aureus* (MRSA) and extended spectrum beta lactamase (ESBL)-producing bacteria.

PCR analysis of raw water showed that ARGs are more often detected in surface water as compared to groundwater. The investigated ARGs could be classified into three categories: i) often detected (blaTEM, ermB), ii) intermedian detected (blaCTX-M-32) and iii) rarely detected (blaNDM-1 and mecA). Analysis of samples using culture based methods showed the presence of ESBL and VRE bacteria in surface water, but also in ground water – even if only in a small portion of the samples was positively tested. However, the resistant bacteria were not always removed completely by soil passage.

The results will help to identify and to avoid the risk of spreading multi-resistant bacteria via the water pathway. According to the UN "sustainable development goals", mitigation of resistances spreading and improvement of raw water quality are encouraged.

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### EMP168

#### Machine-assisted cultivation and analysis of biofilms

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Biofilms are one of the most abundant forms of microbial life in our ecosystem. This extremely robust and vital class of living systems affects various environmental and industrial processes as well as human health. On the one hand biofilms are causing many severe problems, like biofouling or infectious diseases, but on the other hand they are also offering promising possibilities for innovative applications in biotechnology. By taking advantage of their unique properties biofilms could be used for the bioproduction of new pharmaceuticals or the selective removal of unwanted substances from waste. Successfully pursuing this strategy will need a deep understanding of the biology of biofilm formation and growth as well as the interactions within the community.

Microfluidic bioreactors are a promising tool for biofilm studies, since they offer the possibility to better control growth conditions. However, there is urgent need for analytical methods compatible with microfluidic bioreactor systems.

A novel platform was developed for cultivation and analysis of multi-species biofilms under flow. The system allows the control of the flow rate and temperature. The flexible design allows the use of various flowcell geometries, from microfluidic channels to millimeter scaled channels. The flowcells are compatible with analysis instrumentation, such as fluorescence reader, automated microscopes or OCT devices. The system offers high spatiotemporal resolution in the analysis of metabolites and biofilm composition. An automatic sampling system as well as offline assays like FISH have successfully been established for the platform.

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### EMP169

#### Deciphering the interaction between *Rhizoglossum irregulare*, hyphae attached phosphate-solubilizing bacteria and their impact on *Solanum lycopersicum*

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Sustainable agricultural practices are needed to improve plant yield and to solve the global crises of optimal food production in the coming years without further detrimental impact on the environment. Arbuscular mycorrhizal fungi share a symbiotic relationship with the majority of terrestrial plants, and play a key role in improving availability of nutrients and water uptake of plants. In recent years, the knowledge on the interaction of these ubiquitous fungi with rhizospheric bacteria has been improved. Since AM fungi are barely able to solubilize phosphate in significant amounts,

but can aid in the transfer of P from the soil to the plants, it is important to study the interaction between P-solubilizing bacteria and AM fungi, to unravel if the bacteria can improve P nutrition of plants by AMF. One approach for enhancing the effects of such bioinoculants could be co-formulations, which can be achieved once the mechanism of interaction between these two groups of microorganisms is understood in detail. In this study, we isolated AM fungal hyphae-attached P-solubilizing bacteria using *Rhizoglossum irregulare* in two compartment pot system. The bacteria were characterized on the basis of their P solubilization and hyphae colonization efficiency. Based on the results, the genomes of three strains were sequenced and annotated. In order to test the impact of the bacterial-fungal consortium on globally important food crop *Solanum lycopersicum*, plants were inoculated with AM fungi and P solubilizing bacteria alone or in combination. The effect on plant growth and plant P nutrition was monitored. Further studies will be conducted based on the genome sequences of all partners to understand the molecular basis of these beneficial tripartite bacterial-fungal-plant interactions.

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### EMP170

#### **Comparative Transcriptomics and Proteomics of *Dehalococcoides mccartyi* strain CBDB1 Indicates Induction of a 1,2,4-Trichlorobenzene-Dependent Reductive Dehalogenase**

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*Dehalococcoides mccartyi* strain CBDB1 is a strictly anaerobic, obligate organohalide-respiring bacterium encoding 32 non-identical reductive dehalogenase genes (*rdhA*) in its genome. The only functionally characterized *RdhA* is *CbrA*, which dechlorinates the central, doubly-flanked chlorine of 1,2,3-trichlorobenzene (1,2,3-TCB) (1). The aim of the current study was to identify *rdhA* genes with a putative function in the dechlorination of 1,2,4-TCB, which is dechlorinated exclusively at positions flanked by a single C-Cl bond. Strain CBDB1 was grown either with 1,2,3- or with 1,2,4-TCB and cells from the late exponential and early stationary phases were subjected to transcriptomic (RNA-Seq) and proteomic analysis, respectively. Using the RNA-seq data, normalized average expression values for *rdhA* genes were calculated. Peptide lysates were analyzed by LC/MS/MS and identified peptides were median-normalized to allow inter-sample comparison. RNA-Seq identified transcripts of 30 *rdhA* genes, most at relative expression levels < 100. The highest expression levels (~10000) with both compounds were obtained for *cbrA* and *rdhA80*. In contrast, *rdhA1588* was specifically up-regulated (to an expression level of ~4000) with 1,2,4-TCB, and this was confirmed by qPCR analysis. The proteomes reflected the transcription data: whereas *CbrA* and *RdhA80* were the most abundant *RdhAs* under both growth conditions, *RdhA1588* was exclusively detected in 1,2,4-TCB-grown cells. The fact that the *D. mccartyi* strain DCMB5, which also contains orthologs of *CbrA* and *RdhA80*, but not of *RdhA1588*, cannot use 1,2,4-TCB as electron acceptor, supports the conclusion that *RdhA1588* has a function in the dechlorination of singly-flanked chlorines.

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### EMP171

#### **Bacterial potential to produce exopolysaccharides and lipopolysaccharides during the development of biological soil crusts**

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Biological soil crusts (BSC) are communities of bacteria, algae, mosses, lichens and fungi covering the first millimeters of the topsoil. BSC promote the aggregation of soil particles, improving soil stability and resistance to erosion. Especially bacteria produce exopolysaccharides (EPS) and lipopolysaccharides (LPS) which act as "glue" for soil particles. We postulated that the bacterial potential to produce EPS and LPS increases during the development of BSC. To test this hypothesis, we performed a potting experiment where we cultivated BSC on two different soil substrates. Bulk soil was taken from an inland dune near Lieberose, and the artificial catchment "Chicken Creek" near Cottbus (both in Brandenburg, Germany). Samples were taken at the beginning of the experiment, after 4 and 10 months of incubation. DNA was extracted and directly sequenced on a MiSeq sequencer. Functional analysis focused on genes catalyzing the biosynthesis and export of extracellular and capsular polysaccharides, such as alginate, colanic acid and levan, as well as lipopolysaccharides. As postulated, strong differences in the distribution pattern of the analyzed genes were detected between bulk soil and BSC. In bulk soil, the analyzed genes were harbored mainly by Betaproteobacteria, whereas in BSC, the major potential producers of polysaccharides were members of Cyanobacteria, Alphaproteobacteria and Chloroflexi. Interestingly, differences were also found in the diversity pattern of LPS and EPS producers linked to the different substrates. Those were mainly related to Chloroflexi and Cyanobacteria. Overall, our data indicates that BSC harbor a high number of potential producers of polysaccharides and that their diversity is shaped by underlying soil substrate.

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### EMP172

#### **Microbial community in salt-tolerant and autotrophic perchlorate-reducing enrichment culture**

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Perchlorate (ClO<sub>4</sub><sup>-</sup>) is an emerging contaminant that inhibits iodine uptake into the thyroid gland and decreases thyroid hormone production. Previously we enriched microorganisms that could reduce perchlorate through sulfur oxidation in the presence of 5% NaCl. The salt-tolerant and autotrophic perchlorate-reducing culture was used for microbial analysis in this study. Quantitative real-time PCR revealed that the copy number (/ng DNA) of bacterial 16S rRNA genes was approximately 26 times higher than that of archaeal ones, suggesting bacteria were dominant in the culture. PCR-DGGE and pyrosequencing analyses of the enrichment culture showed that microbial profiles of the inoculum and enrichment culture were different from each other and most (82.56%) of the pyrosequencing reads of bacteria was *Thioalbus denitrificans* belonged to the γ-Proteobacteria. Cloning of full 16S rRNA gene of the dominant bacterial population revealed that it was most closely related (99% sequence similarity) with *Thioalbus denitrificans* Su4 in the GenBank database. A phylogenetic tree showed the dominant population belonged to a different clade from known perchlorate-reducing bacteria. On the other hand, the class *Methanobacteria* was most dominant archaea in the culture as determined by PCR-DGGE analysis. Taken

together, this study provides microbial information that can be basis to develop monitoring technology to control and enhance perchlorate-removal efficiency of the process that employs the enrichment culture.

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#### EMP173

##### Amino acid acquisition and longevity of the deep-ocean microbial cells

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Deep, dark seawaters comprise the bulk (>95%) of the World Ocean. These waters are populated and dominated by ubiquitous microbes. How they acquire amounts of organic nutrients sufficient to support their living and thriving in the deep ocean remains an open question. Here we show that compared to microbes living in the surface, sunlit waters the deep-water (1-3 km) microbes, whilst still preferring leucine to lysine at their ambient picomolar concentrations, acquire these amino acids at 100 and 1000 times lower rates, respectively. Assuming that a rate of amino acid uptake indicates a rate of microbial cell growth the deep-water microbes could grow 1000 times slower than the surface-water microbes. If surface-water microbes divide approximately once a day, deep-water microbes divide only once in several years suggesting their unparalleled cell longevity.

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#### EMP174

##### Influence of long-term organic or mineral fertilization practices on the rhizosphere microbiome and plant health

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Soil represents the reservoir for the recruitment of the rhizosphere microbiome conferring important benefits to the plant. Agricultural intensification resulted in severe consequences for soils. In order to ensure soil quality and future crop production, more extensive and sustainable farming strategies are needed. Various studies indicated the effect of agricultural management on soil microbiomes. We assume the existence of a soil memory effect that is conveyed by microbial communities to current crops at their establishment time. We cultivated lettuce (*Lactuca sativa* L.) for 10 weeks under growth-chamber conditions in organic or mineral fertilized soils taken from two long-term field experiments (LTEs; DOK Therwil, HUB Thyrow). 16S rRNA amplicon sequencing showed significant differences in bacterial community compositions between soils from different sites and long-term fertilization. Independent of the field site, a high relative abundance of *Firmicutes* in the rhizosphere of organically fertilized soils was observed. In mineral fertilized soils, more sequences affiliated to *Lysobacter* and *Pseudoxanthomonas* were found in the lettuce rhizosphere than in organic soils. Long-term fertilization affected the diversity and abundance of microbial genes involved in nutrient cycling and biocontrol. Expression

studies showed similar stress responses in plants grown in organic soils, despite of different soil origins. The up-regulation of genes important for stress tolerance in plants grown in organic fertilized soils indicated a defense priming effect. Our results suggest that soil properties are shaped by the legacy of agricultural management affecting the establishment of the rhizosphere microbiome and performance of a subsequent plant generation.

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#### EMP175

##### Could cyanobacteria have made the salinity transition during the late Archean?

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Modern molecular evolutionary studies suggest the freshwater origin of cyanobacteria during the late Archean, about 2.7 Ga ago, with an even earlier evolution of oxidative photosynthesis. The large amount of oxygen required to oxygenate the Earth's atmosphere during the Great Oxygenation Event (GOE) ~2.4 Ga is thought to have been produced by large cyanobacterial blooms in the open ocean. This then poses the question as to whether ancient lineages of cyanobacteria would have survived the salinity transition.

This study investigates the effect of increasing salinity on the photosynthetic efficiency of two modern day descendants of ancient cyanobacteria, *Chroococidiopsis thermalis* PCC7203 and the root species, *Gloeobacter violaceus* PCC7421.

Organisms were cultured in fresh, brackish or sea water analogous media under a present atmospheric level (PAL) atmosphere or an atmosphere with reduced O<sub>2</sub> and elevated CO<sub>2</sub> (rO<sub>2</sub>eCO<sub>2</sub>). The net photosynthesis (NP) rates were determined in liquid cultures, while the O<sub>2</sub> profiles were determined in pseudomats.

While *C. thermalis* PCC7203 was able to grow under increasing salinities under both atmospheres tested, *G. violaceus* PCC7421 could not make the salinity change to sea water. NP rates were reduced for *C. thermalis* under increasing salinities, as were the levels of dissolved O<sub>2</sub> in the media. A gene screen indicated that *C. thermalis* genome carries genes for both sucrose and trehalose synthesis, whereas *G. violaceus* has only the later genetic component, suggesting a mechanism for their differing salt tolerances. This study supports the hypothesis of Cyanobacterial evolution in freshwater environments and their transition into increasingly salty environments during the late Archean, prior to the GOE.

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#### EMP176

##### Isolation and characterization of an anaerobic biofilm-forming microbial consortium from Costa Rica

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Costa Rica is popular for its exceptional biodiversity, represented by a remarkably rich flora and fauna. In contrast, its microbial diversity is mostly undiscovered and information

on strictly anaerobic organisms is mainly based on metagenomic datasets. In order to investigate the country's anaerobic microbial life, samples were taken from selected anoxic habitats in the Cahuita National Park in southeastern Costa Rica. From the anaerobic enrichment cultures, several organisms were selected for single cell isolation via the optical tweezer or underwent several dilution series. The genus of the isolated organisms was determined by 16S rRNA gene sequence analysis using universal bacterial and archaeal primers. Subsequently, physiological tests were made to detect optimal growth temperature, sodium chloride concentration and pH. Cell morphology was analyzed using phase contrast and fluorescence microscopy as well as transmission and scanning electron microscopy (TEM, SEM). 16S rRNA gene sequence analysis of a biofilm-forming consortium from an oil-well borehole revealed the presence of an Archaeum belonging to the genus *Methanobacterium* and a sulfate-reducing bacterium (SRB) from the genus *Desulfomicrobium*. Together with the results of the morphological and physiological tests we conclude that both organisms display novel species. As both organisms are able to grow on pure culture as well as in co-culture, the role of the individual species within the consortium still has to be surveyed. This study not only increases the knowledge on microbial biodiversity in Costa Rica, but also offers new perspectives for the investigation of anaerobic communities. Further studies will focus on the roles of both partners and their interactions within the biofilm.

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#### EMP177

##### Is the lag phase in microbial growth real?

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**Introduction:** According to the text books, during the lag phase, cells are adapting to the new environment by producing enzymes needed for metabolizing new substrates, increasing cell size and biomass but not cell number.

**Objectives:** Here, we challenge the classical hypothesis that during the lag phase all cells in a culture slowly turn on their metabolism and start to grow. As an alternative hypothesis, we want to test at single cell level if the phenomena of the lag phase can be explained by a small population of dividing cells that becomes dominant over time in the background of a majority of inactive cells.

**Materials and Methods:** *P. fluorescens* was grown with M9 medium, fully <sup>13</sup>C-labelled glucose as sole carbon source, and 35 atom % deuterated water. Samples were taken every 30 min for optical density, cell count, Raman Microspectroscopy and stable isotope analysis of CO<sub>2</sub>.

**Results:** As determined by optical density and cell count, lag phase lasted until 240 min. During the lag phase, Raman-Microscopy revealed the incorporation of <sup>13</sup>C and deuterium into single cells indicating active metabolism, distinguishing active cells from non-active cells. Initially very small number of active cells increased during the lag phase from zero to over 90% of the cells. The evolution of labelled CO<sub>2</sub> showed the metabolic activity during lag phase.

**Conclusion:** Our results indicate that the classical explanations of the lag-phase do not hold. In contrast, a small number of individual cells are metabolically active and exponentially dividing during the lag phase. The high background of inactive cells leads to the impression of lag phase of the whole culture. At the start of the lag phase, the

active cells become more abundant providing the impression of starting growth of the "culture"

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#### EMP178

##### The relationship between cable bacteria and submerged plants

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Cable bacteria belonging to the family *Desulfobulbaceae* couple sulfur oxidation and oxygen reduction by a long-distance electron transfer over centimeter distances in marine and freshwater sediments [1]. In such ecosystems, submerged plants are known to actively transport oxygen to the rhizosphere providing a potential habitat for cable bacteria. Here, we investigated if cable bacteria can grow from oxygen released in the rhizosphere and how they are spatially arranged next to the plant roots.

Single roots of the freshwater macrophyte *Littorella uniflora* were placed between two microscope slides containing a suspension of filamentous *Desulfobulbaceae* originating from the iron-reducing culture 1MN [2] and FeS. The microscope slides were placed in sandy sediments amended with FeS, and water was filled up until the plants were fully submerged. The presence and spatial orientation of cable bacteria was investigated by fluorescence in situ hybridization (FISH).

After growing the plants for 13 days, the black FeS next to the roots was oxidized to red iron oxides indicating oxygen release from the roots. Preliminary results from FISH analysis revealed the presence of cable bacteria either in the vicinity of the roots or directly attached to the roots.

These results indicate a mutualistic relationship of cable bacteria and submerged plants. Cable bacteria might shield the plants from toxic sulphide by sulfur oxidation based on long-distance electron transfer to oxygen released by the roots.

#### References:

[1] Burdorf, L.D., et al, Biogeosciences, 2017.

[2] Marozava, S., et al, Biodegradation, 2017.

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#### EMP179

##### Flagellar motility of *Rhodobacteraceae* – Evolution of three flagellar systems and characterization of the archetypical *fla1-type1* flagellum

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Recently, three distinct flagellar geneclusters (FGC) have been identified within the family *Rhodobacteraceae*, designated *fla1-fla3*. *Fla1* is the most abundant flagellar system, but the function of four universally conserved proteins (CP1-CP4) remains unclear. The motile model organism *Phaeobacter inhibens* DSM 17395 harbors the most common *fla1*-type flagellum and was therefore ideal to investigate this FGC. In this study we established a comprehensive phylogeny of the flagellar systems of more than 300 completely sequenced *Rhodobacteraceae* and a phylogenomic reference tree. The analyses clearly showed

that *fla1* is the archetypical FGC of the *Rhodobacteraceae*. Furthermore, motility assays of 100 *Roseobacter* strains revealed functionality of all three FGCs. Extensive screening of more than 12,000 *P. inhibens* transposon mutants on soft agar plates resulted in the identification of 64 genes essential for *fla1* motility, thereunder CP1 to CP4 and all three genes of the CtrA phosphorelay. Genetic complementation of these mutants validated their essential function for swimming motility. An exoproteomic detection of flagellar proteins and electron microscopy suggested that CP1 and CP4 still synthesize a complete but static flagellum. CP2 and CP3 lack secreted flagellar proteins, pointing either towards a structural or a regulatory function of the proteins. Analyses of high throughput Illumina transcriptome data of the CtrA phosphorelay mutants showed a strong downregulation of chemotaxis and flagellar genes. The comparison of these data with the photosynthetic model organism *Dinoroseobacter shibae* revealed a core regulon of only 30 genes mainly composed of flagellar genes. These data clearly show the crucial function of the archetypical *fla1* flagellum for *Rhodobacteraceae*.

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### EMP180

#### Role of (pro)phages in *Shewanella oneidensis* MR-1

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**Question:** The dormant form of temperate phages, prophages, are found ubiquitously in bacterial genomes. Recently it was shown that prophages can be a possible competitive advantage under certain conditions by helping the bacterial population to colonize surfaces and increase the virulence towards their host. We are interested in the mechanism of phage production and the relationship between (pro)phages and their host bacteria.

**Methods:** To better understand timing and mechanism of phage production in *Shewanella oneidensis* MR-1, we introduced an additional copy of the gene encoding the major capsid protein fused to VENUS into the prophage chromosome, yielding highly fluorescent but still active phage particles. We also use marker less in-frame deletions to investigate the role of several clusters of small genes of unknown function within the  $\lambda$ So genome.

**Results:** The fluorescent fusions of the major capsid protein gene allow us to follow the construction of phage particles within the cell and their release upon cell lysis. Per cell, 20 to 50 phage particles start to form around 90 min after induction. We could show that two of the gene clusters we deleted are required for the infectiousness of the phages.

**Conclusions:** *Shewanella* populations show a high heterogeneity regarding prophage induction subsequent phage particle production and need further investigation. Fluorescently labeled  $\lambda$ So are a powerful tool for this. Current experiments also utilize this tool to further elucidate the interaction between bacteriophages and their hosts in biofilm formation. The clusters of small genes of unknown function require further experiments to exactly determine the roles these genes play in the infection mechanism of  $\lambda$ So.

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### EMP181

#### Unraveling the phylum Planctomycetes

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Members of the phylum Planctomycetes, a member of the PVC (Planctomycetes-Verrucomicrobia-Chlamydiae) superphylum, have in the past been described to lack peptidoglycan and to possess a compartmentalized cell plan. However, both assumptions have recently been challenged turning most of the previous hypotheses of the phylum upside down. Nevertheless, Planctomycetes still have many unusual features. They divide FtsZ-independently by an extraordinary polar budding mechanism. Associated with this atypical trait is a life style switch from sessile mother to planktonic daughter cells. Also, planctomycetal genomes encode numerous giant genes and bear the genomic potential for secondary metabolite production. With relatively few isolates being publicly available, the whole phylum is notably under-sampled. Therefore, we focused our efforts on broaden the strain and data basis for future insights.

For this purpose, we sampled multiple aquatic habitats around the globe and isolated about 80 new planctomycetal strains from various biotic and abiotic surfaces. To allow detailed analyses we produced closed as well as high-quality draft genomes of the novel isolates as well as high-quality images.

Most of these strains represent novel taxa up to the order level, capturing an unprecedented degree of diversity. In total, we brought more novel Planctomycetes into pure culture than currently described species of this phylum exist. The exploration of the gained genomic information not only enables deep insights into the taxonomy of the phylum and its subtaxa, but especially into their unique cell biological characteristics and their promising secondary metabolism.

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### EMP182

#### Optimum O<sub>2</sub>:CH<sub>4</sub> ratio promotes synergy between aerobic methanotrophs and denitrifiers to enhance nitrogen removal

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**Question:** The impact of the O<sub>2</sub>:CH<sub>4</sub> ratio on nitrogen removal in the aerobic methane oxidation coupled with denitrification (AME-D) process

**Methods:** Batch experiments were conducted to study the effect of the O<sub>2</sub>:CH<sub>4</sub> ratio (0, 0.05, 0.25, and 1.0) on nitrogen removal in the AME-D process. Triplicate samples for each treatment were incubated on a shaker (180 rpm) in the dark for 60 hours at 28°C. Methane consumption, and denitrification (NO<sub>3</sub><sup>-</sup>-N, NO<sub>2</sub><sup>-</sup>-N and NH<sub>4</sub><sup>+</sup>-N) and turnover of intermediates were monitored in each treatment. After incubation, samples were collected and subjected to DNA extraction followed by PCR-based quantification of functional genes.

**Results:** Methane oxidation activity substantially decreased from 277.80 mmol/gVSS/d to 21.06 mmol/gVSS/d when the O<sub>2</sub>:CH<sub>4</sub> ratio was increased from 0 to 1 ( $p < 0.05$ ). With the exception of treatment 1 (O<sub>2</sub>:CH<sub>4</sub> ratio = 0), *pmoA* gene corresponded well to the methane oxidation activity. In the bulk medium, the three primary metabolites observed in the bulk media were formaldehyde, acetate and citrate. Methanol, formate and butyrate were detected as trace metabolites.

The nitrite removal tremendously increased from 0.53 mmol NO<sub>2</sub><sup>-</sup>-N/gVSS/d to 7.32 mmol NO<sub>2</sub><sup>-</sup>-N/gVSS/d concurrently with the increase in the O<sub>2</sub>:CH<sub>4</sub> ratio from 0 to 0.25.

However, the nitrite removal decreased by 53.8% when the O<sub>2</sub>:CH<sub>4</sub> ratio was further increased from 0.25 to 1 ( $p < 0.05$ ).

**Conclusions:** The greatest nitrite removal and the low contribution of NO-N and N<sub>2</sub>O-N in total reduced nitrogen were observed at the O<sub>2</sub>:CH<sub>4</sub> ratio of 0.25. At this ratio, denitrifying activity reached the highest level of 7.32 mmol NO<sub>2</sub>-N/gVSS/d. Thermodynamic calculations suggest methanol, butyrate and formaldehyde to be the main active intermediates of the AME-D process.

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#### EMP183

##### Comparative genomics of linuron-mineralizing *Variovorax* sp. isolated from geographically distant locations

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**Question:** Linuron is a phenylurea herbicide that is widely used around the world. Several bacterial strains have been isolated that can mineralize this compound, either in consortium or alone. Most degrading strains that were isolated belong to the genus *Variovorax*. This study aims to comparatively analyze the genomes of linuron-degrading bacteria to elucidate the mechanisms behind the evolution and distribution of the catabolic pathways.

**Methods:** The genomes of six linuron-degrading strains were sequenced. For comparison, the genomes of ten non-linuron-degrading *Variovorax* strains were retrieved from the NCBI Genome database. The genomes were phylogenetically classified, and the presence of linuron, 3, 4-dichloroaniline (DCA) and chlorocatechol degradation gene clusters were investigated.

**Results:** Compared to the non-degraders on the genomic level, linuron-degrading *Variovorax* strains form a distinct cluster within the genus. The degrading *Variovorax* either have the *hlyA* or the *libA* linuron amidases. Remarkably, the contigs on which *hlyA* or *libA* are found are present in each respective strain and are 99% identical to each other. The phenotypes of the linuron-degraders match the presence or absence of the 3, 4 DCA degrading cluster, suggesting that some of these strains are adapted to mineralize linuron in a consortium, while others can mineralize it themselves. There is strong evidence that the DCA catabolic cluster is on a mobile genetic element, and transferred within the cluster.

**Conclusion:** Linuron-degrading *Variovorax* strains form a distinct cluster within this genus. Comparative genomics suggests that although geographically distant, the linuron degradation genes are possibly acquired through horizontal gene transfer and are conserved among the species.

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#### EMP184

##### Governing factors of bacterial communities in temperate grassland plant rhizospheres

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The rhizosphere of plants has long been acknowledged as a hotspot of bacterial activity. Bacteria are attracted to this nutritionally rich zone, which is supported by a significant investment of plants via exudation of a myriad of compounds. Complex interactions between plants and bacteria take place in this niche, which have important roles in various ecosystem processes. Nevertheless, very few studies have attempted to analyse the specific plant-bacteria interactions at community level and to pinpoint the specific signals that drive these associations in natural settings.

In this study we used high-throughput Illumina sequencing to investigate the rhizosphere bacterial community of the plants *Dactylis glomerata*, *Arrhenatherum elatius*, *Alopecurus pratensis*, *Plantago lanceolata*, *Achillea millefolium* and *Ranunculus acris*, which occur naturally in German grasslands. We intend to ascertain the major drivers of rhizosphere bacterial community assembly amongst the different plant species in the eight different soil types evaluated. Moreover, we used GC-MS analysis of the plants' root exudates in order to assess their contribution to the development of distinct bacterial assemblages.

We found soil type to be the major driver of rhizosphere bacterial community composition, as opposed to plant species, which seems to have a neglectable effect. Nevertheless plant species have an indirect effect since they are one driver of root exudate composition, which in turn have a modest effect in the bacterial composition. Analysing further the specific relationships, it was clear that distinct bacterial groups are differentially attracted to distinct compounds exuded from particular plant species.

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#### EMP185

##### Microbiological characterization of initial soils on James Ross Island, Maritime Antarctica

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The interaction of biotic and abiotic processes remains one of the fundamental questions in ecosystem research. So far, only few studies exist for polar environments that integrate pedogenic and microbiological research. Due to the absence of vascular plants and burrowing animals, the ice-free areas of James Ross Island offer the exceptional opportunity to improve our understanding of the impact of microbial processes on soil formation in a pristine laboratory. Soil profiles at St. Martha Cove (SMC) and Brandy Bay (BB) on James Ross Island were sampled. These study sites are characterized by similar topographic positions and parent material. The soil samples were investigated through an interdisciplinary approach combining pedological, geochemical and microbiological methods. Prokaryotic communities were characterized with qPCR and Illumina 16S rRNA gene sequencing. Microbial abundances showed a similar trend for both sites with comparable 16S rRNA gene copies in the topmost layer and a substantial decrease with depth. Both profiles were dominated by bacteria and only the upper layers of SMC showed higher proportions of archaeal OTUs. Increasing relative abundances of OTUs related to *Acidiferrobacteraceae*, potential sulfur and/or iron oxidizing bacteria, were observed in deeper layers of BB and SMC.

Multivariate statistics revealed that differences in grain size distribution, and the amount of organic and inorganic carbon have the highest influence on the microbial community structure and explain 52.2% of the variation. The present study gives a first insight in the state of microbial life in initial soils of Antarctica and could help in understanding the future development of Antarctic soil environments and the response of microbial communities to a changing climate.

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#### EMP186

##### >Welcome to the Neighborhood -The Capability of Planctomycetes to shape Biofilms

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In the environment biofilms can be found on almost all surfaces, allowing organisms to colonize even extreme ecological niches. This comes with negative impacts on mankind. Biofilms on wounds or the urinary tract can cause major health issues as antibiotic resistance is strengthened. Additionally, biofilms are major drivers of biofouling causing economic losses. In many biofilms, like on the surfaces of phototrophs, Planctomycetes are abundant. Since these bacteria are rather slow growing, it is still unknown how they maintain their abundance in biofilms. Planctomycetes are amongst the "talented producers" of novel secondary metabolites and we recently elucidated the first planctomycetal bioactive structure. Thus, Planctomycetes have the potential to maintain their position in the biofilm by "chemical warfare". Especially biofilms on algae have been a rich source to isolate novel Planctomycetes. We tested the ability of Planctomycetes to colonize algal structures in an artificial system, where algal powder was added to a planctomycetal culture. The used planctomycetal strain was previously isolated from an algal biofilm and immediately attached to the algal surfaces. Subsequently, we investigated biofilms on seagrass leaves by scanning electron microscopy (SEM), amplicon sequencing and metagenomics. We found complex communities in which each bacterium must stand its ground to thrive. Based on amplicon analyses, Planctomycetes can constitute up to 80% of the analyzed sequences obtained from such biofilms. But how is such a dominance even possible? By evading areas of the biofilm where too many competitors are present e.g. by a nomadic lifestyle? Or by making use of bioactive molecules, to fight against their competitors or invite others to join their neighborhood.

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#### EMP187

##### Does nitric oxide dismutation (NOD) play a role in petroleum-contaminated groundwater treatment system?

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Nitric oxide dismutation (NOD) is a recently proposed addition to the increasingly complex microbial nitrogen cycle. Possibly involved microbial populations could be of significant appeal for novel solutions in pollutant degradation and water treatment. Nitric oxide dismutation genes (*nod*) have been found widely present in contaminated aquifers as well as wastewater treatment systems. However, the identity and functions of the microbes possibly involved are still enigmatic. Here, we present the metagenomic investigation of a microbial biofilm formed in a hydrocarbon-contaminated groundwater treatment system. *nod*-targeted qPCR and sequencing analysis have suggested *nod* genes to be abundantly present in the biofilm samples. Thus we speculate that *nod*-carrying microbes could be important in remediating hydrocarbons. We used PacBio Sequel long-read sequencing to dissect this complex community of aerobic, facultative and strictly anaerobic hydrocarbon-degrading microbes. Well-known hydrocarbon-degrading *Thauera*, *Acidovorax* and *Burkholderia* spp. were amongst the most dominant populations. Iron-respiring populations of *Geobacter* spp. were also resolved at appreciable abundance (~4.8%). The construction of metagenome-assembled population genomes (MAGs) is currently underway to identify taxa possibly harboring *nod* genes. Further analysis will be carried out to identify the possible metabolic pathways of *nod*-carrying microbes, and to determine their contribution to hydrocarbon removal.

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#### EMP188

##### Biodiversity of Nitrate-Reducers in grassland soils by massive cultivation and genomics

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Nitrogen is a limiting nutrient in many grassland soils. Fertilization with reactive nitrogen like nitrate stimulates emission of the greenhouse gas nitrous oxide via denitrification. Denitrification is the sequential reduction of nitrate via nitrite and nitric oxide to nitrous oxide and/or dinitrogen and thus associated with nitrogen loss in soils. Nitrate reduction to ammonia by dissimilatory nitrate reducers (DNRA) competes with denitrification and is associated with nitrogen retention in soils. However, the diversity and regulation of DNRA in grasslands soils remain to be determined. Representative isolates are lacking.

We applied a massive cultivation approach where we isolated 1400 bacteria in anoxic media selective for nitrate reducers. 992 and 476 isolates were obtained from the upper 10 cm of grassland areas in the Nationalpark Hainich (H.) and the Schorfheide Chorin (S.), respectively. Isolates were analyzed with MALDI-TOF fingerprints for first characterization and clustered into phylotypes. App. 50% of isolates were identified on the basis of their MALDI-TOF fingerprints. 59% and 40% of isolates were identified from H. and S., respectively. *Pseudomonas* (22%) and *Rhodococcus* (18%) were the most abundant genera in isolate libraries from H. and S., respectively. 16S rRNA of selected strains is currently sequenced for identification of isolates. However, the high proportion of unidentified isolates also indicates yet unknown microbes necessitating further characterization.

The collective data indicate an impact of sampling area on phylogenetic differences in the cultured fraction of grassland nitrate reducers, extends our basis of nitrate reducing isolates and will thus give future insight into genomics and regulation of nitrate reducers from grassland soil.

## EMP189

### Microplastics harbor non-native bacterial communities, which could develop into different genotypes of bacteria potentially harmful for corals

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Microplastic particles (MP) are colonized by diverse bacterial communities (BCs), which represent a part of the plastsphere. Corals can ingest MP and its associated BCs can negatively affect coral health, which has not been studied so far. A microcosm with corals was used to compare bacterial colonization of MP and sediments after their addition to the system and 12 weeks of incubation. MP and sediments BCs were compared to detritus and particle-attached (PA) and free-living (FL) water-associated BCs (>5 µm and 0.22-5 µm water fractions). Scanning electron microscopy showed particle-specific colonization by complex eukaryotic and prokaryotic communities. DGGE fingerprinting and Illumina MiSeq sequencing of 16S rRNA gene amplicons revealed exclusive and stable BCs on MPs from independent tanks, while sediment BCs were more similar to detritus and PA BCs. A high relative abundance of *Jejundonia*, *Roseivivax*, and *Marinobacter* was determined on MP, which were absent in other samples. Cultivation of abundant bacteria of the 5 sample types was performed. Isolates were identified by 16S rRNA gene sequencing, differentiated at strain level by genomic fingerprinting, and screened for biological activity (quorum sensing, antagonism). Several abundant cultured bacteria represented abundant taxa found in the cultivation-independent approach. Isolates identified as *Vibrio* spp. (potential coral pathogens) of MP exhibited differential genomic fingerprint pattern compared to *Vibrio* spp. from the other samples. The study indicated the development of specific BCs with bacteria of distinct genotypes. The collection of isolates now enables a detailed analysis of the potential coral pathogenicity of MP associated bacteria compared to other surface-colonizing bacterial taxa and genotypes.

## EMP190

### Investigating novel *Dehalococcoides mccartyi* strains from dioxin contaminated river sediments

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Polychlorinated dibenzo-p-dioxins (PCDDs) are among the most notoriously persistent contaminants in the environment. Reductive dechlorination of highly chlorinated dioxin compounds by a group of specialized bioremediating bacteria is a crucial step prior to the aerobic degradation of PCDDs. Our objective was to reconstruct genomes of the two dominant *Dehalococcoides mccartyi* strains enriched from Hackensack (NJ, USA) and Kymijoki (Finland) rivers and amended with 1,2,3,4-Tetrachlorodibenzo-p-dioxin (1,2,3,4-TeCDD) as the only electron acceptor. This helped us to gain a better understanding of their physiology and key genes reductive dehalogenases (rdhA) as well as to anticipate their potentials in bioremediation of PCDD contaminated sites. Genomic DNA was extracted from enrichment cultures and sequenced on an Illumina MiSeq

instrument. Draft genomes of the two predominant *D. mccartyi* strains were reconstructed with 92% and 97% completeness and subjected to comparative genome analysis. High-quality draft genomes of the strains revealed a strictly anaerobic lifestyle, with reductive dehalogenation being the sole mode of energy conservation. Both strains contain distinct suites of rdhA genes, suggesting that they have different substrate ranges permitting them to respire on different chlorinated substrates. However, we also found that they share a rdhA gene with 100% and 99.8% similarity at the amino acid level to cbrA of *D. mccartyi* CBDB1, respectively. Comparative genome analysis allowed us to speculate that cbrA and its four known orthologs contribute to respiratory reductive dechlorination of 1,2,3,4-TeCDD. This gene might therefore be used as a biomarker to determine the potential for the bioremediation of PCDD-contaminated sediments in the future.

## EMP191

### Factors influencing the survival of *Salmonella enterica* in soil and the colonization of crop plants

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Salmonellosis outbreaks are increasingly associated with the consumption of contaminated raw fruits and vegetables. Contamination of produce can occur along the whole production chain also, during the plant growth. However, the knowledge about factors influencing the persistence of *Salmonella* in the plant environment and the associated colonization of plants is scarce. We analysed, the influence of preadaptation, soil type, organic fertilizer amendment and soil sterilisation on the survival of *S. enterica* serovar Typhimurium strains 14028s, and LT2, and *S. enterica* serovar Senftenberg in soil. At the same time, we analysed the colonization of lettuce, corn salad and tomato by *Salmonella* and the related plant immune responses. Preadaptation of *Salmonella* was simulated by cultivation in a new-developed lettuce medium. In summary, despite an initial decline, our data indicated that survival of *Salmonella* was higher in loamy than in sandy soil. Preadaptation promoted the survival of *Salmonella*, while competition by the indigenous soil microbial community reduced its survival. Organic fertilizer amendment had a positive effect on the survival of *Salmonella* in soil. We observed a colonization of plants at a low percentage range that seemed to be affected by the soil type, the plant as well as the *Salmonella* strain. Interestingly, the plants reacted to colonisation with an induction of defence responses that was dependent on the bacterial serovar. Together, our results indicate that *Salmonella* can persist in soil, posing a risk of fresh produce contamination. The fact that *Salmonella* use plants as alternative hosts strongly suggests that plants represent a much larger reservoir for animal pathogens than so far estimated.

## EMP192

### Determination of assimilable organic carbon in anaerobic groundwater

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**Introduction:** The quality of the groundwater (GW), which is the most important drinking water (DW) resource in Germany, is at risk at many sites. To assess and monitor its ecological status, suitable bioindicators and methods are needed. The concentration of easily assimilable organic carbon (AOC) is a measure for the readily available energy for bacteria in GW.

**Objective:** In order to adapt the AOC method for DW by Hammes & Egli (2005) to GW the effects of different incubation conditions and sample treatments on bacterial growth were investigated.

**Materials & Methods:** Two anaerobic GW samples with different iron concentrations (16 and 3.5 mg/L) were incubated aerobically with/without additional nutrients (10 mg/L NO<sub>3</sub><sup>-</sup>; 1 mg/L PO<sub>4</sub><sup>3-</sup>) at varying temperatures (12, 20, 30°C) for five weeks. To determine the growth of the natural autochthonous bacterial population, total cell counts (TCC) as well as the ratio of high/low nucleic acid bacteria (HNA/LNA) were determined by flow cytometry with/without previous ultrasonication.

**Results:** TCC and relative abundance of HNA cells increased with increasing temperature pointing at the growth of distinct subpopulations. The addition of nutrients did not result in higher TCC, indicating an organic carbon limitation rather than a nutrient limitation. Ultrasonic pretreatment of samples caused an increase of TCC for up to 50 %. This effect was more pronounced in the sample with higher iron concentration. AOC-values ranged between 5.5 and 24.3 µg/L.

**Conclusions:** For AOC determination in anaerobic GW we recommend up to 35 d incubation, no nutrient addition, and ultrasonication. Incubation temperature can be adapted to measure either the intrinsic (12°C) or maximum (30°C) growth potential of a water. Further standardization is required.

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## EMP193

### Aluminum hydroxides and iron sulfides promote cell attachment and sulfate reduction at low pH

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Gene sequences of sulfate reducing bacteria affiliated to the genus *Thermodesulfobium* are often found in natural sulfuric environments. This marks them as prospective candidates for the remediation of acidic metal rich waste water. *Thermodesulfobium* strain 3baa was isolated from sediments of an acidic mine pit lake. It was shown to grow in the range of pH 2.6 – 6.6. At low pH, high sulfate reducing activities were accompanied by mineral precipitation which appeared to be a prerequisite for the colonization of solid surfaces. In order to investigate the specific roles of Al hydroxides and Fe sulfides, batch experiments were performed i) with aluminum, ii) with ferrous iron, and iii) without Al/Fe. Each

treatment was run at the initial pH-values 3, 4, and 5. Highest sulfate reduction rates were obtained in the presence of aluminum at pH<sub>ini</sub> 3. Confocal laser scanning microscopy and scanning electron microscopy revealed a thick biofilm consisting of Al hydroxide agglomerates densely covered by cells. Biofilm formation and sulfate removal decreased with increasing initial pH-values. High sulfate reduction rates were determined in all treatments with ferrous iron. A dense crust of iron sulfides and cells formed irrespective of initial pH-values. An overall low activity was observed in the control treatment with only few cells attached to the carrier surface. The results show that cell-mineral interactions may strongly promote cell activity and growth. In case of acidophilic strain 3baa the effect is much stronger than the effect exerted by the pH of the aqueous environment. These findings are significant for the general understanding of sulfate reduction at low pH in both natural and engineered systems.

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## EMP194

### Multi-parametric characterization of methanotrophic bacteria in groundwater treatment

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**Introduction:** Although methane is widespread in anaerobic groundwater, it is often neglected as relevant parameter in drinking water production. After oxygenation, methane can promote the growth of aerobic methane oxidizing bacteria (MOB) in rapid sand filters (RSFs) causing diverse treatment problems.

**Objectives:** The quantification of MOB in filter material is a prerequisite to identify them as a cause for treatment difficulties. Therefore, we tested a multi-parameter approach combining quantification of MOB cells by fluorescence *in situ* hybridization (FISH) and quantitative PCR (qPCR) with MOB activity measurements.

**Materials & Methods:** Filter material was sampled in RSFs of a groundwater treatment plant receiving raw water with different methane concentrations (0.16-5.50 mg L<sup>-1</sup> vs. 0.05-0.17 mg L<sup>-1</sup>). MOB in filter material were quantified by FISH (probes My84+My705, My669, Creno445, Ma450) and qPCR targeting MOB 16S rRNA genes (primers U785F, MethT1bR, MethT2R). The methane oxidation potential was determined in batch experiments following methane degradation over time by gas chromatography.

**Results:** Multi-parametric analysis revealed a reduced quantity of active MOB in the RSF fed with raw water low in methane. Independent from raw water methane concentration, MOB abundance and activity decreased with increasing filter bed depth.

**Conclusion:** The combined application of FISH, qPCR and activity measurements gives a broad picture of MOB in RSFs as a function of operation conditions. To make optimal use of the advantages of the single methods, we advise the application of (1) FISH to estimate relative MOB abundance, (2) qPCR to describe the vertical distribution of MOB by absolute results, (3) MOX to verify the quantitative results by an activity-dependent parameter.

## EMP195

### Old yellow flavoenzymes: Typical enzymes involved in the anaerobic degradation of polycyclic hydrocarbons

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**Question:** Polyaromatic hydrocarbons (PAH) are pollutants of high persistence and their removal is of great concern due to their toxicity and potential cancerogenicity during long-term exposure. Degradation of PAH in oxygen-rich environments is well understood, while mechanisms of enzymatic anaerobic PAH-degradation remain elusive. The so far only characterized enzyme involved in anaerobic PAH degradation is the 2-naphthoyl-CoA reductase (NCR) from the enrichment culture N47. After activation of naphthalene to 2-naphthoyl-CoA (NCoA), NCoA is reduced by NCR at an unusually low potential of  $E^{0'}$  = -493 mV, a key step in the anaerobic degradation of the model compound naphthalene.<sup>1,2</sup> The product 5,6-dihydro-2-naphthoyl-CoA (DHNCOA) is further reduced by 5,6-DHNCOA reductase (DHNCR), an NADH dependent reductase that is homologous to NCR.

**Methods:** We expressed both proteins in *E. coli* and the absolute product configuration was solved by vibrational circular dichroism spectroscopy after conversion in D<sub>2</sub>O. The structure of NCR was solved at 2.5 Å.

**Results:** We identified the absolute configurations of NCR and DHNCR products. A structure-based mechanism for NCR is presented. We propose that NCR/DHNCR operate at an inverse order of electron and proton transfer events.

**Conclusion:** A complex I-like NADPH:acceptor oxidoreductase component is suggested as electron bifurcating electron donor for NCR. We propose that similar old yellow enzymes are involved in the anaerobic degradation of PAH with more than two fused rings.

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## EMP196

### Characterization of MbdNOPQ, a novel type of class I benzoyl-CoA reductase

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Anaerobic biodegradation of many monoaromatic compounds proceeds via the central intermediate benzoyl-CoA (BCoA), which is initially reduced by dearomatizing benzoyl-CoA reductases (BCRs). For ATP-dependent class I BCRs, so far only the BCR from denitrifying *Thauera aromatica* has been isolated and biochemically characterized. A "Birch-like" reaction mechanism coupled to ATP hydrolysis was proposed to overcome the highly

negative redox potential for reduction of BCoA<sup>1</sup>. The *Thauera*- and *Azoarcus*-type of class I BCRs have been distinguished based on subunit architecture and amino acid sequence similarity<sup>2</sup>.

During studies on 3-methylbenzoate degradation in several denitrifying bacteria a novel type of class I BCR was identified, encoded in the *mbdNOPQ* genes<sup>3,4</sup>. In this work, the *mbdNOPQ* product was heterologously produced in *Escherichia coli* and biochemically characterized. In comparison to *Thauera*-/*Azoarcus*-type class I BCRs it exhibited an extended substrate spectrum towards halogenated and methylated BCoA analogues. Biochemical characterization together with homology modelling with the structure of the 2-hydroxyisocaproyl-CoA dehydratase<sup>5</sup> provided evidence for a radical-based mechanism with substrate being ligated to an [4Fe-4S]-cluster via the thioester-carbonyl.

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## EMP197

### The glyoxylate cycle speeds up C2-assimilation in the ethylmalonyl-CoA pathway-employing

#### Alphaproteobacterium *Paracoccus denitrificans*

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Bacteria have to balance the flux of their metabolites between the two processes of energy conservation and biomass formation. Serving both, acetyl-CoA resembles a control point in metabolism. In *Escherichia coli*, assimilation of acetyl-CoA proceeds via the glyoxylate cycle. An alternative route for this is the recently discovered ethylmalonyl-CoA pathway, which is used instead of the glyoxylate cycle by many environmentally abundant organisms. The Alphaproteobacterium *P. denitrificans* possesses genes encoding for both of the above mentioned pathways. This makes the organism an ideal model to study different assimilation strategies in one system.

In this study, we aimed to shed light on the regulation of energy and carbon metabolism in *P. denitrificans* by clarifying the roles both assimilation pathways assume in the cell.

Proteomic analyses and enzyme activity assays in cell-free extracts were used to investigate the activity pattern of both pathways during growth of *P. denitrificans* under different conditions. We show that the glyoxylate cycle and the ethylmalonyl-CoA pathway are active during growth of *P. denitrificans* on acetate as the sole source of carbon. However, while the glyoxylate cycle seems to be activated as a specific response to growth on acetate, the ethylmalonyl-CoA pathway is constitutively expressed by the cell also during growth on other carbon sources. Knock-out studies show that only one pathway is sufficient for growth on

acetate, which highlights an unexpected metabolic flexibility of our model organism. Further studies aim at understanding the specific flux through one or the other pathway in changing environmental conditions.

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#### EMP198

##### **Potential soil management dependent priming capacity through barley rhizosphere bacterial communities**

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The plant defense capacity can be enhanced by beneficial interactions between a plant and its rhizomicrobiota. So called primed plants have a stronger and faster defense response to biotic and abiotic stresses compared to unprimed plants. We aim to investigate the ability of soil management dependent microbial communities to enhance the resistance of barley against *Blumeria graminis*, a powdery-mildew causing fungus. Agricultural management is assumed to be an important factor shaping soil microbial communities. We hypothesize that soils under different long-term soil management or fertilization treatments will harbor different microbial communities that may influence the priming capacity. The rhizomicrobiota of barley grown at a long-term field trial with four different treatments was investigated. Significant differences in the bacterial rhizomicrobiota analyzed by 16S rRNA gene fingerprints were only revealed between the soil managements. Currently ongoing 16S rRNA gene amplicon sequencing will provide further insights into the bacterial community structure.

An experimental approach to test the priming capacity of soils was developed. The rhizomicrobiota detached from barley grown in field soil was transferred to autoclaved substrate/sand mixture or not and planted with barley. The rhizosphere bacterial community composition of barley infected or not with *B. graminis* was analyzed by fingerprinting of 16S rRNA gene fragments. Identification of the priming efficiency was performed by a detached leaf assay. Although resistance against *B. graminis* was not improved, a strong influence of both the rhizosphere inoculant and the presence of the fungal leaf pathogen *B. graminis* on the bacterial rhizosphere community could be shown by 16S rRNA gene fingerprints.

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#### EMP199

##### **A novel methylotrophic Roseobacter group isolate with an unusually large genome**

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The *Roseobacter* group comprises a significant group of diverse and abundant marine bacteria which are involved in the global cycles of carbon and sulfur. They are considered representative for niche adaptations in marine habitats due to their large functional and metabolic versatility, and methylotrophy has been observed in some members. Methylotrophs employing the methanol dehydrogenase XoxF have been shown to require rare earth elements for growth on methanol, providing an option for targeted isolation approaches. Addition of lanthanum to methanol enrichments of coastal seawater facilitated the isolation of a novel methylotroph within the *Roseobacter* group: *Marinibacterium* sp. strain La 6. Sequencing and genome analyses revealed

an exceptionally large genome of 7.2 Mbp, the largest so far reported for the *Roseobacter* group. Furthermore, core and pan genome analyses suggest unique genetic adaptations distinguishing this strain from its closest sequenced phylogenetic relatives. The genomic and physiological data of this isolate provide new insights into methylotrophy in the ocean and broaden our understanding of niche adaptation within the *Roseobacter* group.

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#### EMP200

##### **Inoculation with plant growth promoting *Pseudomonas* sp. RU47 shifts phosphomonoesterase gene abundances and activities in the tomato rhizosphere**

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The limitation of plant-available phosphorous in soils is a major constraint for crop production and necessitates extensive P fertilization. Plant growth promoting bacterial inocula supporting P mineralization from soil are discussed as an alternative. In the frame of the "BIOFECTOR" project, funded by the European Commission, the strain *Pseudomonas* sp. RU47 was investigated in its abilities to promote and contribute to P mineralization in soil via phosphomonoesterases (PM).

Tomato plants were grown in a greenhouse experiment at low or high P fertilization and inoculated with RU47. To differentiate between direct and indirect effects of the strain, controls included treatments with heat-killed RU47 cells, unselectively cultivated microbes from the soil, and no inoculation. 50 days after sowing, potential alkaline and acid PM activities and the corresponding *phoD* and *phoN* gene abundances were quantified in bulk soil and rhizosphere samples.

At P-deficient conditions, treatments with living RU47 lead to a significant higher P uptake in plant shoots in comparison to the non-inoculated control. Both, potential alkaline and acid PM activities were higher in the rhizosphere as compared to bulk soil and alkaline PM activity increased overall with RU47. Although the specific contribution of RU47 to PM activities remains ambiguous, our data shows that inoculation lead to a shift in the bacterial community towards higher proportion of *phoN* and a reduced relative abundance of *phoD* at higher P fertilization levels.

Still, the mechanism behind the observed plant growth promotion through RU47 might directly be linked to P mineralization, but likely includes further aspects such as hormonal stimulation and interactions with the indigenous microbial community.

## EMP201

### A non-bifurcating electron transferring flavoprotein involved in coupling fatty acid oxidation to biogas formation

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**Introduction:** The formation of biogas from primary fermentative products, e.g. in the digestion tower of waste water treatment plants, involve the syntrophic association of a secondary fermenting bacterium with a methanogenic archaeon. Here, short chain fatty acids are oxidized to acetate and the electrons are used to reduce protons to H<sub>2</sub> and CO<sub>2</sub> to formate. The latter serve as electron donors for the methane formation by the archaeon. Fatty acid oxidation in the fermenting syntroph require the formation of acyl-CoA and its subsequent degradation to acetate via  $\beta$ -oxidation. Here, acyl-CoA dehydrogenases transfer electrons to electron transferring flavoproteins (ETFs) at a redox potential of around 0 mV. The reduction of CO<sub>2</sub> or proton, however, requires electrons at a redox potential of at least -280 mV under syntrophic conditions.

**Objectives:** In literature, two mechanism for the endergonic electron transfer from acyl-CoA oxidation to CO<sub>2</sub> or proton reduction are discussed: Electron confurcation from acyl-CoA and ferredoxin to NAD<sup>+</sup> mediated by an ETF as well as a redox loop driven by the membrane potential. Our goal was to find evidence for one or the other mechanism in the model organism *Syntrophus aciditrophicus*.

**Methods:** Enrichment and *in vitro* characterization of the only ETF of *S. aciditrophicus*.

**Results:** ETF showed interaction with a representative acyl-CoA dehydrogenase of *S. aciditrophicus*, but exhibited almost no electron transfer activity from NADH to INT. LC-MS analysis showed a cofactor content of one FAD and one AMP.

**Conclusion:** The lack of NADH-INT activity and the missing second FAD contradict an electron confurcation via ETF. Thus, the redox loop is the more plausible mechanism for the endergonic electron transfer.

## EMP202

### Effect of Sodium Chloride on Cell Viability and Respiratory Rate by Moderately Thermophilic Iron & Sulfur Oxidizing *Sulfobacillus thermosulfidooxidans*

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Biomining is the utilization of acidophilic iron- and/or sulfur-oxidizing microorganisms for metal extraction from low-grade sulfide ores and mineral concentrates. This microbial process of metal recovery has been proven to be a cost-effective and sustainable technology. However, biomining operations are challenged by the presence of chloride ions through the dissolution of silicate minerals or use of saline/seawater. Acidophilic microorganisms are extremely sensitive to chloride ions. The growth of *Acidithiobacillus ferrooxidans* and *Leptospirillum ferriphilum*, for example, is completely inhibited in the presence of 6 gL<sup>-1</sup> and 12 gL<sup>-1</sup> NaCl, respectively. This study aimed to investigate the influence of increasing concentrations of sodium chloride on cell viability and iron respiratory rates of *Sb. thermosulfidooxidans* (DSMZ 9293). In addition, cell viability

and respiratory rate by cell grown in the absence of NaCl and cell adapted to sodium chloride under chloride stress are also compared. The most probable number method was used to examine cell viability in the presence of different sodium chloride concentrations, ranging from 0 to 500 mM NaCl and The respiration rate with iron (II) ions as a substrate in the presence of sodium chloride was measured using optical oxygen meter FireSting O2 with 4 oxygen channels (Pyro Science). This study has shown that sodium chloride has inhibitory effects on cell viability and respiratory rate of *Sb. thermosulfidooxidans* and the inhibitory concentration depends strongly on pH values. In addition, cells of *Sb. thermosulfidooxidans* proved capable of adapting to low concentrations of sodium chloride.

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## Poster Session 2

### Gene Regulation (GRP)

17 April 2018 • 14:30–17:00

#### GRP241

##### Osmosensing by the bacterial PhoQ/PhoP two-component system

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The PhoQ/PhoP two-component system plays an essential role in the response of enterobacteria to the environment of their mammalian hosts. It is known to sense several stimuli that are potentially associated with the host, including extracellular magnesium limitation, low pH and the presence of cationic antimicrobial peptides. Here, we show that the PhoQ/PhoP two-component systems of *Escherichia coli* and *Salmonella* can also perceive an osmotic upshift, another key stimulus to which bacteria become exposed within the host. In contrast to most previously established stimuli of PhoQ, the detection of osmotic upshift does not require its periplasmic sensor domain. Instead, we show that the activity of PhoQ is affected by the length of the transmembrane helix as well as by membrane lateral pressure. We therefore propose that osmosensing relies on a conformational change within the transmembrane domain of PhoQ induced by a perturbation in cell membrane thickness and lateral pressure under hyperosmotic conditions. Furthermore, the response mediated by the PhoQ/PhoP two-component system was found to improve bacterial growth recovery under hyperosmotic stress, partly through stabilization of the sigma factor RpoS. Our findings directly link the PhoQ/PhoP two-component system to bacterial osmosensing, suggesting that this system can mediate a concerted response to most of the established host-related cues.

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#### GRP242

##### CRISPR interference in *Paenibacillus riograndensis*

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*Paenibacillus riograndensis* SBR5 is a candidate for application as a plant growth promoting rhizobacterium. Although tools for gene expression have been established in SBR5 [1], a method for genome regulation is still missing. To facilitate the mapping of gene expression levels in order to better characterize SBR5, we present the first step in the development of a CRISPR interference (CRISPRi) plasmids using deactivated Cas9 (dCas9) to repress genes in this organism. Single-guide RNA (sgRNA) directing dCas9 to

specific targets in plasmid level (*gfpUV*) was first designed to proof the functionality of the CRISPRi plasmid. The shuttle vector pNW33N backbone was utilized to construct the plasmid pdCas9-*gfpUV* containing *dcas9* gene expression driven by the mannitol inducible promoter (m2p) from *Bacillus methanolicus* [2] and the sgRNA targeting *gfpUV*. In this study, a toxic effect of *dcas9* gene was observed, reducing the plasmid-harboring *Escherichia coli* cells viability. As m2p promoter presented high *dcas9* background expression in *E. coli*, the operator sequence of the *E. coli lac* operon was inserted in the m2p promoter sequence, which reduced toxicity and *dcas9* background expression in *E. coli*. Furthermore, *E. coli* DH5 $\alpha$ (pBV2mp-*gfpUV*) harboring pNW33N or pdCas9-*gfpUV*(laclop) plasmids were cultivated in LB media with or without addition of inducer (Isopropyl  $\beta$ -D-1-thiogalactopyranoside) and analyzed by flow cytometry. Inducing the expression of the plasmid-borne *dcas9* and sgRNA resulted in reduced GfpUV production in 50% as compared to the non-induced treatment. This approach can be applied for SBR5 and other bacilli metabolic engineering.

#### References:

[1] Brito et al (2016) doi:10.1007/s00253-016-7999-1

[2] Irla et al (2016) doi:10.3389/fmicb.2016.01481

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#### GRP243

##### EnuR – The chief regulator of Ectoin catabolism in *Ruegeria pomeroyi* DSS3

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Ectoine and its derivative hydroxyectoine are synthesized as osmoprotectants by a wide range of microorganisms. Upon their release into the environment, other microbes can use ectoines as nutrients. The marine *Roseobacter* species *Ruegeria pomeroyi* can use ectoines as carbon and nitrogen sources<sup>1</sup>. The genes used in the catabolism of ectoines are controlled by a complex regulatory scheme comprising three regulatory proteins, EnuR (GabR/MocR-type), AsnC (feast and famine-type) and NtrYX (two-component system). EnuR contains a covalently bound pyridoxal-5'-phosphate (PLP) as a cofactor in its aminotransferase domain. The PLP-bound protein binds to a specific operator region and then serves as a transcriptional repressor. The specific ectoine metabolite  $\alpha$ -ADABA serves as an inducer and binds to PLP, whereupon repression of the operon is relieved.

This work focusses on new insights in the working mechanism of EnuR and the way it interacts with its specific operator regions and its ligand  $\alpha$ -ADABA. Ligand binding studies by microscale thermophoresis showed that EnuR binds its effector molecule  $\alpha$ -ADABA with a KD of 1.76  $\mu$ M. We identified two EnuR binding-sites upstream of the ectoine-catabolic genes and we investigated the DNA binding mechanism of EnuR to the operator sites via bandshift analysis and site-directed mutagenesis.

These findings point to a new regulatory mechanism of EnuR in which it may work both as an activator and as a repressor. Database searches suggest that our findings have important

ramifications for an understanding of the molecular biology of most microbial consumers of ectoines.

#### References:

[1] Schulz A. *et al.* (2017) Transcriptional regulation of ectoine catabolism in response to multiple metabolic and environmental cues. *Environ Microbiol* 19:4599-4619.

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#### GRP244

##### The aldehyde dehydrogenase AldA contributes to the hypochlorite defense and is redox-controlled by protein S-bacillithiolation in *Staphylococcus aureus*.

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**Introduction:** Bacillithiol (BSH) is the major low molecular weight thiol in *Staphylococcus aureus*. BSH maintains the reduced state of the cytoplasm and forms mixed disulphides with protein thiols to protect them against overoxidation under hypochlorite stress.

**Objectives:** In this study, we aimed to investigate the expression, function and redox regulation of AldA from *S. aureus* under thiol-stress conditions.

**Materials and Methods:** We have used Northern blot analysis to analyze the transcription of *aldA* under different thiol-specific stress conditions. Furthermore, we constructed a *aldA* mutant to investigate the role of AldA in the defense against NaOCl in *S. aureus*.

**Results:** Northern blot analysis revealed SigmaB-independent induction of *aldA* transcription under formaldehyde, methylglyoxal, diamide and NaOCl stress. The *aldA* deletion mutant showed an NaOCl-sensitive phenotype in growth and survival assays. Detailed kinetic analysis showed broad substrate specificity for oxidation of several aldehydes, including formaldehyde and methylglyoxal. In the presence of BSH, AldA was reversibly inhibited due to S-bacillithiolation. Using molecular docking and molecular dynamic simulation, we further show that BSH occupies two different positions in the AldA active site depending on the AldA activation state. The apo-enzyme forms the BSH mixed disulfide with Cys279 in the "resting" state position, but the holoenzyme favours the disulphide bond formation with BSH at Cys279 in the "attacking" position close to the substrate-binding site.

**Conclusion:** In conclusion, we show here that AldA is an important target for S-bacillithiolation in *S. aureus* that is up-regulated under NaOCl stress and functions in protection under hypochlorite stress.

## GRP245

### Transmembrane Redox Signaling and Proteolysis of CSS Domain c-di-GMP Phosphodiesterases in Bacterial Biofilm Formation

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The nucleotide second messenger c-di-GMP nearly ubiquitously promotes bacterial biofilm formation, with enzymes that synthesize and degrade c-di-GMP being controlled by diverse N-terminal sensor domains<sup>1,2</sup>. Here we describe and compare a novel class of widely occurring c-di-GMP phosphodiesterases (PDEs) that feature a periplasmic 'CSS domain' flanked by two transmembrane regions (TM1 and TM2) and followed by a cytoplasmic EAL domain with phosphodiesterase activity.

Using PdeC of *E. coli* as a model, we show that DsbA/DsbB-promoted disulfide bond (DSB) formation in the CSS domain inhibits PDE activity. By contrast, the free-thiol form is enzymatically active, which depends on TM2 as a dimerization domain. Moreover, this form is processed by periplasmic proteases DegP and DegQ, yielding an irreversibly activated TM2+EAL fragment slowly removed by further proteolysis. This versatile interplay of redox control and proteolysis of PdeC regulates the production of amyloid curli fibres and cellulose, i.e. major biofilm matrix polymers in *E. coli*.

#### References:

[1] Hengge, R. Principles of c-di-GMP signalling in bacteria. *Nat Rev Microbiol* **7**, 263-273, doi:10.1038/nrmicro2109 (2009).

[2] Povolotsky, T. L. & Hengge, R. Genome-Based Comparison of Cyclic Di-GMP Signaling in Pathogenic and Commensal *Escherichia coli* Strains. *J Bacteriol* **198**, 111-126, doi:10.1128/JB.00520-15 (2015).

## GRP246

### The complex regulation of the unique microbial volatile compound sodorifen of *Serratia plymuthica*

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It is well established that bacteria produce a wealth of small secondary metabolites, but so far little thoughts and data are available regarding the regulation of microbial volatile compounds (mVOCs). Subsequently, special impetus is needed to explore the different regulatory pathways, particularly those which are involved in the emission of novel mVOCs such as sodorifen.

Sodorifen is emitted in very high quantities by the rhizobacterium *Serratia plymuthica* 4Rx13 amounting to almost 50 % of the total volatile bouquet. It was found to be a polymethylated, bicyclic sesquiterpene. Transcriptome analysis revealed a four-gene cluster indispensable for the biosynthesis of this unique VOC. Interestingly, the genetically closely related strain *S. p.* AS9 also contains the sodorifen cluster but does not emit sodorifen. RT-PCR and Northern blot could prove that the sodorifen cluster in AS9 is silent indicating transcriptional regulation. To assess potential transcriptional regulators, the 5'-UTR of the sodorifen cluster was analysed. Thereby, a  $\sigma^S$ -dependent promoter was identified which is specifically responsible for the expression of genes necessary during stationary phase growth. Moreover, a  $\sigma^S$ -negative mutant exhibited a sodorifen-

negative phenotype. Furthermore, the genome of *S. p.* 4Rx13 was screened for potential quorum sensing systems. One *luxI/R*-like system was found (*esal/R*) which was successfully mutagenized and displayed a significantly reduced sodorifen emission, proving quorum sensing to be a second supervisory authority in sodorifen biosynthesis. A third regulatory entity is carbon catabolite repression regulating sodorifen emission which makes sodorifen a highly interesting compound for further functional investigations.

## GRP247

### Effect of an altered 5'-UTR in *Serratia plymuthica* AS9 on its capability to produce sodorifen

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Sodorifen is a novel volatile compound, of which until now only five isolates, all belonging to the species *Serratia plymuthica*, are known as producers. Although its function is still unknown, a gene cluster involved in the biosynthesis of sodorifen could be identified recently (sodorifen cluster). This cluster is present in the sodorifen producer strain *Serratia plymuthica* 4Rx13 as well as in the non-producer strain *Serratia plymuthica* AS9, but is only expressed in 4Rx13. Thus, it was suggested that the differential expression of the cluster is mediated by regulatory mechanisms.

Earlier experiments indicated a potential role of carbon catabolite repression (CCR) on the sodorifen emission, since the application of glucose lead to a significantly reduced emission. Subsequent construction of deletion mutants lacking the central CCR genes *cya* and *crp* showed the same result. Additionally, two potential binding sites of the cAMP/CRP complex were identified in the 5'-UTR of the sodorifen cluster (CRE1/2). Upon deletion of CRE1 in 4Rx13 a reduction in sodorifen emission as well as cluster expression was observed. Interestingly, the CRE1 sequence is altered in the sodorifen non-producer *S.ply.* AS9 compared to 4Rx13.

To further investigate the role of CRE1 as a regulatory element in sodorifen biosynthesis, the wildtype sequence of the non-producer strain AS9 was replaced with the sequence from 4Rx13 via homologous recombination and the volatiles were analysed. However, the mutant did not produce any sodorifen, proving that the CRE1 sequence alone is not sufficient to induce sodorifen cluster expression. Additional factors still have to be determined to fully understand the regulation of the cluster and, moreover, to reveal the function of the novel compound sodorifen.

## GRP248

### Characterization of the LOV (light-oxygen-voltage) histidine kinase Dshi\_1135 and its role in the light-dependent regulation of bacteriochlorophyll a biosynthesis in *Dinoroseobacter shibae*

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**Introduction:** The marine bacterium *Dinoroseobacter shibae* possesses a gene cluster, encoding the structural components of the photosystem and the light harvesting pigments spheroidenone and bacteriochlorophyll a (Bchl<sub>a</sub>), to perform aerobic anoxygenic photosynthesis. Transcriptome analyses revealed a light dependent

expression of this gene cluster. Screening of the transposon mutant library of *D. shibae* for negative pigmentation and Bchl<sub>a</sub> phenotypes identified the gene locus Dshi\_1135, encoding a potential blue-light dependent LOV (light, oxygen, voltage) histidine kinase (HK).

**Objectives:** Determination of the role of Dshi\_1135 for light-dependent regulation of Bchl<sub>a</sub> biosynthesis in *D. shibae*.

**Materials & Methods:** The *D. shibae* Dshi\_1135 protein was heterologously produced and purified under red light conditions. UV/ Vis spectroscopy was used for cofactor determination. The nature of the cofactor was studied under different light conditions. An autophosphorylation assay was established, using radioactive [ $\gamma$ -<sup>32</sup>P] ATP, to proof a blue-light activated kinase activity of Dshi\_1135.

**Results:** UV/ Vis measurement of purified Dshi\_1135 showed two maxima at 380 nm and 450 nm, typical for a FMN cofactor. Exposure of the protein to blue light led to quenching of the absorption. Absorption could be restored by returning the sample back into the dark. In contrast, red light had no effect on the absorption spectrum of the protein. Thus, the FMN cofactor bound to Dshi\_1135 undergoes a reversible blue light driven photocycle. Moreover, autophosphorylation of Dshi\_1135 was only determined under blue light conditions.

**Conclusion:** The LOV-HK Dshi\_1135 undergoes a blue-light driven photocycle and gets subsequently phosphorylated.

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#### GRP249

##### Regulatory features of colibactin production in *Escherichia coli*

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**Question:** Commensal and pathogenic *Escherichia coli* strains can carry a genomic island coding for the non-ribosomal peptide/polyketide colibactin biosynthetic machinery. The biological role of colibactin is unclear. Colibactin production could be related with probiotic as well as genotoxic effects. The genotoxicity results from DNA damage due to DNA double strand breaks. Since colibactin purification remains challenging, our approach to understand its biological function is to unravel regulatory features of colibactin expression, incl. the role of the essential positive regulator ClbR.

We want to gain insights into the regulatory network affecting the expression of the colibactin determinant. Especially, we investigate the impact of the transcriptional activator ClbR on the transcription of the colibactin operon and on polyketide expression.

**Methods:** To analyze *clbR* transcription, a *clbR*-promoter-luciferase operon fusion was cloned into the *attB* locus of *E. coli* strain M1/5. With this reporter fusion, we tested the influence of different growth conditions, of a variable number of tandem repeat region (VNTR) located upstream of *clbR* and of the ClbQ protein which facilitates colibactin synthesis. Also colibactin production was measured indirectly by cytopathic effect.

**Results and Conclusion:** We identified differential *clbR* transcription in response to different growth media and describe an effect of the VNTR. Furthermore, we observed

that overexpression of ClbR or ClbQ affected *clbR* transcription and colibactin expression. Our findings demonstrate that fine-tuned colibactin expression responds to different regulatory features incl. the *clbR* promoter activity, the size of the VNTR region upstream of *clbR* as well as the amount of colibactin produced.

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#### GRP250

##### Footprints in bacteria – Ribosome covered mRNAs pieces are better indicators for protein expression than RNAseq data

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**Introduction:** Some gut bacteria are facultative anaerobic, like *Escherichia coli* LF82. Cultivation under anaerobic or aerobic conditions *in vitro* leads to expression of different metabolic pathways. To analyse differential gene expression, often the transcriptome is measured using microarray or RNAseq; the proteome is evaluated by mass spectrometry.

**Objectives:** None of these above-mentioned methods deciphers the translome, i.e. mRNA at the point of protein translation. However, translation can be measured by ribosomal footprinting (RIBOseq), analysing which protein is translated at the moment of harvest.

**Materials & methods:** Using RIBOseq for *E.coli* LF82 cultivated under anaerobic or aerobic conditions, the positional information of ribosomes on the mRNA was used to delineate the translome. The transcriptome was measured at the same time using conventional RNAseq.

**Results:** Sequencing results show upregulated or downregulated gene expression between both cultivation conditions. At the same time, transcriptional and post-transcriptional regulation can be distinguished.

**Conclusion:** These findings demonstrate that RIBOseq is useful for detecting and comparing condition-specific protein translation and obtain a more accurate picture of gene expression regulation compared to RNAseq alone.

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#### GRP251

##### GTP-Cyclohydrolase I: The second member of the Zur-regulated *cobW1*-gene cluster

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Zinc is an important trace element and about 6% of all proteins in prokaryotes are zinc-binding proteins, even though zinc is toxic in high concentration (1). The  $\beta$ -proteobacterium *Cupriavidus metallidurans* can cope well with both zinc abundance and starvation conditions (2). To understand the mechanism of zinc homeostasis, we characterized operons that are regulated by Zn<sup>2+</sup>-dependent repressor Zur. The Zur-dependent *cobW1* gene cluster contains six genes, which are strongly expressed under zinc starvation conditions and contains two Zur-boxes in the operator region (3). The first gene of this cluster is *cobW1*, which encodes a zinc chaperone of the G3E family of P-loop GTPases. The genes downstream of *cobW1* encode paralogs of zinc-dependent proteins. *Rmet\_1099* or *foIE2* is the gene directly downstream of *cobW1*. *Rmet\_1099*

is a putative class I GTP-cyclohydrolase (GCHY I) converting GTP into 7,8-dihydropteryne, which subsequently enters different metabolic pathways, e.g. leading to tetrahydrofolate or modified tRNA nucleosides such as queuosine and archaeosine. *C. metallidurans* contains three GTP-cyclohydrolases, the type IB proteins Rmet\_1099 and Rmet\_2614, and the type IA protein Rmet\_3990 (FoIE). While usually IA-type enzymes are Zn<sup>2+</sup>-dependent and constitutively expressed, type IB proteins may use other transition metals (4). We investigated if under zinc starvation conditions (i) CobW1 delivers zinc to the gene products encoded downstream of *cobW1* or (ii) if CobW1 delivers this metal to the paralogs of these proteins while the products of the *cobW1* cluster are able to function with other metal cations.

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## GRP252

### Tracking gene expression and oxidative damage of O<sub>2</sub>-stressed *Clostridioides difficile* by a multi-omics approach

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**Introduction:** *C. difficile* is a vast problem in human health care; as it causes serious and recurrent inflammation of the intestinal epithelium often with a lethal outcome. *C. difficile* is known as strict anaerobe bacterium, so that the presence of oxygen and reactive oxygen species in the human intestinal tract should hamper its growth. Remarkably, a high tolerance of the pathogen to oxygen was recently described.

**Approach:** To understand this high tolerance of *C. difficile* to oxidative conditions, we stressed *C. difficile* 630Δerm with micro-aerobic conditions (5% O<sub>2</sub>) and monitored its response at 15 and 60 min via a multi-omics approach, including transcriptomics, metabolomics and thiol-redox-proteomics.

**Results:** *C. difficile* grows to the same extent independent of the cultivation under anaerobic or micro-aerobic conditions for the tested duration. However, an essential change could be identified in the gene expression by RNA-seq. More than 250 genes were affected after 15 min and even 600 genes changed their expression with a factor two or higher after 60 min of 5% O<sub>2</sub>. A non-targeted metabolomics analysis revealed changes for selected metabolites in the intra- and extracellular metabolome during micro-aerobiosis. Furthermore, mass spectrometry-based redox proteomics facilitated determination of a drastic increase in the redox state of cysteine in more than 800 peptides.

**Conclusion:** Abrogating *C. difficile* adaptation to oxygen and reactive oxygen species could be a starting point for the development of novel treatment strategies. This study indicates that the tolerance of *C. difficile* is not an effect of few detoxifying enzymes, but a complex interplay of changes in gene expression, in the redox state of proteins and the metabolome.

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## GRP253

### A novel antisense overlapping gene completely embedded in EDL933\_1238 of *Escherichia coli* O157:H7 strain EDL933 is regulated under sodium chloride stress conditions

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Bacterial open reading frames overlapping in *antisense* (shadow ORF, sORF) to annotated genes (mother ORF, mORF) are short and often excluded from genome annotation. Thus, they seem to be a rather rare phenomenon and the existence of such sORFs can only be supported by a functional characterization. Here we report on the experimental and bioinformatic characterization of a short 264 bp sORF of the pathogen *Escherichia coli* O157:H7 strain EDL933 (EHEC). RIBO-seq data show that the sORF mRNA is translated. Competitive growth experiments of EHEC expressing this sORF and its translationally arrested mutant show a strong phenotype in the presence of sodium chloride. RT-PCR confirms the transcription in EHEC cells grown in sodium chloride and Luria broth medium (LB). In cells adapted to sodium chloride, the sORF is regulated in comparison to the LB control dependent on the growth rate. There are no annotated homologous proteins in NCBI, but homologous sequences are distributed among gammaproteobacteria, many of them with internal stop codon. Two of the non-intact homologues were tested and do not show the sodium chloride induced phenotype. We conclude that this sORF is a novel, NaCl responsive protein encoding gene.

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## GRP254

### CRIMoClo: conditional-replication, integration, and modular cloning plasmids for synthetic biology applications in *Escherichia coli*

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Synthetic biology heavily relies on novel techniques for DNA manipulation and assembly, like ligase cycling reaction (LCR), Gibson assembly and the Modular cloning (MoClo) system, all of which allow for fast, multi-fragment DNA assembly. MoClo in particular allows for simple library propagation and combinatorial assembly of genetic circuits from reusable parts. One limitation of the MoClo system is, however, that all circuits are assembled on single and multi-copy plasmids, while a rapid route to chromosomal integration is lacking. To overcome this bottleneck, we here took advantage of the conditional-replication, integration, and modular (CRIM) plasmids, which can be integrated in single copies into the chromosome of *Escherichia coli* and related bacteria by site-specific recombination at one of four different phage attachment sites (Haldimann and Wanner, 2001). Integrands are stably maintained even in the absence of antibiotic selection, and eventually, they can be easily excised from the chromosome. By combining their features with the modularity of the MoClo system we created a set of modified CRIMoClo plasmids and benchmarked their suitability for synthetic biology approaches. To this end we evaluated gene expression in four phage attachment sites and show full insulation from the cellular genetic context. Next we assembled and genomically integrated genetic circuits up to 12kb with high efficiency, and show that the growth defects observed for circuits encoded on medium-copy plasmids were abolished for chromosomally integrated circuits. We conclude that CRIMoClo plasmids provide a rapid route to constructing and integrating large DNA assemblies into the chromosome of *E. coli*.

## GRP255

### Expansion, promoter prediction and genomic context of the extracytoplasmic function (ECF) sigma factor family

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ECFs are the simplest members of the  $\sigma 70$  family of  $\sigma$  factors. Previous work showed that the contacts between ECFs, their target promoter and their negative regulators are orthogonal and host-independent, which makes ECFs particularly suited for synthetic biology applications in heterologous organisms (Rhodius *et al.*, 2013). Since their first description, about 90 phylogenetically distinct ECF groups have been identified, enabling a group-specific study of their mechanism of action (Staroń *et al.*, 2009, Jogler *et al.*, 2012, Huang *et al.*, 2015). However, to date the classification of ECF groups has been limited to only about 500 organisms out of the more than 60,000 genomes currently annotated. We designed a computational framework based on Hidden Markov Models (HMMs) that automatically extracts new ECFs from the current set of annotated genomes and clusters them into new ECF groups. As a result, the ECF library was expanded by 50-fold. Besides the identification of novel ECF groups, group-specific analysis of the genomic context of ECFs allowed for the identification of their cognate regulators and target promoter. This work provides an expansion of the available sets of transcriptional activators, target promoters and negative regulators that can be used for the implementation of complex synthetic circuits.

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## GRP256

### Structural dynamics in DcuS signal transduction in *E. coli*

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The DcuSR two component system regulates aerobic and anaerobic C4-dicarboxylate (C4DC) metabolism in *E. coli* [1]. Stimulation of the membrane integral C4DC-sensitive sensor histidinekinase DcuS causes autophosphorylation and activation of the associated response regulator DcuR [2]. The dimeric DcuS contains the transmembrane helices 1 and 2 (TM1, TM2). Periplasmic signal input and signal conversion in the cytoplasm of DcuS is physically connected by TM2, whereas TM1 is the N-terminal membrane anchor. Changes in membrane localization upon activation indicate a primary piston type movement of TM2 in direction of the periplasm [3].

DcuS interaction has been investigated with respect to TM-sequence motifs indicating an indispensability of TM2 dimerization for DcuS functionality. Intermolecular oxidative cysteine crosslinking of TM1 and TM2 single-cysteine variants revealed the helical interface of the TM-homodimers in both the inactive/responsive and stimulated state of DcuS. Additionally, cysteine crosslinking extends knowledge on DcuS structural dynamics in signal transduction.

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- [1] Zientz *et al.* 1998. *J. Bacteriol.* 180 (20):5421-5
- [2] Scheu *et al.* 2010. *Future Microbiol.* 5(9):1383-402
- [3] Monzel and Uuden 2015. *Proc. Natl. Acad. Sci.* 112 (35):11042-7

## GRP257

### Regulation of Mo- and Fe-nitrogenase genes in *Rhodobacter capsulatus*

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Diazotrophic bacteria reduce atmospheric dinitrogen (N<sub>2</sub>) to bioavailable ammonia (NH<sub>3</sub>) and thus, play a central role in the global nitrogen cycle. Biological N<sub>2</sub> fixation is catalyzed by nitrogenase enzymes. The photosynthetic alphaproteobacterium *Rhodobacter capsulatus* synthesizes two nitrogenases, a *nifHDK*-encoded Mo-nitrogenase and an *anfHDKG*-encoded Fe-nitrogenase. Activity of both nitrogenases strongly depends on additional *nif* genes, which are required for cofactor biosynthesis and electron supply. Expression of *nif* and *anf* genes is strictly repressed by ammonium. In the absence of ammonium the superior regulator NtrC activates expression of the *nifA1*, *nifA2*, and *anfA* genes coding for the transcriptional activators of Mo- and Fe-nitrogenase genes, respectively. Both NifA and AnfA activate their target genes in concert with the alternative sigma factor RpoN. Deletion of *nifA1* and *nifA2* greatly diminishes *rpoN* transcription demonstrating that AnfA does not mediate substantial *rpoN* expression. Consequently, NifA1 and NifA2, the direct activators of *nifHDK*, are also required for maximal transcription of *anfHDKG*. At the same time, AnfA affects transcription of NifA-dependent genes. Hence, the two nitrogenase systems mutually control their expression.

In addition to classical *nif* genes, NifA activates transcription of the *cooA* gene, whose product, in turn, activates *cowN* expression with increasing carbon monoxide concentrations. CowN protects Mo-nitrogenase against CO inactivation. Besides its role in *cowN* activation, CooA represses transcription of several *nif* genes including *nifHDKG*. However, CooA disruption strongly hinders diazotrophic growth suggesting that increased *nif* expression does not enhance nitrogenase activity, but instead affects the fitness of cells.

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## GRP258

### Metagenomic and mutagenesis studies uncover novel features for regulating RubisCO expression in an uncultured deep-sea vent bacterium.

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The Calvin-Benson-Bassham (CBB) cycle accounts for almost all the CO<sub>2</sub> assimilated on earth. It is the most widespread pathway among the aerobic members of the  $\alpha$ -,  $\beta$ - and  $\gamma$ -proteobacteria and the only autotrophic carbon fixation route operating in cyanobacteria, algae, and terrestrial plants. The central carboxylation reaction in the CBB cycle is catalyzed by one of two key enzymes, namely the Ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO). Because of RubisCOs primary role in global biomass production, but the generally known catalytic imperfection enormous research interest persists on improving RubisCOs efficiency. Therefore it becomes crucial to understand how RubisCOs gene expression is regulated and how the enzyme is activated.

Given the tremendous potential among autotrophic unculturable, we used a metagenomic approach to investigate genes and enzymes associated with RubisCO expression and activation from an uncultured deep sea bacterium. Using a recently established activity-based screen we detected several RubisCO active fosmid clones

and sequenced their inserts. One metagenomic DNA insert was investigated more intensely. Its DNA originates from a deep-sea hydrothermal vent field along the southern Mid-Atlantic Ridge. It encodes two different RubisCO forms (*cbbL/S* and *cbbM*) alongside with two transcriptional regulators (*lysR1* and *lysR2*), and four genes whose products are expected to function as RubisCO activating enzymes (*cbbO-m*, *cbbQ-m*, *cbbQ-1*, and *cbbO-1*). In silico analyses, mobility shift assays and transposon mutagenesis studies unfold novel features behind expressing a fully functional RubisCO enzyme. A model summarizes our findings and predicts putative interactions of the different proteins influencing RubisCO gene regulation and activation.

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## GRP259

### **Psp<sub>ACT</sub> is a dual function domain within PspA**

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The phage shock protein (Psp) system is highly induced under manifold stress conditions and may stabilize the cytoplasmic membrane in bacteria. PspA is one of its key components and therefore in focus of many studies concerning the Psp regulatory cascade. Due to its oligomerization and aggregation properties, PspA is experimentally difficult to handle. Nevertheless, by using a fragmentation approach, we recently solved the structure of a completely soluble PspA fragment covering 2/3<sup>rd</sup> of the whole protein, demonstrating the *in silico* predicted coiled-coil organization [1]. This soluble fragment tightly associates with the enhancer binding protein PspF and thereby strongly downregulates the *pspA* promoter activity. Here, we focus on the C-terminal part of PspA (= Psp<sub>ACT</sub>) whose structure is unknown. We could show that recombinant Psp<sub>ACT</sub> is a soluble and a dual function domain that induces the Psp response in a *psp* wildtype background and, surprisingly, silences the *psp* gene expression in a *pspA* deletion mutant. Constitutive expression of Psp<sub>ACT</sub> also enhances the effect of a Psp-response-triggering signal, possibly due to its contribution to PspA oligomerization. Together, our data indicate a pivotal role of Psp<sub>ACT</sub> in the signaling cascade of this stress response system.

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## GRP260

### **Transcriptional regulation during NO-induced dispersal of *Pseudomonas aeruginosa* biofilms**

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Biofilms are surface-associated microbial communities with great medical relevance. As an opportunistic human pathogen *Pseudomonas aeruginosa* causes a broad range of acute and chronic infections often involving the formation of biofilms. Therefore, understanding of the mechanisms leading to biofilm dispersal may help to combat biofilms in the future. Several environmental cues have been identified to trigger biofilm dispersal and have been linked to cause low intracellular c-di-GMP (bis-(3"-5"-)cyclic dimeric guanosine monophosphate) concentrations generated by the c-di-GMP degrading phosphodiesterases (PDEs). Non-lethal concentrations of nitric oxide (NO) trigger PDE activity in *P. aeruginosa* and lead to detachment of motile cells from the

biofilm. The membrane-anchored protein NbdA (NO-induced biofilm dispersion locus A) with the domain organisation MHYT-AGDEF-EAL was shown to be a major player in the NO-induced biofilm dispersal response of *P. aeruginosa* (Li *et al.*, 2013). While the main hypothesis postulates direct NO sensing via the MHYT-domain resulting in increased PDE activity, initial qPCR experiments also suggested regulation at the transcriptional level [1]. Within this study we further examined the transcriptional regulation of *nbdA* using transcriptional promoter *lacZ*-fusions. In order to investigate whether endogenous NO has an impact on *nbdA* transcription a markerless *nirS* mutant strain, deficient in dissimilatory nitrite reductase, has been generated. Additional experiments aimed at elucidation of a predicted RpoS consensus sequence in the promoter region of *nbdA*. The obtained data will be presented and discussed with respect to the current model of NO-induced biofilm dispersal in *P. aeruginosa*.

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## GRP261

### **Cyclic di-AMP mediated control in *Corynebacterium glutamicum*: Functional analysis of two putative c-di-AMP dependent riboswitches**

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The nucleotide second messenger c-di-AMP is involved in the control of sporulation, potassium uptake and cell wall homeostasis in Gram-positive bacteria. In *C. glutamicum*, enzymes for c-di-AMP metabolism have been identified and c-di-AMP was shown to be involved in control of K<sup>+</sup>-homeostasis.

We investigated two putative c-di-AMP-dependent riboswitches (RS), one located upstream of *rpf1*, encoding a resuscitation promoting factor, and one upstream of an operon of seven genes, the first two of them encoding NlpC/P60 endopeptidases involved in cell growth and cell separation. Reporter plasmids carrying a putative RS upstream of a fluorescence reporter gene were constructed. In an *E. coli*-based system, reporter expression was strongly inhibited in presence of intracellular c-di-AMP for the plasmid harbouring the RS of the *nlpC* operon. In contrast, no effect was observed for the putative RS from *rpf1* in presence or absence of c-di-AMP. These results indicate that c-di-AMP is involved in control of the *nlpC* operon, whereas the putative RS from *rpf1* might not be functional. Analyses of the *nlpC* promoter region revealed the presence of two binding sites for the response regulator MtrA upstream of two transcriptional start sites (Brocker *et al.* 2011. *J Bacteriol* 193:1237-1249). The two-component system MtrAB controls expression of genes for cell wall homeostasis negatively (*mepA*, *ppmA*) and genes for uptake of compatible solutes positively. In difference to *mepA*, expression of *nlpC* was only slightly increased upon deletion of *mtrAB*, which might be caused by the c-di-AMP RS. The consequences of the interplay between the c-di-AMP-riboswitch and MtrAB for the control of the *nlpC* operon will be discussed.

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## GRP262

### **Characterization of new genes regulated by the sigma factor PfrI in *Pseudomonas putida* KT2440**

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*Pseudomonas putida* is a Gram-negative soil bacterium that colonizes the rhizosphere of plants. The fluorescent

bacterium synthesizes a siderophore called pyoverdine to acquire iron. The metabolically costly synthesis of pyoverdine is strictly regulated. The ferric uptake regulator Fur is the key component of the regulatory network. Among others, it controls the sigma factor Pfrl (PvdS), which is required for the expression of genes involved in pyoverdine synthesis and secretion (1). However, until this day, there is no complete information about the genes controlled by Pfrl.

The aim of this work was to identify new genes regulated by the sigma factor Pfrl in *Pseudomonas putida* KT2440, and to investigate their function and significance. For this purpose, we compared the transcriptome of a strain constitutively expressing *pfrl* with the transcriptome of a *pfrl* deletion mutant. The approach was complemented by gene expression analyses involving qRT-PCR and reporter gene fusions. Selected genes were deleted, and resulting mutants were phenotypically characterized.

The transcriptome comparison identified 72 upregulated and 17 downregulated genes with a minimum 5fold change of the expression level. Out of the upregulated genes, 15 genes/operons are under the direct control of Pfrl. Our analysis also showed that most of the upregulated genes are involved in the synthesis, maturation or secretion of pyoverdine. Two new upregulated genes of yet unknown function were further analyzed and shown to affect pyoverdine production. These results contribute to a better understanding of the complex regulatory mechanisms underlying adaptation to iron limitation.

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[1] Matthijs, S. *et al.* (2009) *Biometals* **22**, 951-964

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#### GRP263

##### **Photooxidative stress-response in *Rhodobacter capsulatus*: from physiology to omics**

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Alphaproteobacteria of the genus *Rhodobacter* are Gram-negative, mostly aquatic species with high metabolic versatility. The switch between aerobic respiration and phototrophy is controlled by a tight regulation of gene expression, as the simultaneous presence of light, oxygen and photopigments causes photooxidative stress: excited bacteriochlorophyll a (BChl a) in the photosystems in *Rhodobacter* can transfer energy to molecular oxygen, producing cytotoxic singlet oxygen (<sup>1</sup>O<sub>2</sub>). While studies have outlined the <sup>1</sup>O<sub>2</sub>-response in *Rhodobacter sphaeroides*, preliminary work based on RNA-Seq data hint at salient differences in the closely related *Rhodobacter capsulatus*. This study aims to unravel the <sup>1</sup>O<sub>2</sub>-response in *R. capsulatus* and to compare it to the response in *R. sphaeroides* on the levels of physiology, transcriptomics and proteomics.

Exponentially growing cultures of the wild-types strains *R. capsulatus* SB1003 and *R. sphaeroides* 2.4.1 were shifted from semiaerobic conditions in the dark to photooxidative stress via aerobic conditions with methylene blue and high light (800 W m<sup>-2</sup>). During this process, we monitored physiological parameters (e.g. growth, pigmentation) and analysed *R. capsulatus*-samples via RNA-Seq and mass spectrometry.

Upon <sup>1</sup>O<sub>2</sub>-stress, only *R. capsulatus* showed a consistently rising ratio of <sup>1</sup>O<sub>2</sub>-quenching carotenoids to <sup>1</sup>O<sub>2</sub>-producing BChl a. The proteome response showed other differences: for instance, proteins for oxidative stress response increased only in *R. capsulatus* after 90 min of stress, whereas a reduction of photosynthesis-related proteins as observed in *R. sphaeroides* was missing.

As our data underlines differences in the <sup>1</sup>O<sub>2</sub>-response in *R. sphaeroides* and *R. capsulatus*, further studies on differentially expressed genes are underway.

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#### GRP264

##### ***In vitro* analysis of xylose repressor XylR from *Bacillus megaterium***

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**Introduction:** Within the last years Gram positive *Bacillus megaterium* was systemically developed for the production of recombinant proteins using the strong xylose-inducible promoter. This system is based on a multicopy plasmid containing the gene encoding the xylose repressor XylR with its corresponding promoter and the promoter P<sub>xyIA</sub> followed by a multiple cloning site. It is postulated that in the absence of xylose XylR binds to the operator and prevents expression of the following genes, while in its presence xylose binds to XylR losing its affinity for the operator and expression is possible. So far this model was not confirmed using *in vitro* analyses with purified XylR.

**Objectives, materials & methods:** The recombinant production and purification of XylR<sub>B.meg</sub> with and without bound xylose and subsequent electrophoretic mobility shift assay (EMSA), footprinting assays and *in vitro* transcription analyses should be established to investigate regulation model.

**Results:** EMSA with purified XylR with/without bound xylose showed two different mobility complex bands indicating different oligomeric states depending on xylose. With this it was shown for the first time that both forms of XylR binds to the same DNA-region. Footprinting assays confirmed that XylR is able to bind to the same operator sequence independently of xylose but clearly indicated a different binding behaviour of both forms. This could lead to DNA organization like loop structures causing repression of genes in the absence of xylose. *In vitro* transcription with purified XylR with bound xylose gave the first hints that transcription occurred with DNA-bound XylR.

**Conclusion:** In summary, the postulated model of DNA-bound XylR without xylose and free XylR with xylose was adapted.

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#### GRP265

##### **The MarR-type regulator MaiR is involved in stress-responsive cell envelope remodeling in *Corynebacterium glutamicum***

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**Introduction:** In former studies we described the small nucleoid-associated protein CgpS as an essential silencer of cryptic prophage elements in *Corynebacterium glutamicum*

[1]. Using DNA affinity chromatography with selected CgpS target promoters, several transcriptional regulators were identified which potentially interfere with the activity of CgpS and represent further players in the regulatory interaction of prophage and host.

**Objectives:** This study focused on the characterization of the MarR-type regulator MalR and its impact on prophage induction in *C. glutamicum*.

**Methods:** We used ChAP-Seq experiments in combination with EMSA studies to provide genome wide insights into the MalR regulon. Furthermore, a comprehensive phenotyping of the *malR* mutant was performed using phenotypic microarrays.

**Results:** ChAP-Seq analysis revealed that MalR binds to several targets inside CGP3 as well as to different host promoters, *i.a.* its own promoter region. Overproduction of MalR causes severe growth defects and elongated cell morphology. Furthermore, transcriptome analysis of the overexpression strain emphasized a central role of MalR in cell envelope remodeling in response to environmental stresses. Prominent MalR targets are for example involved in peptidoglycan biosynthesis and synthesis of branched-chain fatty acids. Phenotypic microarrays suggest an altered sensitivity of a  $\Delta malR$  mutant towards selected antibiotics.

**Conclusion:** In conclusion, our studies underline MalR as a regulator targeting stress-responsive remodeling of the cell envelope of *C. glutamicum*. The multitude of MalR binding sites within the CGP3 prophage suggests a link between cell envelope stress and the control of phage gene expression.

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[1] Pfeifer *et al.*, *Nucleic Acids Res.*, 2016. 44(21):p. 10117-10131

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#### GRP266

##### **Plant infection-related micro-proteins in *Sinorhizobium fredii* NGR234 are involved in symbiotic plasmid copy number regulation**

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Since their discovery, micro-proteins or small ORFs were demonstrated to have several cellular functions. Nevertheless, they were long time neglected, overseen and are now not in the data bases to be used as templates for functional analysis.

*Sinorhizobium fredii* NGR234 is a plant symbiont which is able to form nitrogen-fixing nodules in over 120 plant genera. Its genome encodes for two autoinducer (AI) systems of which the Tral system is localized on the symbiotic plasmid pNGR234a. The second AI synthase NgrI is encoded on the chromosome. As we are mainly interested in micro-proteins related to plant infection, we focused our analyses on micro-proteins that are located on pNGR234a.

By using deep RNAseq, we were able to compare detailed transcription profiles of the wildtype and a deletion mutant of both AI systems. We identified over 200 small ORFs on pNGR234a that showed altered transcription levels in the background of an AI double deletion mutant compared to the wildtype. Furthermore, the AI mutant showed an up to eight-fold increased copy number of its symbiotic plasmid. Thereby we identified a small ORF that encodes a 51 aa micro-protein and that is located in-between the AI synthases gene *tral* and the known *repABC* genes on pNGR234a. We designate this micro-protein RepX. We speculate that RepX is involved in the plasmid copy regulation and has an influence on the symbiotic interaction as well.

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#### GRP267

##### **The DNA-methyltransferase M.Ssp6803II modifying the motif GGm4CC is involved in DNA repair and replication of *Synechocystis* sp. PCC 6803**

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Abstract has been withdrawn.

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#### GRP268

##### **PAS4-LuxR solos as inter-kingdom-signaling receptors in *Photorhabdus luminescens***

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It is well known that the cell-cell-communication systems of many Gram-negative bacteria contain LuxR-type receptors that detect specific communication molecules to regulate expression of specific target genes. LuxR-type receptors consist of a C-terminal DNA binding domain (DBD) and an N-terminal signal binding domain (SBD). The Gram-negative entomopathogen *Photorhabdus luminescens* harbors the remarkably high number of 40 LuxR-type receptors, most of them harbor a "PAS4"-SBD. Knockouts of *PAS4-luxR* gene clusters showed a specific decrease in pathogenicity against larvae from different insect species. Therefore, the PAS4-LuxR receptors are supposed to play a central role in inter-kingdom-signaling between the bacteria and their eukaryotic hosts. Conspicuously, the PAS4 domains of the LuxR solos Plu2018 and Plu2019 are structurally homologous with the "PAS3"-SBD of the Met regulator from *Drosophila melanogaster*, which binds insect juvenile hormone 20-ecdysone as signal. This fact suggested that the Plu2018/Plu2019 PAS4 domains might also sense insect hormone-like molecules. We identified *plu0258*, among other genes, as a putative target gene of Plu2018 and/or Plu2019 via comparative proteome analysis. P<sub>plu0258</sub> activity could be specifically induced by *Galleria mellonella* insect homogenate revealing that the signal, which is sensed by Plu2018/Plu2019, is derived from this eukaryotic host. We then isolated the Plu2018/Plu2019 signaling molecule from *G. mellonella* insect homogenate and curtailed the number of possible ligands to a few comparatively hydrophobic small molecules. Currently we perform mass spectrometry and NMR in order to identify the chemical nature of the first PAS4-LuxR solo inter-kingdom-signaling molecule.

## GRP269

### Regulation of Polar Flagellar Assembly in *Shewanella putrefaciens* CN-32

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Flagella are organelles of locomotion and multiprotein complexes whose assembly and positioning requires complex spatiotemporal control. Depending on the bacterial species, the complete flagellar structure consists of about 25 different structural units in various stoichiometries. The construction and operation of a flagellum is a costly investment for the cell and thus strictly regulated.

*Shewanella putrefaciens* CN-32 has a transcriptional regulatory mechanism like other monopolarly flagellated species such as *Pseudomonas* and *Vibrio*. However, only the regulation of flagellar genes at mRNA level and not at protein level is known for all species. Therefore, we have studied the regulation of polar flagellar genes by the regulators FlrA, RpoN ( $\sigma_{54}$ ) and FliA ( $\sigma_{28}$ ) on mRNA level by RNAseq and protein level via mass spectroscopy with the corresponding knock-out strains of *Shewanella*. Also, some flagellar proteins were fused with a FLAG tag in the respective wildtype or mutant strains of *Shewanella* and directly determined protein levels by immunoblotting.

The major regulators, FlrA and RpoN, activate the transcription of the basal body building blocks, the flagellar export gate and the further regulator FliA. However, only a few of these early flagella proteins are regulated and the others are present at normal levels in the absence of the master regulators. The sequential expression ensures that the subsequent transcription of genes activated by FliA and coding for the main filament subunit Flagellin is only transcribed after completion of the hook arrangement.

These findings provide new insights into the transcriptional regulation and assembly of polar bacterial flagella.

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## GRP270

### The role of the small protein RSP\_6037 in the oxidative stress response in *Rhodobacter sphaeroides*

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The facultative phototrophic bacterium *Rhodobacter sphaeroides* is able to adapt its life style to changing environmental conditions. In the past our group has elucidated the role of protein regulators and small, regulatory RNAs (sRNAs) in the stress responses, particularly in oxidative stress response, in *R. sphaeroides*. Quite recently it became evident that bacterial genomes encode many small proteins, here defined as proteins of 50-70 amino acids or less in the absence of processing. These small proteins were previously overlooked due to the small size of the open reading frames (ORFs), their annotation and their complexity of biochemical characterization. RNAseq and Northern Blot analysis of transcripts unveiled an ORF, which encodes the small protein RSP\_6037. This small ORF encodes a 70 amino acid protein, which contains a conserved domain of unknown function (DUF1127) and is structurally related to eukaryotic RNA binding proteins. The corresponding gene is

cotranscribed with a downstream repeat of the four homologous sRNAs CcsR1 to CcsR4 (conserved CCUCCUCCC motif stress-induced RNA). These sRNAs are generated from the primary transcript by RNase E - dependent processing and the expression of this operon is controlled by a RpoHI/RpoHII- dependent promoter, which leads to induction under multiple stress conditions like oxidative and heat stress. A plasmid-derived overexpression of RSP\_6037 was used to investigate the cellular influence of the small protein on CcsR1-4 RNA-levels. The results demonstrate that the abundance of CcsR1 and the resistance to oxidative stress are influenced by the presence or absence of RSP\_6037. We test the hypothesis that binding of the RSP\_6037 with the DUF1127 domain influence the stability and processing of RNA transcripts.

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## GRP271

### A SigB-dependent antisense RNA affects osmotic induction of *opuB* expression in *Bacillus subtilis*

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**Introduction:** The soil bacterium *B. subtilis* uses compatible solutes like glycine betaine (GB) to cope with hyperosmotic stress. GB can be accumulated directly from the environment or synthesized from choline that is imported via the ABC transporters OpuB and OpuC [1]. In a transcriptome study under various environmental conditions [2], we noted differential induction of the *opuB* and *opuC* operons in response to either suddenly imposed or sustained salinity. These data also revealed a SigB-dependent antisense RNA (S1290) covering the *opuB* operon, which was induced by osmotic stress.

**Objectives:** Based on the observation that an osmotic upshift does not lead to a rapid induction of the *opuB* operon we wanted to analyze the impact of asRNA S1290 on *opuB* expression and choline utilization.

**Methods:** We performed time-resolved analyses of different transcripts in response to osmotic upshifts in the absence or presence of GB and choline. To construct an S1290 promoter mutant we altered the -10 and -35 regions without changing the amino acid sequence of OpuB.

**Results:** S1290 RNA levels peaked at 10 or 30 min after osmotic upshift, depending on the NaCl concentration; *opuB* expression started only at subsequent time points. This time-delayed induction of *opuB* was not observed in the S1290 promoter mutant. These cells showed a higher expression of the *gbsAB* genes encoding the choline-to-GB pathway in the presence of both compounds compared to the wild type.

**Conclusion:** Expression of an asRNA delays the osmotic induction of the genes encoding the choline-specific transporter OpuB. Our results suggest that this regulation contributes to the preferred use of preformed osmoprotectants.

References:

[1] Hoffmann *et al.*; J. Bacteriol. 2011

[2] Nicolas *et al.*; Science 2012

## GRP272

### Characterization of the transcriptional landscape of *Escherichia coli* by genome-wide *in vitro* transcription RNA-Seq (ROSE)

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The bacterial transcription process is driven by sigma factor-dependent recognition of conserved promoter elements. Most commonly, sigma factor regulons are studied by differential transcriptome sequencing (RNA-Seq). However, it is known that in many cases sigma factor regulons overlap and are affected by additional transcriptional regulators, which leads to inconclusive results in current *in vivo*-based approaches.

Therefore, the ROSE (Run-Off transcription/RNA-Sequencing) method, which couples genome-wide *in vitro* run-off transcription with RNA-Seq, has been developed. RNA is transcribed *in vitro* with a single, specific sigma factor bound to the *Escherichia coli* RNA polymerase and subjected to sequencing library preparation. Via the enrichment of primary transcripts it is possible to determine transcriptional start sites (TSS) with single nucleotide resolution after mapping of the reads to the reference genome.

Here, 2712  $\sigma^{70}$ -dependent TSS in the *E. coli* MG1655 genome have been identified in a single ROSE experiment and validated by promoter motif analysis and comparison to literature. About 780 novel TSS, many of which are negatively regulated *in vivo* and therefore hidden in conventional RNA-Seq studies, have been found.

Further, by the addition of the DksA protein and the alarmone ppGpp, the first level of stringent response-associated transcriptional regulation could be determined on a genome-wide level in conformity with the processes described for the *in vivo* regulation.

These findings prove that ROSE is a powerful method for the elucidation of transcriptional regulation in bacteria and that it can be extended to simulate complex *in vivo* situations with multiple regulators.

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## GRP273

### Establishing CRISPRi in *Halomonas elongata* to uncover metabolic pathways

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The discovery of the CRISPR-Cas systems revolutionized genetic engineering. Here we are using CRISPR-mediated interference (CRISPRi) for controlled gene repression in the halophilic bacterium *Halomonas elongata*. *H. elongata* synthesizes and accumulates organic osmolytes to cope with osmotic stress in saline environments. The osmolyte ectoine possesses protecting properties and stabilizes proteins and whole cells against stresses such as UV radiation. *H. elongata* is the industrial producer strain of ectoine, which is produced on an annual scale of tons and processed into cosmetics and pharmaceuticals. Still, the metabolic traits and the factors for optimum ectoine production remain to be fully explored. Therefore, we have established the CRISPR-Cas9 system in *H. elongata* to investigate metabolic pathways and subsequently optimize ectoine production. With the application of CRISPRi we are expanding the molecular toolbox for *H. elongata*. This tool allows sequence-specific

repression of gene expression. The catalytically inactive dCas9 was integrated into the genome under the constitutive expression of the teaABC operon. GuideRNA constructs were added to trigger site-directed repression of target genes. The first target was one of the ectoine synthesis genes, ectA. The repression of ectA resulted in a decrease of ectoine production. Additionally, changes in gene expressions levels were determined via qRT-PCR. The second target was the Entner-Doudoroff pathway of glucose degradation, which allow us to determine the preferred glycolytic strategy of *H. elongata* under varying salt conditions. This successfully established CRISPRi system is a valuable tool that allows us to further investigate metabolic pathways to keep optimizing ectoine production and excretion in *H. elongata*.

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## GRP274

### Investigation of pristinamycin signalling molecules

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The biosynthesis of the streptogramin antibiotic pristinamycin in *Streptomyces pristinaespiralis* is governed by a complex signaling cascade that involves seven transcriptional regulators (SpbR, PapR1-PapR6). Three of them - SpbR, PapR3 and PapR5 - belong to the TetR-like family. SpbR is a  $\gamma$ -butyrolactone (GBL) receptor protein, whereas the ligand binding capacity of PapR3 and PapR5 is not known so far. TetR-like regulators, derived from antibiotic gene clusters, can either act as "real" GBL receptors or as "pseudo"-GBL receptors. "Real" GBL receptors accept GBLs as ligands, whereas "pseudo"-GBL receptors bind antibiotics or intermediates thereof. Gelshift shift assays (EMSA) revealed that GBL-like molecules, but not pristinamycin or its intermediates, serve as effectors for the *S. pristinaespiralis* TetR-like regulators. Thus, SpbR, PapR3 and PapR5 act as "real" GBL receptors. In accordance with these data, we found that the addition of synthetic 1,4-GBL to the *S. pristinaespiralis* culture leads to an increase of pristinamycin production, which demonstrates that 1,4-GBL is an inductor of pristinamycin biosynthesis.

So far, the GBL biosynthetic gene(s) is/are not known. A putative pristinamycin effector biosynthesis gene, *snbU*, has been identified within the pristinamycin gene cluster, encoding a putative cytochrome P450 monooxygenase. Inactivation of *snbU* led to an increase of pristinamycin biosynthesis, which indicates that SnbU has a regulatory effect on pristinamycin production. EMSA studies showed that culture extracts from the *snbU* mutant do not prevent the above mentioned regulator-DNA binding, suggesting that SnbU is involved in pristinamycin effector biosynthesis. Additional putative effector biosynthesis genes (*snbS, T, V, W*) are currently under investigation.

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## GRP275

### Transcriptional analysis of magnetosome gene cluster expression in *Magnetospirillum gryphiswaldense*

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*Magnetospirillum gryphiswaldense* aligns itself along the earth's magnetic field by means of specific organelles, the magnetosomes, which consist of membrane-enveloped magnetite crystals<sup>1</sup>. The >30 genes controlling magnetosome biosynthesis are organized in operons *mamABop*, *mamGFDCop*, *mamXYop*, *mms6op* and

*feoABop*, which are clustered in a single chromosomal region called Magnetosome Island (MAI) extending over 100 kb<sup>1</sup>.

We used up-to-date transcriptome techniques, such as Cappable-sequencing and whole transcriptome shotgun sequencing (WTSS), to investigate the MAI transcriptional organization under optimal conditions for magnetosome biosynthesis. Preliminary data revealed primary transcription start sites (TSSs) in front of each operon. In case of the large *mamAB* operon, which comprises 16 of the most crucial magnetosome genes, two additional putative internal TSS within the coding region of two different genes were detected, hinting towards two suboperons with five to seven genes in addition to the single large 16 kb transcript. Fluctuating transcription levels within *mamABop* seem to be consistent with the presence of these internal promoters. Also in the small (5 kb) *mamXYop*, one internal TSS was detected, subdividing it in two suboperons. Furthermore, a strong termination signal followed by an internal TSS could be detected within *mms6op*, again suggesting a separation into two suboperons. The organization of *mamGFDCop* as a single transcript driven from one promoter could be verified. Altogether, these data hint towards a more complex transcriptional organization of magnetosome gene clusters as previously assumed. Further investigations, as for instance by qRT-PCR and reporter gene fusions are in progress.

References:

[1] Uebe R. and Schüler D. (2016) *Nat. Rev. Microbiol.* 14:621–637.

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## GRP276

### Functional analysis of magnetosome biosynthesis and gradual genome reduction by large-scale deletion mutagenesis in *Magnetospirillum gryphiswaldense*

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In the  $\alpha$ -Proteobacterium *Magnetospirillum gryphiswaldense*, magnetosome biosynthesis is controlled by >30 magnetosome-specific proteins encoded in several operons that are clustered in a single genomic region of ~100 kb, the so-called Magnetosome Island (MAI).<sup>1</sup> The regions flanking and interspersing magnetosome gene clusters are particularly rich in pseudogenes and mobile genetic elements responsible for genetic instability of the MAI. In addition, bioinformatics, transposon mutagenesis and proteomic analysis predicted further auxiliary candidate genes localized outside the MAI that might be putatively involved in magnetosome biosynthesis. In order to optimize expression of magnetosome biosynthesis and to identify the minimal gene set sufficient to sustain this synthesis, we started to systematically delete large regions comprising flanking or interspersing of the MAI. Using RecA-mediated homologous recombination, regions up to 65 kb could be deleted, thereby eliminating large parts of irrelevant or problematic gene content. All deletions comprising the known magnetosome clusters were impaired in magnetosome biosynthesis, whereas elimination of flanking regions had no effect on the magnetosome phenotype. Non-magnetic mutants grew faster compared to the wild type. In addition, >15 candidate genes with so far unknown functions were deleted outside the MAI. However, none of these mutants had a discernible effect on magnetosome biosynthesis, growth and cell morphology. Based on this results, we expect an enhanced understanding of genetic complexity of magnetosome biosynthesis, and systematic large-scale genome reduction

may help to increase genetic stability, growth and magnetosome expression in *M. gryphiswaldense*.

References:

[1] Uebe R., Schüler D. (2016) *Nat. Rev. Microbiol.* 14:621–637.

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## GRP27+7

### The targeted GudB degradation in *Bacillus subtilis*

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**Introduction:** *Bacillus subtilis* 168 encodes two glutamate dehydrogenase, RocG and the intrinsically inactive GudB. Inactivation of GudB is caused by a duplication of three codons in the catalytically active center (Belitsky *et al.*, 1998). Interestingly, GudB was found modified by a remarkable high number of arg-Ps (Elsholz *et al.*, 2012; Schmidt *et al.*, 2014) and it was suggested that arg-P serve as degradation tag (Trentini *et al.*, 2016).

**Objectives:** The aim of this study was to examine the role of arg-Ps for GudB degradation and to identify the proteolytic system.

**Materials and Methods:** Previously identified GudB arg-P sites were substituted by site-directed mutagenesis (R-K). Radioactive pulse-chase labelling and immunoprecipitation were utilized to follow the fate of GudB in different isogenic mutant strains or R-K substituted mutants. Furthermore, Western-blot analyses were performed to estimate the amount of GudB in the cytosolic and aggregate protein fraction.

**Results:** First of all, the previously described destabilizing effect of McsB on GudB was confirmed by radioimmunoprecipitation. Furthermore, ClpCP was found involved in the degradation of GudB. Subsequently, putative argP-sites were substituted against lysine (R-K;) and tested for their influence for proteolysis. Surprisingly, selected R-K mutations of GudB revealed only a slight stabilization effect suggesting a fine-tuned role of arg-P for substrate recognition. Nevertheless, inactive GudB appears constitutively synthesized and proteolyzed during all growth phases.

**Conclusion:** Our results show that arginine kinase McsB orchestrates GudB degradation in the regulated Clp-dependent proteolytic network of *B. subtilis*.

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## GRP278

### *yfeS* mutant of *Escherichia coli* is comparatively hypersensitive to boric acid and may play a role in boron stress response

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**Introduction:** Boron is an important semi-metal in biological life forms. While being essential or beneficial at low concentrations, it becomes toxic and harmful at higher levels. Thus, excess boron causes cellular stress and the organisms must employ specific molecular players to combat such threats in order to survive. The objective of the study is to

determine hypersensitive mutants to boric acid. The finding would indicate direct or indirect involvement of the particular gene with the phenotype of boron tolerance-sensitivity.

**Methods:** We conducted screens using a number of 114 mutants under different boron concentrations. Following screens, spot tests, MIC determinations and growth curves were also applied to confirm the results. Additionally, complementation studies were performed to link the phenotype to the specific gene in question.

**Results:** *E.coli* wild type cells were shown to tolerate up to 100-120mM boric acid. However, the results repeatedly showed that the growth of *yfeS* mutant was inhibited by 50mM boric acid compared to the wild type. Additional results obtained from the two other experimental approaches confirmed the tolerance levels. *yfeS* mutant and the wild type have the MIC values of 50mM and 100mM boric acid, respectively. The complementation of the *yfeS* mutant by a plasmid-encoded *yfeS* gene restored the ability of the mutant to grow in higher concentration of boron.

**Conclusion:** The function of this protein is unknown and to our knowledge, no publications exist regarding this gene in the literature. Therefore, we propose for the first time that the novel activity of YfeS is potentially involved in boron sensitivity-tolerance in bacteria.

**Acknowledgement:** We thank the Scientific and Technological Research Council of Turkey (114Z987) for support.

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#### GRP279

##### Reengineering of a synthetic toehold switch for posttranscriptional activation of gene expression in *Synechocystis* sp. PCC 6803

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Cyanobacteria show a wide range of developing tools concerning environmental questions. In the context of synthetic biology, which gives new opportunities in customizing living organisms with artificial tools, we gain a huge amount of possibilities.

Riboregulators, which offer diverse functions, have been designed synthetically for various applications. Their operating principle is based on binding to a signal nucleic acid molecule by base pairing. Thus, they are able to regulate positively/negatively i.e. in a posttranscriptional manner. However, despite the abundance of natural riboregulators in cyanobacteria, there are few synthetic devices published.

Based on the toehold switch system originally published by Green et al. in 2014 for *E. coli*, which is characterized by its high dynamic range and strong positive regulation, we modified one of their toehold devices to be investigated in *Synechocystis* sp. PCC 6803.

Unlike the Green et al. system, the trigger sRNA is controlled by the Co2+ inducible promoter PcoaT. In order to use this promoter, 5'RACE was performed to map its transcriptional start site. In addition to the original construct, we added three other slightly modified versions of the device for optimization of the RBS.

The devices were analyzed using fluorescence, Northern Blot and qPCR measurements.

Our preliminary results show that this device is able to be switched on in *Synechocystis*. Strikingly, the different modifications showed very different behavior regarding their fluorescence.

According to these results, toehold sequences for *Synechocystis* could be further optimized, provided that further tools such as strongly inducible promoters are developed for cyanobacteria.

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#### GRP280

##### *Burkholderia glumae* CRISPR-Cas gene cluster expression depends on quorum-sensing

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*Burkholderia glumae* PG1 (BGPG1) is a plant pathogenic  $\beta$ -proteobacterium that infects rice plants in the flowering stage. Since the organism produces highly versatile lipases and other secreted enzymes it is of high relevance to biotechnology. Recently, we have established the genome sequence of this organism. Interestingly, we found complete CRISPR-Cas gene cluster of the I-F (*Yersinia pestis*) subtype within the BGPG1 genome, whereas other *Burkholderia* species carry no or only incomplete CRISPR-Cas systems. Using RNA-seq we were the first to observe that the expression of the *cas/csy* genes is regulated in a quorum sensing-dependent manner. Thereby it is notably that BGPG1 codes for three autoinducer (AI) synthases (*bgal1-bgal3*), which are all involved in the synthesis of N-acyl-homoserine-lactones (AHLs) with varying chain length. This is an unusual feature within the species *B. glumae*, since the genomes of all other currently sequenced strains contain only a single AHL synthase gene. Mutations in each of the three loci resulted in the up to 10-fold altered expressions of the respective *cas/csy* genes as well as some of the several CRISPR arrays in BGPG1.

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#### GRP281

##### Identification and analysis of mRNA levels of an antiporter, excinuclease subunit and endonuclease genes in response to boron in a *Pseudomonas* isolate

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**Introduction:** Presence of high levels of boron in different parts of the world is harmful to life and hence an economic and scientific issue. Identification of bacteria and genes is an important step in finding solutions. Boron rich soils and mines enable us to obtain model bacterium isolates and quest for the genes within. In this study, a boron tolerant bacterium isolated from a boron mine in Turkey was used and the initial studies have set forward three candidate genes that may be utilized by the bacterium to grow in such harsh conditions of extreme boron levels. We also aimed to investigate the regulation of these genes in response to availability of increasing levels of boron by analyzing mRNA levels using qPCR analyzes.

**Methods:** Three candidate genes were identified using gene libraries and enrichment. The primers were designed for the genes. The isolate was grown to early logarithmic phase and exposed to 0, 50 and 150mM boric acid for 10, 30 and 60 min. The cells were harvested and total RNA was isolated (Ambion). cDNAs were synthesized (ThermoScientific). Real

Time PCR analyzes were performed using SYBR Green PCR mix in a Roche 480 II instrument. Fold changes were calculated based on Ct values.

**Results:** We find that mRNA levels of Ca<sup>2+</sup>/Na<sup>+</sup> antiporter, excinuclease ABC subunit B and endonuclease III are differentially regulated in the *Pseudomonas* isolate by varying levels of boron.

**Conclusion:** We propose that the antiport membrane protein may be used in getting rid of excess boron, while the latter two activities may be employed in protecting the cell against DNA damage caused likely by oxidative stress probably originated from excess boron.

**Acknowledgement:** We thank the Scientific and Technological Research Council of Turkey (114Z987, 112T614)

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## GRP282

### The HTH-type regulator MalT is a transcriptional activator of a large genomic region in *Actinoplanes* sp. SE50/110

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Acarbose is used in the treatment of diabetes mellitus type II and is produced by *Actinoplanes* sp. SE50/110 [1]. Although the biosynthesis of acarbose is intensively studied, profound knowledge about transcription factors involved in acarbose biosynthesis and their binding sites is missing until now. In contrast to acarbose biosynthetic gene clusters in *Streptomyces* spp. [2,3], the corresponding gene cluster of *Actinoplanes* sp. SE50/110 lacks genes for transcriptional regulators. In this study, we identified the transcriptional regulator MalT as an activator of some acarbose biosynthesis genes. Furthermore, MalT is involved in the activation of a large genomic region around the *malT* gene itself.

The transcriptional activator MalT was identified through the analysis of co-transcribed genes. Bioreactor cultivations were conducted to maintain controlled cultivation conditions and microarrays were used in a high temporal resolution to study transcriptional changes during growth. A hierarchical cluster analysis of the data was performed to elucidate co-transcribed genes, which were discussed and analyzed for conserved regulatory sequences. It could be shown, that the acarbose biosynthesis genes *acbD* and *acbE* were co-transcribed with the genes surrounding *malT*.

The gene for *malT* was deleted using CRISPR/Cas9 [4] and characterized through cultivation experiments, genome-wide microarray hybridizations, and RT-qPCR as well as an *in-silico* binding motif analysis.

This study characterized a further transcription factor of the acarbose biosynthetic genes *acbD* and *acbE* in *Actinoplanes* sp. SE50/110 [5] and is an outstanding example for the transcriptional regulation of large genomic regions in bacteria.

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## ePoster Session 2

### Biochemistry (BCP)

17 April 2018 • 14:30–17:00

#### BCP024

##### Aldehyde:ferredoxin oxidoreductase (AOR): purification, characterisation and maturation factors

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Tungsten-dependent aldehyde:ferredoxin oxidoreductases (AOR) are able to directly interconvert aldehydes and the corresponding carbonic acids. In addition to the previously known archaeal AORs [1], similar enzymes are also present in bacteria, but differ in their subunit composition and other properties. In preliminary work, AOR from the betaproteobacterium *Aromatoleum aromaticum* was purified and found to consist of three subunits. It forms a complex consisting of two AOR-type subunits carrying a *W-bis*-MPT cofactor and an Fe-S cluster, two electron transfer subunits carrying four Fe-S clusters and one FAD-containing subunit. The corresponding genes are organized in an operon [2]. This structure resembles that of a previously reported orthologue from *Moorella thermoacetica* which still lacks thorough characterisation because it could not be sufficiently purified [3]. This was also initially observed with *A. aromaticum*, but AOR can now be reproducibly purified in significant amounts from a constructed mutant which depends on this enzyme for anaerobic Phe degradation [4]. The project is centered around two major questions: (i) the biochemistry of bacterial AOR itself, including the catalytic properties, structure-function relations, functional relevance of the additional subunits, Fe-S-clusters and the reason of its unusual oxygen resistance and (ii) the maturation pathway of the *W*-cofactor in AOR-type enzymes.

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### BCP025

#### Understanding the Role of HypD in Diatomic Ligand Biosynthesis of NiFe-Hydrogenases

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[NiFe]-hydrogenases are enzymes that reversibly activate hydrogen. They have a NiFe(CN)<sub>2</sub>CO-cofactor whereby the iron ion ligates the diatomic ligands. Six conserved Hyp proteins are needed to synthesize the cofactor. HypD is an FeS-cluster-containing protein with a central role in biosynthesis of the Fe(CN)<sub>2</sub>CO group of the cofactor. The CN<sup>-</sup> ligands are derived from carbamoylphosphate and current evidence suggests that the CO ligand is generated from endogenous CO<sub>2</sub> bound to an iron ion coordinated by the HypD-HypC complex; HypC and its paralogue HybG are chaperones suggested to deliver the Fe-CO<sub>2</sub> moiety to HypD. The nature of the electron donor to HypD is unknown and our aim, therefore, is to determine whether Fdx is a candidate electron donor to HypD. To study this, we have cloned and over-expressed the *fdx* gene and the *hypDEF-hybG* genes and purified Fdx and the HypD-HybG<sub>strep</sub> complex anaerobically from *E. coli* strains BL21 (DE3) and MC4100. These purified proteins were then analyzed by UV/Vis, Raman and infra-red spectroscopies. It was possible to identify signals corresponding to the CN<sup>-</sup> and CO ligands, as well as the FeS cluster in the HypD protein. The influence on the ligand signals of adding reduced ferredoxin provided initial evidence that Fdx might indeed act as an electron donor in NiFe-cofactor synthesis. Using these spectroscopic methods, we aim to determine how the HypD-HybG complex synthesizes the CO ligand, what the order of diatomic ligand addition is and how the complex transfers the Fe(CN)<sub>2</sub>CO group into the active site of the apo-hydrogenase.

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### BCP026

#### Hydrogen-Dependent Organohalide Reduction Without Quinone-Involvement by a Soluble Multiprotein Complex from *Dehalococcoides mccartyi* Strain DCMB5

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*Dehalococcoides mccartyi* belongs to the phylum *Chloroflexi* and conserves energy exclusively by H<sub>2</sub>-dependent organohalide reduction. The bacterium is remarkable because it performs electron transport phosphorylation without quinone involvement. Recently we identified a protein complex isolated from the membrane of strain CBDB1 that catalyses H<sub>2</sub>-dependent reduction of 1,2,3,-trichlorobenzene

(1,2,3-TCB) *in vitro*. To determine whether such a respiratory complex is common to other *D. mccartyi* strains, we analyzed H<sub>2</sub>-dependent 1,2,3-TCB reducing activity in solubilized membrane fractions from strain DCMB5. Combined size-exclusion chromatography, in-gel hydrogenase activity-staining, immunological analysis and mass spectrometry identified a large molecular mass protein complex that catalyzed this reaction. A strong correlation was observed between H<sub>2</sub>-dependent 1,2,3-TCB reduction and the presence of a catalytic subunit of an iron-sulphur molybdoenzyme (OmeA), the chlorobenzene-specific reductive dehalogenase, CbrA, and the NiFe-hydrogenases HupLSX and VhuAB. A particularly strong correlation between electron-transfer activity and the ferredoxin-like protein HupX and the integral membrane protein OmeB was observed. Together with the other components of this complex, OmeB and HupX are proposed to be crucial for the postulated proton translocation activity of the complex. Our data reveal that this unusual protein-based electron-transfer complex is widespread among *D. mccartyi* strains.

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### BCP027

#### Nitrogenase-like biosynthesis of bacteriochlorophylls

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**Introduction:** The biochemistry of nitrogenase plays a fundamental role for the formation of chlorophylls (Chls) and bacteriochlorophylls (Bchls). Biosynthesis of Chls and Bchls requires the stereospecific reduction of the C17-C18 double bond of ring D of protochlorophyllide which can be catalyzed by the nitrogenase-like enzyme dark-operative protochlorophyllide oxidoreductase. The biosynthesis of all Bchls requires a second nitrogenase-like enzyme termed chlorophyllide oxidoreductase (COR) for the stereospecific reduction of the C7-C8 position of chlorophyllide. It was demonstrated that the COR enzyme from *Rhodospseudomonas palustris* additionally catalyzes the reductive 1,2 protonation at C8<sup>1</sup>-C8<sup>2</sup>. The COR enzyme from *Hellobacterium modesticaldum* is able to perform an alternative 1,4 protonation at C7-C8<sup>2</sup>.

**Objectives:** Crystallization and mechanistic understanding of COR enzyme. Engineering of a chimeric nitrogenase reductase.

**Materials and methods:** Anaerobic protein production and purification, crystallization screening, activity assays, mutagenesis, protein interaction.

**Results and conclusion:** We established the production and purification of the COR subcomplexes X<sub>2</sub> and (YZ)<sub>2</sub> from the two organisms and characterized the respective proteins using spectroscopic approaches and activity assays. Proteins have been subjected to initial protein crystallization experiments. Furthermore, we have established a production and purification system for an engineered L<sub>2</sub> protein containing a docking interface which mediates the interaction with the catalytic component of nitrogenase.

## BCP028

### Structure and function of novel bilin biosynthesis enzymes from marine metagenome

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All functional light-harvesting or light-sensing bilin chromophores are derived from heme. The heme oxygenase (HO) catalyzed cleavage of heme at the  $\alpha$ -carbon position yields the first open-chain reaction product biliverdin IX $\alpha$  (BV). BV is further reduced by ferredoxin-dependent bilin reductases (FDBRs) to the specific pigments. During a deep screening of the VirMic dataset, a new family of putative FDBRs (designated PcyX) as well as a new family of HOs (designated HemO) was discovered that each group together as a new branch in a phylogenetic tree. The VirMic dataset [1] includes scaffolds from the "global ocean sampling expedition" [2] that are considered to be of viral origin but contain microbial gene clusters. In order to determine whether HemO and PcyX are functional enzymes, synthetic genes were expressed in *E. coli*. Assays with affinity purified proteins and the respective substrates showed that both are functional enzymes. We were able to identify the reaction product of HemO as BV and established that PcyX is a FDBR catalyzing the reduction of BV to phycoerythrobilin via the intermediate 15,16-dihydrobiliverdin. To gain insights into the PcyX reaction mechanism the crystal structure was solved at a resolution of 2.2 Å. Furthermore, mutagenesis experiments as well as electron paramagnetic resonance measurements were performed. These experiments revealed that PcyX adopts an  $\alpha/\beta/\alpha$ -sandwich fold typical for all FDBRs. Moreover, we were able to show that the reaction proceeds through a radical mechanism and identified a conserved histidine-aspartate pair as crucial for the activity of the enzyme.

#### References:

[1] Sharon, I., et al. (2011) *The ISME journal*, 5(7), 1178-1190.

[2] Rusch, D. B., et al. (2007) *PLoS biology*, 5(3), e77.

## BCP029

### Reaction mechanism of corrinoid-reducing metallo-ATPases

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**Introduction:** Acetogens like *Acetobacterium dehalogenans* can use phenyl methyl ethers as carbon and energy source. In the methylotrophic metabolism, the corrinoid cofactor of O-demethylase corrinoid proteins (CP) plays an important role as methyl group carrier. Due to the low redox potential of the [Co<sup>II</sup>]/[Co<sup>I</sup>]-couple, the inactive [Co<sup>II</sup>]-form of the corrinoid cofactor can be formed as a result of autoxidation. The reactivation to [Co<sup>I</sup>] is catalyzed by a RACE protein (reductive activator of corrinoid enzymes), named activating enzyme (AE) in *A. dehalogenans*. This metallo-ATPase couples the exergonic hydrolysis of ATP to the unfavorable electron transfer to [Co<sup>II</sup>]-CP for its reduction to active [Co<sup>I</sup>]-CP.

**Objectives:** The aim is to elucidate the reaction mechanism of the ATP-dependent corrinoid reduction catalyzed by AE.

**Material & Methods:** The redox potential of the protein-bound corrinoid cofactor was determined via redox titration in the absence or presence of AE. Ti(III) citrate or Eu(II) chloride were used as electron donors and were stepwise added to the sample prepared in 50 mM Tris-HCl pH 7.5. In parallel to the measurement of the redox potential, UV/Vis spectra were recorded.

**Results:** We determined the midpoint potential of the protein-bound corrinoid cofactor. The titration behavior led to the assumption that conformational changes in CP, in its cofactor and/or AE occur during reductive activation. We were also able to crystallize a complex of CP and AE without and with AMP-PNP, a non-hydrolyzable ATP analogue. The crystals will be used for structural analysis.

**Conclusion:** Based on the new results, a tentative reaction scheme for ATP-dependent corrinoid reduction catalyzed by AE was developed.

## BCP030

### Structure of a Unique 3-hydroxyacyl Dehydratase (FabZ) and its Complex with an Acyl Carrier Protein (ACP) from Anammox Bacteria

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Ladderane lipids are found exclusively in the membranes of anaerobic ammonium-oxidizing (anammox) bacteria. These lipids contain highly unusual hydrocarbon moieties consisting of three or five linearly fused cyclobutane rings which are linked via a glycerol backbone to polar headgroups that are also present in common phospholipids (Sinninghe Damsté, 2002; Boumann, 2006). Several gene clusters putatively involved in ladderane biosynthesis were put forward based on genome analyses (Rattray, 2009). One of these putative operons, which encodes enzymes of canonical bacterial fatty acid biosynthesis (FASII) as well as an S-adenosylmethionine (SAM) radical protein, is most highly conserved among different anammox genera. We heterologously expressed several of these genes including an acyl carrier protein (amxACP) and a unique 3-hydroxyacyl-(ACP)-dehydratase (amxFabZ). We determined the structure of amxFabZ from the anammox organism *Scalindua brodae* at 1.8 Å resolution by X-ray crystallography. Like other FabZ homologues this protein assembles into a trimer of dimers. Preliminary biochemical assays with amxACP-bound 3-hydroxy fatty acids confirmed the dehydratase activity of amxFabZ. To further elucidate whether the active site of amxFabZ could accommodate lipid intermediates containing one or more cyclobutane rings we formed a complex of amxFabZ with an amxACP-bound cyclobutane-containing fatty acid *in vitro*. Crystals of this complex diffracted to 3.5 Å and revealed an amxFabZ hexamer bound to six amxACP molecules.

### BCP031

#### Biophysical properties of TsdA from *Allochrochromatium vinosum* and *Campylobacter jejuni*

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Thiosulfate dehydrogenases (TsdA) are periplasmic c type diheme cytochromes which catalyse thiosulfate oxidation and tetrathionate reduction [1]. The preferred reaction directionality varies dependent on the source organism [1,2]. TsdA from *Allochrochromatium vinosum* primarily oxidises thiosulfate [1], whereas TsdA from *Campylobacter jejuni* is biased towards tetrathionate reduction [2]. TsdAs exhibit an unusual His/Cys ligation at the active site heme [1]. Heme 2 is ligated by His164/Lys208 in oxidised AvTsdA crystals [1]. In CjTsdA, Heme 2 exhibits His207/Met255 ligation [2].

To get further insight into their biochemical and biophysical properties, we analysed Av and CjTsdA and variants thereof. Substrate binding was studied for CjTsdA variants in which a positively charged amino acid residue residing in the potential substrate binding pocket, Arg134, was exchanged by Ala, Gln or Lys. Enzyme activity drastically decreased in both catalytic directions for all variants, especially when the distal ligands of Hemes 1 or 2, Cys138 or Met255, were additionally replaced by glycine. This indicates that Arg134 plays a pivotal role in stabilisation of the negatively charged substrate thiosulfate. Both TsdAs were analysed by protein film electrochemistry using graphite electrodes. While CjTsdA displayed the expected form of catalytic waves at pH 5 as well as at pH 7, AvTsdA worked well at pH 5 but appeared to lose activity over time at pH 7. Addition of tetrathionate or sulfite to electrode-bound AvTsdA resulted in inhibition of thiosulfate oxidation. Micromolar concentrations of sulfite proved inhibitory and primarily increased the S0.5 value of the enzyme, indicating a competitive mode of inhibition.

#### References:

[1] Brito *et al.* (2015) JBC 290, 9222

[2] Kurth *et al.* (2015) JACS 137, 13232

### BCP033

#### New insights into the catalytic mechanism of ATP-dependent ketone carboxylation

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Abstract has been withdrawn.

### BCP034

#### Reconstitution of a proteolytically active Clp complex of streptomycetes *in vitro*

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Clp protease is one of the major degradation machineries in eubacteria. Clp is composed of a proteolytic core, ClpP, which acts in concert with specific hexameric unfoldases, the Clp-ATPases, in the turnover of misfolded proteins and regulatory proteolysis. ClpP assembles into two stacked heptameric rings with the proteolytic chamber secluded

inside the assembled tetradecamer [1]. The access to the proteolytic chamber is tightly controlled by Clp-ATPases, which recognize Clp substrates and unfold them in an ATP-dependent manner [2]. In contrast to firmicutes, which encode only one non-essential *clpP*, streptomycetes rely on a functional Clp system for viability and contain up to five *clpP* genes organized in two bicistronic and one monocistronic operons [3].

So far, knowledge on the interaction of the Clp proteins from streptomycetes is rare, and a functional Clp system has not been achieved to be reconstituted *in vitro* which may be owed to its increased complexity. To shed light on the interaction and functional cross talk between the ClpP proteins, we expressed the ClpP proteins and the ClpP-ATPases in *Escherichia coli* and purified them via anionic exchange or affinity chromatography. Studying the purified Clp proteins in *in vitro* activity assays, including degradation assays using casein as a substrate, we could for the first time reconstitute a proteolytically active Clp system from streptomycetes *in vitro* which is an essential step forward to decipher the composition and function of these complex proteolytic machineries in detail.

#### References:

[1] Wang *et al.* (1997) *Cell*, 91: 447-456

[2] Sauer & Baker (2011) *Annu. Rev. Biochem.*, 80: 587-612

[3] Viala *et al.* (2000) *Mol. Microbiol.*, 38(3), 602-612

### BCP035

#### New enzymes discovered in anaerobic metabolism of phenylalanine and aromatic amines in the denitrifying betaproteobacterium *Aromatoleum aromaticum*

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Abstract has been withdrawn.

### BCP036

#### Uncommon Mechanism of a PKS II-derived Aryl Polyene Pigment produced by *Xenorhabdus doucetiae*

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**Introduction:** A widely distributed class of bacterial polyketides are arylpolyene (APE) pigments. Recently, APE biosynthetic gene clusters (BGCs) were shown to be one of the most common BGCs in Gram-negative bacteria. Nevertheless, the biological function and biosynthesis of APE are not yet fully elucidated.

**Objectives:** By heterologous expression of the APE gene cluster from *X. doucetiae*, APE compounds were produced and identified by HPLC-MS and their structure were confirmed by feeding experiments. Enzymes involved in the biosynthesis were isolated and an *in vitro*-assay was established. The protein bound intermediates were identified by HPLC-MS (Pant-ejection assay). Protein interactions were investigated by pull-down-experiments and ESI-MS experiments of the intact protein complexes.

**Results:** One of the yellow APE pigments was identified, thus it was confirmed that these genes are sufficient for APE production. By Pull-down-experiments stable heterodimeric complexes were identified (KS, CLF, ACP, DH, TE) by measuring the intact proteins with ESI-MS. We were further able to reconstitute the APE biosynthesis *in vitro* by analyzing the ACP-bound intermediates in each step of the biosynthesis and could detect different APE chain-length. Interestingly different chain-length were produced by using different combinations of KS and/or CLF.

**Conclusion:** The uncommon biosynthetic mechanism of the APE pigment was elucidated *in vitro* being the basis for future research on this highly interesting compounds. It was further shown that there exist other stable protein-protein interactions, beside the "normal" KS/CLF-interaction, in PKS II systems.

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## ePoster Session 2

### Food Microbiology (FMP)

17 April 2018 • 14:30–17:00

#### FMP204

##### Proficiency-testing scheme for Hepatitis A, Norovirus GI and Norovirus GII in strawberry

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Norovirus GI, Norovirus GII and Hepatitis A are the leading causative agent of foodborne disease outbreaks worldwide. The number of laboratories detecting these viruses has gradually increased in recent years to answer the growing demand of food routine controls. However, due to their low infectious doses and low concentrations in food samples, these analyses are a challenge for the laboratories, which need to prove the reliability of their results to obtain recognition of their analytical procedures by costumers and accreditation bodies. To meet this demand, BIPEA organizes regular proficiency-testing schemes (PTS) for the analysis of viruses in food. In July 2017, a PT was conducted on strawberries contaminated with Noroviruses GI and GII and Hepatitis A at 4 different levels of concentration. Samples were prepared by spiking strawberries, free from any viruses, with suspensions of Noroviruses GI and GII and Hepatitis A in well controlled proportions. According to the requirements of the ISO 13528, the homogeneity and the stability of the samples was verified by experimental studies. The participating laboratories were required to analyze samples according to the standard methods or to the alternative ones and were invited to return their results on a dedicated website. The statistical treatment of the data was performed according to ISO 13528. Assigned values were calculated from the participants' results and the performances of the laboratories could be evaluated individually and collectively according to ISO 17043. This PT allowed laboratories to draw up a general inventory of their analytical skills and was a very useful tool to verify the reliability of their results as well as to detect bias or non-compliant results for each tested concentration of viruses.

#### FMP205

##### Comparison of *Vibrio cholerae* non-O1, non-O139 from aquatic environments and from seafood in Germany with clinical strains

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Non-toxicogenic *Vibrio cholerae* bacteria are present in German coastal waters and can be isolated from seafood. Most of the bacteria lack the cholera toxin gene (*ctx*) and the genes for the toxin-coregulated pilus (*tcp*). They are collectively designated non-O1, non-O139 strains. However, some strains can cause gastroenteritis and extraintestinal infections. In the present study, we investigated German isolates to find out if some of these isolates could pose a risk for public health.

We selected 100 environmental strains from the North Sea and Baltic Sea and 30 isolates from seafood. The strains were characterized by MLST and examined for the presence of cholera toxin gene and other virulence-associated factors including hemolysins, RTX toxins, pandemic islands and type III secretion system. Phenotypic assays for hemolytic activity were also performed.

Genotyping results showed that none of the isolates contained the cholera toxin (*ctxA*) and genes of the *ctx* associated element as well as those for the toxin-coregulated pilus. The presence of other toxins showed a strain specific pattern. Based on MLST analyses, the phylogenetic relationship of strains was characterized. Nearly all strains showed clear hemolytic activity against human and sheep erythrocytes.

Our study indicates the need for continued surveillance of *Vibrio* spp. in Germany as *Vibrio* infections are predicted to increase due to global warming.

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#### FMP206

##### Monitoring the microbial status of raw and pasteurized milk from milk filling stations in Brandenburg, Germany

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In Germany, ready-to-consume milk may only be offered as certified raw milk ("Vorzugsmilch") or heat-treated milk. On the other hand, raw milk can be delivered directly from dairy farms' milk filling stations to the final consumer. However, this raw milk is not intended for direct consumption. Milk filling stations are an increasingly popular form of milk marketing for direct sellers offering raw milk or pasteurized milk. The number of milk filling stations in Brandenburg has almost tripled in 2017.

Since September 2017, more than 100 raw milk and pasteurized milk samples from 19 milk filling stations have been examined. It was shown that in about one third of all samples selected reference values for the total bacterial count were clearly exceeded. Increased bacterial counts of *Enterobacteriaceae* were detected in about 50% of the samples. In order to assess a health risk under the assumption of direct consumption, the raw milk samples were additionally examined for *Salmonella*, VTEC, *Campylobacter* and *Listeria monocytogenes*. Pathogens were detected in about 25% of these samples.

Furthermore, the dominant microorganism flora from the total bacterial count as well as technical details of the machines and the cleaning and disinfection processes were surveyed, in order to identify sources of contamination and to give advice to the farms with regard to their milk and milk vending machines.

From this data, problems with milking hygiene, interruptions of the cold chain or faults in the cleaning and disinfection processes of the milk vending machines can already be deduced. Thus, countermeasures were indicated and will be monitored in the upcoming months.

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#### FMP207

##### Microbiological analysis of packaged lamb's lettuce for total viable count, yeasts and total coliforms

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Packaged fresh-cut vegetable can be purchased in many supermarkets. Since these salads are only minimally processed, raw consumption can pose a health threat due to the potential contamination by human pathogens. The objective of this study is to investigate the microbial load of two different packaged fresh-cut lamb's lettuce varieties and the antimicrobial susceptibility of isolated *Escherichia coli*. A total of 10 samples - including 5 samples packaged in protective plastic bowls and 5 samples packaged in plastic bags - were checked quantitatively for total mesophilic aerobic counts, yeasts and coliform counts with the spread plate method. Determination of *E. coli* was performed with biochemical analysis in order to examine the antimicrobial susceptibility of *E. coli* which was carried out using the standardized disk diffusion technique. A high microbial load was shown in all analyzed lamb's lettuce samples. Microbial counts result in up to  $9,0 \times 10^8$  colony forming units per grams (cfu/g) for aerobic mesophilic germs,  $1,0 \times 10^8$  cfu/g for yeasts and  $1,4 \times 10^5$  cfu/g for total coliforms. Total mesophilic aerobic counts are up to eighteen times higher and yeast counts are up to a thousand times higher than reference values published in 2014 by the German Society for Hygiene and Microbiology (DGHM). No resistances to the tested antibiotics could be determined for the isolated *E. coli*. Conclusively, additional investigations are necessary to get a comprehensive overview about the specific microflora of raw eaten food such as salads. Experiences in this field may then serve later as a prerequisite to recommend hygiene measures along the food production chain.

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#### FMP208

##### Towards a functional CRISPRi system for *Listeria monocytogenes*

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*Listeria monocytogenes* (*Lm*) is an important human, food-borne pathogen and a model organism for intracellular pathogenesis. Important genetic features relevant to food safety and human health including biofilm formation and virulence are well characterized in *Lm*. Although a wide range of molecular tools are available and *Lm* is genetically well accessible, analysis of essential genes is still challenging. Here, we describe a system for CRISPR

interference (CRISPRi) for conditional repression of gene expression in *Lm*.

For conditional gene silencing, the plasmid pMZ-dCas9 was cloned. This plasmid allows single copy, stable chromosomal integration strictly controlled, anhydrotetracyclin (Atc)-inducible expression of the *Streptococcus pyogenes*-derived dCas9 protein in a dose-dependent manner. In combination with plasmid-based, Atc-inducible expression of single guide RNAs (sgRNAs) efficient and target-gene specific transcriptional repression can be achieved. Functionality of the system was shown by a reduction in expression of *secA2* by 99% following Atc-induced expression of dCas9 and a *secA2* specific sgRNA in *Lm* ScottA. Reduced levels of *secA2* mRNA were accompanied by increased chain formation in exponential growth phase suggesting a defect in cell separation and resembling the phenotype of a *secA2* deletion mutant. Similar observations were made using a sgRNA targeting *iap*, a gene with a *secA2*-like phenotype. Interestingly, no difference in target gene repression was observed between *Lm* EGDe and ScottA despite the fact that strain EGDe harbours genes for two recently described antiCRISPR proteins.

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#### FMP209

##### Bacterial diversity of high bacterial count raw cows' milk from the bulk tanks of different dairy farms in Germany

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The bacterial load of raw milk directly affects quality and shelf life of the processed milk and dairy products. Psychrotrophic bacteria pose a serious problem because they are able to grow rapidly under cold storage and contribute to spoilage by proteolytic or lipolytic activity. In this study, we characterized the dominating contaminants from 48 raw milk samples with high microbial load ( $>100.000$  cfu/mL) from different dairy farms. Total bacterial counts and bacterial diversity was determined by a cultivation approach at 30°C and 10°C. Additionally, diversity was determined by culture-independent analyses of 16S rRNA sequences from DNA extracts. Bacterial diversity of raw milk samples was calculated with Shannon-Weaver and Shannon-Evenness indices. The cultivation approach was used to assess the product spoilage potential of the isolates based on their proteolytic and lipolytic activity.

Results of the culture-dependent and -independent approach revealed two different types of raw milk microbiota: microbiota with low diversity and dominating bacterial taxa and microbiota with higher diversity and without dominating bacterial taxa. As dominating taxa of the first group, species of the phyla Gammaproteobacteria and Firmicutes were identified. The psychrophilic Gammaproteobacteria showed spoilage potential by lipolytic or proteolytic activity at 10°C and/or 4°C and were able to grow rapidly under cold conditions. The mesophilic species of the phylum Firmicutes were identified as potentially pathogenic bacteria, known to cause mastitis in dairy cows. In this study, we demonstrated that both groups, psychrophilic and mesophilic bacteria could represent the dominating contaminant in raw cows' milk from bulk tanks.

## FMP210

### Milking machine biofilms: Bacterial community composition and prevalence of antibiotic resistance

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Biofilms on milking machines are a source of contamination of raw milk and its products. Moreover, biofilms can facilitate the transmission of mastitis pathogens within herds. Due to the close contact of bacterial cells within a biofilm, we analyzed the relation between the density of bacterial populations and the abundance of antibiotic resistance genes to reveal the impact of biofilms on horizontal gene transfer.

In this work, swab samples of different parts of the milking machine of a dairy farm were investigated by culture-dependent and -independent methods. Spots in the milking system with enhanced microbial colonization were identified by colony counting on selective and non-selective media. The fraction of antibiotic resistant cells was quantified on media containing different  $\beta$ -lactams and tetracycline. Isolates were identified by 16S rRNA sequencing to assess the bacterial diversity and to identify dominating bacterial groups and antibiotic resistant isolates. DNA was extracted directly from each swab sample and different groups of antibiotic resistance genes were quantified in these extracts by RT-qPCR.

Different parts of the milking machine displayed high biofilm cell density. A high bacterial diversity, also of antibiotic resistant strains, was detected for the phyla *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria*. Different antibiotic resistance genes were detected and quantified by RT-qPCR and correlated with bacterial densities at the sampling points.

Differences between cultural vs. molecular microbial counts will be elucidated. The impact of bacterial cell density on the abundance of resistant cells and antibiotic resistance genes will be discussed.

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## FMP211

### Increased isoprenoid quinone concentration modulates membrane fluidity in *Listeria monocytogenes* at low temperatures

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*Listeria monocytogenes* is well known as a food pathogen capable of growing at a broad temperature range, from 50 °C down to refrigerator temperatures. A key function in bacterial survival at low temperatures is the ability to adjust lipid composition in order to maintain membrane fluidity. We analysed fatty acid profiles of eight *L. monocytogenes* isolates from food and two reference strains from culture collections as a function of growth temperature (37°C versus 6°C). Different extent of fatty acid profile adaptation for these *L. monocytogenes* strains indicated the presence of a second adaptation mechanism.

Quantitative analyses of respiratory quinones revealed a clear increase of menaquinone concentrations under low temperature growth conditions for those strains with low adaptive response in their fatty acid profiles. The supporting function of menaquinones in membrane fluidization for *L.*

*monocytogenes* was confirmed by *in vivo* membrane fluidity analyses by measuring generalized polarization and anisotropy with the fluorescent dyes laurdan and TMA-DPH, respectively.

Strains with increased quinone concentration showed an expanded membrane transition phase in contrast to those strains with pronounced adaptation of fatty acid profiles. The correlation between quinone concentration and membrane transition phase expansion was confirmed by suppression of the quinone synthesis. This finding revealed an additional mechanism improving the adaptation to temperature shifts for *L. monocytogenes* strains.

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## FMP212

### Inhibition of isoprenoid quinone synthesis reveals the function of menaquinones in membrane adaptation to low growth temperatures for *Listeria monocytogenes*

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*Listeria monocytogenes* is one of the major food-related pathogens and causes listeriosis. One of the most challenging tasks during growth at low temperatures is maintaining cytoplasmic membrane fluidity by modification of lipid membrane composition. For *L. monocytogenes* the dominating adaptation effect is shortening of fatty acid chain length. However, some strains are known, which showed weaker adaptive response in their fatty acid profiles to low growth temperature. For these strains we could demonstrate an increase in respiratory quinone concentration during growth at low temperatures. The strains showed even a higher quinone content after growth at 6°C than after 37°C, which is contradictory to the supposed reduced respiratory activity at lower growth temperatures.

In this study quinone content for these *L. monocytogenes* strains was lowered by supplementation with aromatic amino acids. This supplement caused a feedback inhibition of the quinone synthesis pathway. For these quinone-reduced *L. monocytogenes* strains *in vivo* analyses of the membrane fluidity by measuring generalized polarization and anisotropy, revealed a change of the transition phase. Artificial reduction of the quinone content resulted in a narrower transition phase of the cytoplasmic membrane. Correspondingly, strains with higher quinone content showed an expanded membrane transition phase. Complementary experiments with vitamin K<sub>1</sub> supplemented dipalmitoyl phosphatidylcholine (DPPC) vesicles confirmed the transition phase modifying potential of menaquinones. The increase of this neutral membrane lipid produced a fluidization of the membrane under low temperature conditions and therefore represents a fatty acid-independent adaptation mechanism to low temperatures.

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## FMP213

### The effect of UV-C radiation between 253.7 nm and 285 nm on food-relevant moulds and yeasts

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In food industry, alternatives to reduce the contamination with moulds and yeasts have been considered particularly important such as UV-C radiation with microbicidal effects. This study investigates the efficiency of wavelengths of

different UV-C radiation sources and its impact on food relevant moulds and yeasts.

The radiation sources used in this study are a mercury lamp (253.7 nm) and LED lamps (255 nm, 265 nm, 275 nm and 285 nm). The test devices were Pearlbeam™ by aqisense, mercury lamp by Orca GmbH and the dosimeter UV Touch by sglux GmbH. Prior to irradiation, 50 µl (equivalent to ~ 100 spores) of the serial dilution of mould and yeast spores from the second subculture were spread on MEA agar plates. The radiation intensity of the different wavelength ranges was adjusted through different distances over time. The radiation time was regulated with a shutter. In the experiment, the moulds *P. brasilianum* and *A. phoenicis* and the yeasts *C. albicans* and *Z. bailii* were used. After irradiation, the agar plates were incubated for 48 h (yeasts) or 72 h (moulds), the number of colony forming units (CFU) was determined and the germination rate and the D90 dose were calculated.

It is evident that the effect of the irradiation and thus the microbicidal effect of the UV-C radiation depend on wavelength and on radiation intensity. Using a higher wavelength, the radiation intensity must be raised to achieve the same microbicidal effect on moulds and yeasts.

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#### **FMP214** **Comparing different media to detect *Pseudomonas aeruginosa* in Water Flow Meter using GreenLight® System**

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A new rapid test device is the GreenLight® 930 series of instruments developed in cooperation by Luxcel Biosciences® and Mocon Inc®. GreenLight® is an assay that can relate oxygen depletion to microbial load using a fluorescent oxygen sensor located at the base of each vial filled with a specific nutrient broth. According to the manufacturers' data this system is capable of determining the Total Viable Count (TVC) within 1-16 hours depending on the initial bacterial load.

A rapid assay based on the GreenLight® system was developed to determine *P. aeruginosa* in water flow meters comparing the results with the traditional plate count method. CFC-, CN (Merck) and Pseudalert®250 (Idexx) were used as *Pseudomonas spp.* selective broths. The GreenLight tubes were filled with 9 ml broth and 1 ml of dilution from 105 to 100 CFU/ml. In addition, water samples were mixed with the broths and were transmitted into the Greenlight® with an incubation temperature of 37 °C and a threshold of 32 µs. Simultaneously, the CFU was determined by plate count. A non-target organism control was performed with a mixture of *S. enterica*, *E. hirae* and *E. coli*.

The results show good correlations (CN: R2 = 0.98, Pseudalert® R2 = 0.99, CFC: R2 = 0.99) with the bacterial load in the range of 101 to 105 CFU/ml. The non-target microorganisms show no influence on the results. The time to threshold differs depending on the bacteria count and the different broths. For example, the sample (102 cfu/ml) with Pseudalert reached the threshold after 20 h and the sample with CN-broth after 12 h. Using predetermined criteria such as fail and pass, the system can be a suitable rapid method for determination of *Pseudomonas spp.* in the field of quality control of water flow meters.

#### **FMP215**

#### **The effect of UV-C radiation in a range of 253.7 to 285 nm on food-relevant gram-negative and gram-positive bacteria**

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In order to prolong shelf life, UV-C irradiation has become more important in the recent years. Especially the UV-C LEDs are gaining importance. UV-C irradiations emitted by LEDs in the wavelength range from 265 to 285 nm and a mercury lamp (253.7 nm) have proven various influences on the mortality rate. The effectiveness of different UV-C irradiation wavelengths has been examined.

Prior to the UV-C irradiation, 50 µl of the serial dilution from the second subculture of cultivated microorganisms (*E. coli*; *S. enteritidis*; *P. aeruginosa*; *E. hirae*; *S. aureus*; *B. subtilis*) were spread on TSA agar plates. The test organisms were irradiated with two (265 and 285 nm) or three (253.7, 265 and 285 nm) wavelengths. The test devices were Pearlbeam™ by aqisense, mercury lamp by Orca GmbH and the dosimeter UV Touch by sglux GmbH. The radiation intensity of the different wavelength ranges was adjusted through different distances over time and a shutter was used for the radiation time. After irradiation, the agar plates were incubated for 24 and 48 h and the number of colony forming units (CFU) was determined according to DIN 10161-1 and the D90 dose was calculated.

First results showed that D90 doses differed among each bacterial species depending on the wavelength. The highest effectiveness to inactivate *E. coli* and *S. enteritidis* was achieved using the mercury lamp and the UV-C LED with 265 nm. *S. enteritidis* showed the highest sensitivity to inactivation via radiation in the group of bacteria. Comparing the effects of the 265 nm and 285 nm LEDs on gram-positive bacteria, the 265 nm LED showed the best performance. In the future, LEDs should be considered as an effective alternative to mercury lamps.

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#### **FMP216**

#### **Advanced qPCR innovations and validation for microbial testing in water and food**

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Abstract has been withdrawn.

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#### **FMP217**

#### **Improvement of cell extraction from filters after bioaerosol sampling**

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Bioaerosols have been reported to act as risk factors for human health, especially in some occupational environments. Therefore, well-characterized sampling methods are required to achieve a reliable assessment of bioaerosol exposure. Thus, the aim of this study was to investigate recovery efficiencies of microorganisms after filtration.

Cells of five different microbial species were applied on polycarbonate (P) filters as well as glass (G) and quartz (Q)

fiber filters. The bioaerosol sampling was simulated and cells were extracted from filters with a paddle blender. The recovery efficiency was determined via total cell counting and quantification of extracted DNA. Scanning electron microscopy (SEM) was performed to investigate detachment of bacteria from the filter surface.

Significant species-specific differences in recovery efficiencies were revealed. The recovery efficiencies ranged from < 1 % to 45 %. For *Pseudomonas nitroreducens* applied onto P-filters it was shown that the low recovery rates were mainly the result of insufficient extraction from the filter and attachment of cells to the extraction bags. SEM pictures revealed bacteria penetrating deeply into the G/Q-fiber filters. However, incubation of exposed G-fiber filters with Proteinase K directly before DNA extraction increased the recovery rates of *P. nitroreducens* cells from < 1 % to 58 %.

Our study clearly shows the necessity to investigate bioaerosol sampling characteristics for efficient monitoring of airborne microorganisms and depicts a well improved method for cell extraction from filters.

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#### FMP218

##### AprA peptidase production of *Pseudomonas* isolates from raw milk

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**Introduction:** Several psychrotolerant *Pseudomonas* species produce heat-resistant peptidases that can cause premature spoilage of UHT milk or milk products. So far the only known extracellular metallopeptidase in *Pseudomonas* is AprA, located in the *aprA-lipA2* operon. The *aprA-lipA2* operon was characterized in *P. fluorescens* as a gene cluster comprising eight genes, which encode for a peptidase (AprA), an inhibitor (AprI), a type I secretion system (AprDEF), two putative autotransporters (PrtAB) and a lipase (LipA2).

**Methods:** NGS was performed to sequence the whole genome of 50 raw milk isolates, belonging to eight *Pseudomonas* species. To determine the proteolytic activity of isolates, strains were grown in 1,5% UHT milk at 6 °C and 10 °C, filtrated and the supernatant was used for an enzyme assay using azocasein as substrate.

**Results:** Sequence analysis revealed species- and partially strain-dependent differences in the existence and localization of the different *aprA-lipA* operon genes leading to a classification into four main operon types. In general, the isolates with the operon structure *aprAIDEF prtAB lipA2* showed significantly higher peptidase activity than the ones with other operon types. The presence of the gene cluster *prtAB lipA2* in the *aprA-lipA* operon commonly lead to a strong increase of peptidase activity with rising temperatures from 6 °C to 10 °C, whereas the effect was weaker in strains lacking *prtAB lipA2*.

**Conclusion:** A clear correlation between *aprA-lipA* operon structure and AprA activity at rising growth temperature was observed for some *Pseudomonas* milk isolates. However, the fundamental regulatory mechanisms on transcriptional or posttranscriptional level need to be elucidated to understand strain-specific AprA expression in *Pseudomonas* spp.

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## ePoster Session 2

### Microbial Cell Biology II (MCBP)

17 April 2018 • 14:30–17:00

#### MCBP370

##### Product formation of an aerotolerant mutant of *Clostridium acetobutylicum* under oxidative stress

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*Clostridium acetobutylicum* is considered as an obligate anaerobe, e.g. oxygen is harmful or lethal to this bacterium. Nevertheless, it is known that it can survive limited exposure to air, and eliminates oxygen or reactive derivatives (ROS) via NADH consuming reactions. The peroxide repressor PerR regulates this defense mechanism and its deletion triggers aerotolerance in this anaerobe by overexpression of several enzymes which detoxify O<sub>2</sub>- (desulfoferredoxin, Dfx), H<sub>2</sub>O<sub>2</sub> (reverse ruberythrin, Rbr3) or O<sub>2</sub> (flavoprotein A1 and A2, FprA1 and FprA2). In these reduction reactions NADH:rubredoxin oxidoreductase transfers electrons from NADH to rubredoxin. Reduced rubredoxin is then the electron donor for Dfx, Rbr3, and FprA1 or FprA2. The aim of this study was to analyze whether growth of *C. acetobutylicum* under oxidative stress influences the product pattern. Therefore, the  $\Delta$ perR mutant was grown in a chemostat under acid (pH 5,7) or solvent (pH 4.5) producing conditions. The culture vessel (1.5 l volume) was aerated at different rates (0 - 5.5 l x h<sup>-1</sup>). At pH 5.7 acetate and butyrate were the major products. With increasing aeration rate we observed a slight decrease in butyrate and an increase in acetate concentration (29 mM without aeration, 39 mM with 5.5 l x h<sup>-1</sup> air). Thus, when electrons are consumed for the reduction of oxygen or ROS, *C. acetobutylicum* produces preferentially more oxidized products, e.g. acetate, which might result in higher energy conservation from the substrate. Interestingly, under solvent producing conditions, no steady state could be established under aeration. So far, it can only be speculated that under these conditions the need for electrons for the formation of reduced products and the reduction of oxygen cannot be matched.

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#### MCBP371

##### Tolerance of *E. coli* against oxidative stress generated by atmospheric pressure plasmas

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Atmospheric-pressure plasmas formed by ionization of a gas are of increasing interest in the biomedical and clinical field and already used e.g. for enhancing wound healing or for sterilizing purposes. Driven in ambient air, they consist of a versatile mixture of reactive oxygen and nitrogen species to which microorganisms are more susceptible than eukaryotic cells. However, the molecular mechanisms of bacterial inactivation are not elucidated completely and potential issues related to resistance developing upon uncontrolled clinical use are hard to predict.

In a genome-wide screening using a single-gene knockout library (KEIO collection), 85 genes were identified, which are crucial for survival of low doses of plasma. In a second subsequent screening, mutants were exposed to stressors mimicking plasma components like H<sub>2</sub>O<sub>2</sub>, O<sub>2</sub><sup>-</sup>, or peroxydinitrite to assign plasma factors to which the genes mediate tolerance. Additionally, in a rational approach the stress-activated protein Hsp33<sub>Ec</sub> (heat shock protein 33) was investigated in depth. It was found to prevent plasma-based protein aggregation *in vitro*. Further, Hsp33<sub>Ec</sub> was not inactivated by plasma as most proteins analyzed so far, but activated. In a third line of experiments, bacterial strains exhibiting a significantly higher plasma tolerance than wild-type cells were obtained by directed evolution and repetitive plasma exposure.

The identification of genes mediating plasma tolerance, the discovery of Hsp33<sub>Ec</sub> preventing plasma-induced protein aggregation, and the successful increase in tolerance by directed evolution necessitate to rethink long-term applications of plasmas in clinic. Unregulated use might result in bacterial strains with a plasma tolerance comparable to eukaryotic cells limiting future applications.

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### MCBP372

#### The DUF1127 protein family in *Agrobacterium tumefaciens*

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The genome of *A. tumefaciens* encodes four annotated hypothetical proteins belonging to the widely distributed, but still uncharacterised DUF1127 family (1, 2). This conserved domain, usually composed of 40 to 50 amino acids (aa), can be found in several thousand hypothetical proteins from numerous species, mainly bacteria. It either depicts the entire protein or the C-terminus only. (3, 4, 5) We were able to bioinformatically find an additional family member and we could assign two already annotated genes to the DUF1127 family. Regarding their length those seven proteins can be divided into three subclasses: three "short ones" (ca. 50 aa), two "middle-sized ones" (ca. 75 aa) and two "big ones" (ca. 100 aa). Especially the "short ones" caught our interest since they show a high sequence similarity to each other and to numerous homologues in other Rhizobiaceae. We could show that proteins from that family typically are rich in arginines which appear to be conserved in their abundance but not in position. A strong induction by heat stress could be shown for four of the proteins. In contrast, two showed an induction under cold shock conditions. Despite their high similarity in aa sequence the three short DUF1127 proteins do not show the same expression pattern over the growth curve. From pull down assays we obtained a number of proteinaceous interaction partners. For further characterisation the protein structure will be determined and deletion strains will be constructed and tested.

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- [1] Winans SC (1992) *Microbiol Rev* 56(1): 12-31.
- [2] Gelvin SB (2003) *Microbiol Mol Biol Rev* 67: 16-37.
- [3] Goodner B, et al. (2001) *Science* 294(5550): 2323-8.
- [4] Wood DW, et al. (2001) *Science* 294(5550): 2317-23.
- [5] Mitchell A, et al. (2015) *Nucleic Acids Res.* 43(D1): D213–D21.

### MCBP373

#### (p)ppGpp binding targets and influence on conditional proteolysis in *Escherichia coli*

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The signaling molecule (p)ppGpp act as global regulator in *Escherichia coli*. Accumulation of (p)ppGpp modulates the specificity of the RNA polymerase in order to inhibit general cellular processes (cell division, replication) and, moreover, promotes the expression of genes for several biosynthetic pathways (1, 2, 3). We found that (p)ppGpp affects degradation catalyzed by FtsH or Lon. For instance, the growth rate-controlled degradation of LpxC, the key enzyme of LPS biosynthesis (4). Further identified FtsH an Lon substrates are being tested to determine whether their degradation is altered by presence or absence of (p)ppGpp. Initial *in vivo* degradation experiments revealed an accelerated degradation in a (p)ppGpp-deficient *E. coli* strain (5, 6). However, stability of several other protease substrates remained virtually unchanged in the mutant strain. This aspect indicate a more substrate-specific influence of (p)ppGpp in regulated proteolysis rather than a general modulation of protein turnover. Regarding to these results we started to examine whether (p)ppGpp directly binds to the proteases, their substrates or if it acts indirectly by DRaCALA experiments. Regarding FtsH dependent LpxC degradation our preliminary results indicate that both proteins were unable to bind the alarmone. However, several other enzymes involved in lipid IV<sub>A</sub> biosynthesis, antigen biosynthesis and LPS-modification pathway show ability to interact with ppGpp.

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- [6] Bittner et al. (2015) *J. Biol. Chem.* 290, 19367-19378

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### MCBP374

#### Stochastic modelling of the growth behavior of *Corynebacterium glutamicum* on single cell level

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Since the work of Monod and Schaechter during the middle of last century, the laws of bacterial growth are a fundamental tool to understand the dynamics of microbial growth behavior on a macroscopic scale. Since a few years, modern techniques enabled microbiologists to study these dynamics on a single cell level, which resulted in the generally accepted, so-called "adder"-model. Here we present our work on the stochastic modelling of the growth mode of the apically elongating, (fractional) asymmetrically dividing, Gram-positive *Corynebacterium glutamicum*.

In order to obtain data on the spatio-temporal development of single *C. glutamicum* cells, we used a ready-made microfluidic device together with epifluorescence microscopy. The mCherry-tagged topology protein DivIVA provides a marker for cell cycle progression. We compared the wild type like strain with a deletion mutant of the peptidoglycan glycosyltransferase RodA, both in rich and minimal medium supplemented with acetate, respectively.

Since the manual analysis of time-lapse images often is challenging, we developed a semi-automated framework for the data-acquisition from multichannel fluorescence time-lapse micrographs using the open-source solutions FIJI and R.

This framework provides data on length- and volume development, as well as, on the dynamic localization of fluorescent fusion-proteins of single cells during the cell cycle within the genealogic context of a growing bacterial colony. The acquired data then is used to perform a stochastic modelling of the overall growth behavior.

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### MCBP375

#### **AmiC, a peptidoglycan hydrolase with unusual function in multicellular cyanobacteria of the order Nostocales.**

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Multicellularity requires efficient cell-cell communication to coordinate the activities of all individual cells. Filamentous heterocyst-forming cyanobacteria developed a unique system to enable the transfer of small molecules along the trichome. Numerous nanopores in the septum between two adjacent cells allow the formation of cell joining structures. Nanopores result from the hydrolytic activity of AmiC homologues<sup>1,2</sup>. In unicellular bacteria, AmiC splits septal PG for daughter cell separation and is tightly regulated by NlpD removing an inhibitory  $\alpha$ -helix<sup>3</sup>. Structural examinations on AmiC2 from *N. punctiforme* demonstrate that the inhibitory  $\alpha$ -helix is missing<sup>4</sup> which points to an unknown regulation mechanism of AmiC that exhibits a novel function in cell wall modulation. We investigated the role of AmiC in multicellular cyanobacteria by characterizing the substrate-binding of AmiC2 by site-specific mutations. We focused on the identification of possible cleavage sites for AmiC by elucidating the so far unknown PG composition. We showed for the first time that *Anabaena* 7120 produces a complex network of different cross-linked and amidated muropeptides. Site-directed mutagenesis revealed that several residues are important for the recognition and binding of PG. Mutations of those AAs cause dramatic effects on the catalytic activity and the binding capability of AmiC2. In future prospects, septa of wildtype and *amiC* mutant strains will be enriched from sacculi preparations to find possible cleavage sites for AmiC. Moreover, we will perform interaction studies between AmiC mutant variants and different muropeptides to characterize the substrate specificity.

#### **References:**

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### MCBP376

#### **The role of the septal protein SepJ in pore formation and cell-cell communication in filamentous cyanobacteria of the order Nostocales**

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A peptidoglycan (PGN) layer is present between the cells of filamentous cyanobacteria and encompasses the entire trichome. The PGN needs to be highly dynamic to allow both, the growth of the filament and differentiation from vegetative cells into heterocysts, akinetes and hormogonia. Another requirement for proper cell differentiation is the intercellular communication within the filament. Pores forming a nanopore array which allow such communication have been discovered in the septal PGN and are drilled by the cell wall lytic amidase AmiC2 in *Nostoc punctiforme* (*Np*)<sup>1</sup>.

Various proteins have been suggested to be involved in the formation of septal junctions that penetrate the nanopores. One of those proteins, SepJ, localizes in the septum and is essential for filament integrity in *Anabaena*<sup>2</sup>. Furthermore, it was shown that the intercellular molecular exchange in an *Anabaena sepJ* mutant is slower than in the wild type (WT)<sup>3</sup>. Similar to *Anabaena*, a *sepJ* mutant of *Np*<sup>4</sup> cannot grow diazotrophically and shows a fragmentation phenotype when grown on N<sub>2</sub>. We could show that the intercellular calcein exchange of this mutant is ~6 fold reduced compared to the WT. Sacculi of the *Np* WT and the *sepJ* mutant were analyzed in vegetative cells and hormogonia regarding their nanopore array. Both cell types of the mutant showed a significant lower number of pores compared to the WT. Furthermore, we found via semi-quantitative RT-PCR that *sepJ* is more abundant in vegetative cells than in hormogonia. In contrast, *amiC2* transcripts are more abundant in hormogonia than in vegetative cells. Future studies attempt to show the direct interaction of SepJ and AmiC in filamentous cyanobacteria.

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[1] Lehner et al. 2013

[2] Flores et al. 2007

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### MCBP377

#### **The ultimate steps of Riboflavin biosynthesis in *Bacillus subtilis* occur in a single and dynamic cluster of microcompartments**

D. A. O. Rotter<sup>\*1</sup>, C. Heger<sup>1</sup>, S. L. Dersch<sup>1</sup>,

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Riboflavin (RF) is the precursor of FMN and FAD, indispensable cofactors for redox reactions. The four enzymes responsible for RF biosynthesis have been studied in considerable detail in *Bacillus subtilis* *in vitro*. The last two steps in RF biosynthesis occur in the heavy RF synthase (HRS), which resembles a capsid with an outer shell made of RibH and an inner core of RibE, and therefore allows substrate channelling. However, data describing their localisation and dynamics *in vivo* have been missing.

We have characterised localisation and diffusion by single particle tracking (SPT) using functional fluorescent mVenus-fusions (mV). Our analysis enables discrimination of diffusive species of the same protein as well as determination of dwell times and heat maps.

RibH-mV foci diffuse very slowly, much less than anticipated for single HRS, and confined, mostly close to the cell poles or septa. After cell division HRS assemblies are formed *de novo* in one of the daughter cells. We also detected diffusive RibH-mV adding to existing foci. Stimulated emission depletion microscopy (STED) revealed single RibH-mV foci of divergent size per cell, whose size measured by full width half-maximum (FWHM) was much larger than expected for single HRS. Notably, we also found freely diffusing RibE-mV in the cytoplasm. In contrast to the RibH-mV assemblies, the two bifunctional enzymes catalysing the first four reactions (RibAB-mV, RibDG-mV) are diffusive in the cytoplasm with some dwell events for RibAB-mV, indicating possible interactions with HRS microcompartments.

We conclude that RF biosynthesis occurs in a freely diffusive manner for the first two reactions, while a dynamic assembly of microcompartments, which may further enhance substrate channelling, mediates the last two reactions.

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#### MCBP378

##### Sensitivity of FtsZ to ADEP-activated ClpP peptidase

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FtsZ is the central pace maker protein of cell division in most bacteria. Its polymerisation at midcell into the so-called "Z-ring" is the committed step which leads bacterial cells into the division event. The Z-ring then forms the scaffold which other cell division proteins adhere to, sequentially assembling the divisome that synthesises the new cell membrane and cell wall (1). We previously showed that antibiotic acyldepsipeptides (ADEP) activate bacterial ClpP peptidase for untimely proteolysis of FtsZ thereby preventing Z-ring assembly, cell division and eventually cell death. Noteworthy, stably folded FtsZ emerged to be a significantly vulnerable target for ADEP-activated ClpP compared to other tested proteins (2).

In this study, we aimed at elucidating the reasons for this sensitivity. Our results indicate that it is the overall fold and structure of FtsZ that conveys increased degradation sensitivity, rather than a specific degron sequence. The crystal structure of FtsZ (3) reveals two independently folding domains as well as a central core helix H7 with adjacent T7 loop. The latter is essential for establishing contact of one FtsZ monomer with the preceding monomer during polymerisation. The N-terminal domain harbours a GTP-binding site and the C-terminus contains a long protruding tail that is an important interaction interface for cell division proteins. By analysing mutant proteins of *Bacillus subtilis* FtsZ, we investigate the role of the different structural features in the proteolytic event to identify the reasons for increased sensitivity of FtsZ to proteolysis.

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[2] Sass et al. 2011. PNAS 108(42):17474-17479

[3] Löwe & Amos. 1998. Nature 391, 203-206

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#### MCBP379

##### Conjugative DNA transfer in *Streptomyces*

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Conjugative DNA-transfer in the Gram+ mycelial soil bacterium *Streptomyces* is a unique process, resembling the segregation of chromosomes during bacterial cell division. The initial transfer from the plasmid carrying donor to the recipient is mediated by a single plasmid-encoded protein TraB. TraB is a FtsK-like DNA-translocase, which recognizes a specific plasmid sequence via interaction with specific 8-bp repeats and translocates double-stranded plasmid DNA. Subsequently, the newly transferred plasmids colonize the recipient mycelium very efficiently probably by invading neighboring compartments separated by cross walls. To test the concept of plasmid spreading, we constructed an eGFP-encoding reporter plasmid. Mating experiments with differentially labeled recipient strains allowed the visualization of conjugative DNA-transfer by fluorescence microscopy. Plasmid transfer was detected at the lateral walls of donor and recipient hyphae. Observation of the eGFP signal in the recipient far away from the contact site to the donor demonstrated spreading of the plasmid within the recipient mycelium. Genetic crosses with various mutants complemented by Tra either in the donor or the recipient revealed that plasmid spreading in the recipient depends on the presence of *tra* and plasmid-encoded *spd* genes. Moreover, interaction of Spd-proteins and Tra in bacterial two-hybrid studies suggested a multi-protein complex involved in intramycelial plasmid spreading. The two-step conjugation mechanism of *Streptomyces* plasmids is an adaptation to the filamentous lifestyle of their host. Following the initial plasmid transfer at the lateral wall, plasmids invade neighboring mycelial compartments thereby rapidly colonizing the recipient mycelium before the development of sporogenic hyphae.

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#### MCBP380

##### A small alarmone synthetase and a hydrolase extend (pp)pGpp metabolism in *Corynebacterium glutamicum*

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Stringent response is the answer of bacteria and plant chloroplasts to nutrient deficiencies reprogramming the transcriptional landscape by the production of the small alarmones guanosine pentaphosphate and guanosine tetraphosphate ((p)ppGpp). For decades the bifunctional Rel protein seemed to be the only (pp)pGpp synthase and hydrolase in the actinobacterium *C. glutamicum*

We recently identified genes encoding short alarmone synthetases (SAS) and hydrolases (SAH) in *C. glutamicum* by bioinformatic analyses, named *relS*, *relP* and *relH*. Deletion mutants of *C. glutamicum* and complementation of (pp)pGpp<sup>0</sup>-mutants of *E. coli* assigned (pp)pGpp synthesis to the RelS protein, whereas (pp)pGpp hydrolase activity was shown by RelH. The RelP protein turned out to be not part of stringent response. Furthermore, enzyme activity assays revealed the ability of RelS to synthesize not only pppGpp from GTP and ATP and ppGpp from GDP and ATP, but also pGpp from GMP and ATP. Moreover, these enzyme characteristics were the prerequisite to analyze the RelH enzyme *in vitro*, since standards of pGpp, ppGpp, or pppGpp were either not available commercially or of pure quality.

Production of hyperphosphorylated radioactively labelled standards enables us now to investigate (pp)pGpp

metabolism in *C. glutamicum in vivo* being the basis to decipher the interconnection of the Rel protein to the new SAS and SAH enzymes.

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#### MCBP381

##### Inhibition and recovery of cell division in ADEP-treated *Bacillus subtilis*

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Antibiotics of the acyldepsipeptide class (ADEPs) exert prominent antibacterial activity against many Gram-positive bacteria by dysregulating the bacterial protease Clp [1]. At inhibitory ADEP concentrations close to the MIC, rods form long filaments and cocci develop into enlarged spheres indicating considerable remaining biosynthetic capacity. This filamentation phenotype is due to the delocalization of FtsZ, the pace-making protein of cell division, as FtsZ is a particularly sensitive target for proteolytic attack by ADEP-activated ClpP [2].

In this study, we aimed at gaining insights into how bacterial cells react to such an exceptional antibiotic mechanism. Using high- and super-resolution as well as time-lapse fluorescence microscopy we characterized the fate of proteins linked to cell division - during ADEP treatment, when FtsZ is depleted, as well as after removing ADEP from the growth medium. During ADEP exposure, FtsZ-dependent divisomal proteins failed to localize to the future division site. Upon prolonged ADEP treatment, also nucleoid segregation was impaired while the bacterial membrane remained intact in viable cells. Surprisingly, even ADEP-treated cells with a length of more than 100 µm, lack of divisomes and extensively disorganized nucleoids were able to recover chromosome segregation, cell division and growth after removal of ADEP from the growth medium. Our study shows a remarkable capability of bacteria to re-organize important cellular structures and machineries from otherwise lethal conditions.

#### References:

- [1] Brötz-Oesterhelt *et al.* Nature Med., 2005, 11:1082-87  
[2] Sass *et al.* PNAS, 2011, 108(42):17474-17479

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#### MCBP382

##### Differentiation of small non-coding and small coding RNAs in *Bacillus subtilis*

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Full genome and high-throughput RNA sequencing techniques provide raw nucleotide sequences of an organism's DNA as well as its transcriptome profiles. However, further analysis is needed to provide the biological meaning to these vast data sets, starting with a very basic classification into coding, non-coding and/or regulatory DNA and RNA features. Importantly, this provides the basis for further functional approaches leading to a profound understanding of life processes. Unfortunately, unambiguous differentiation of small RNAs into coding (scRNA) and non-coding (sncRNA) is still a challenge. Therefore, we created a plasmid based screening system pMAX-GFP that covers important aspects like (i) fast and efficient cloning of desired features via the In-Fusion system, (ii) very tight target promoter repression for characterization of potentially toxic products, (iii) stable host maintenance, (iv) direct visual screening and real time quantification using GFP, (v) as well

as rapid and highly efficient purification of proteins/ peptides by TWIN-STREP-tag (IBA) for final identification and characterization by mass spectrometry. To validate the pMAX system we used three small RNAs of *B. subtilis* with unknown function. Two of them were induced, translated, purified and finally identified by mass-spectrometry to verify the new proteins and genes. The third RNA did not mediate translation initiation but seemed to be an active regulatory RNA causing a detrimental growth phenotype upon induction. We have shown that pMAX-GFP can be utilized for the quick classification of sRNAs providing the basis for a comprehensive screening and characterization of arrays of newly identified RNA features of so far unknown function.

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#### MCBP383

##### Interactomic studies of proteins involved in the Stickland fermentation of *Clostridium difficile*

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The anaerobic, Gram-positive bacterium *Clostridium difficile* is causing a severe diarrhoea which leads to several thousand deaths per year in Germany. Stickland fermentation of amino acids represents the most prominent energetic generation process for *C. difficile* growth. It has been unequivocally correlated to toxin production. This metabolic pathway employs pairs of amino acids as electron acceptor-donor systems for generating ATP. The proton motive force and its consequent ATP synthesis appears on the proton-translocating ferredoxin:NAD<sup>+</sup> oxidoreductase complex (Rnf), whose proton pump functioning is coupled to the reduction performance of reductases. Hence, our group strives toward the elucidation of protein-protein-interactions involved in the Stickland pathway. For that, interactomic techniques encompassing affinity co-purification of bait-prey complexes and proteomics-based strategies for the identification of interaction partners were chosen as scientific approach. At first the membrane associated RnfC subunit, which is part of the Rnf pump and the cytoplasmic PrdA monomer of the D-proline reductase complex will be subjected to interactomic studies. Their strep-II tagged versions will be employed as baits to capture the potential preys stabilizing the transient protein-interactions by *in vivo* cross-linking. Preliminary experiments have been conducted leading to the construction of *C. difficile* 630  $\Delta$ erm strains harbouring pMTL82151rnfC-strep and pMTL82151prdA-strep whose genes are flanked by their naturally predicted promoter regions. Moreover, the initial complementation growth experiments followed by Western-blot analysis for the production of the fusion proteins were performed.

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#### MCBP384

##### Polar localization of the phosphodiesterase PdeB

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Bacterial cells are highly organized organisms that require proper spatiotemporal regulation. Cyclic diguanylate (c-di-GMP) has found to be an important regulator of several cellular processes in gram-negative bacteria, such as motility, biofilm and cell cycle. The intracellular c-di-GMP concentration is regulated by a huge number of proteins some of which localize to specific positions, which raises the question if there are c-di-GMP gradients within the cell. The messenger is synthesized by diguanylate cyclases which

contain a specific GGDEF-domain and degraded by phosphodiesterases which have an EAL or HD-GYP domain.

Using transposon mutagenesis we identified a protein that harbors an EAL and a GGDEF domain, called phosphodiesterase biofilm protein (PdeB). We were able to show that the protein affects bacterial motility and biofilm formation. Fluorescence microscopy and genomic in-frame deletions revealed that PdeB localizes at the flagellated cell pole in dependence of the polar marker HubP. However, PdeB does not only affect the polar but also the lateral flagella system, suggesting that the localization may be important for inheritance to daughter cells, but not involved in generating local c-di-GMP gradients. Remarkably, the PdeB GGDEF-domain is responsible for the polar targeting, showing that these domains may have more diverse functions than currently expected. Using several methods we found that the GGDEF-domain directly interacts with the HubPs c-terminal FimV-domain. This interaction may not only be important for proper polar targeting but could also influence the PdeB enzymatic activity. Future studies will focus on determining the enzymatic activity of PdeB and elucidating if the interaction with HubP does have an effect on the phosphodiesterase activity.

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### MCBP385

#### Dynamics and regulation of native and engineered multi-replicon systems in *Sinorhizobium meliloti*

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A share of plant-associated  $\alpha$ -proteobacteria, e.g. *Sinorhizobium*, *Agrobacterium* or *Rhizobium* species, maintain large multipartite genomes consisting of a primary chromosome and up to six secondary replicons. These replicons are termed secondary chromosomes, chromids, or megaplasmids, and vary significantly in their size and number of essential genes. However, they are usually characterized by a combined replication and partitioning *repABC* locus. While RepC most likely acts as replication initiator, the RepABS system is required for vertical transmission of duplicated replicons. The nitrogen fixing  $\alpha$ -*Rhizobium Sinorhizobium meliloti* possesses a tripartite genome composed of a main chromosome (3.65 Mb) and the RepABC-type megaplasmids pSymA (1.35 Mb) and pSymB (1.68 Mb). Previously, a replicon labelling system was established, and a highly ordered coordination of chromosomal and megaplasmid replication origins throughout the entire cell cycle was discovered [1]. To promote studies of the chromosome and secondary replicon organization and maintenance, a Cre/lox toolbox for large-scale genome engineering, including recombinase mediated cassette exchanges and whole replicon fusions, and an enhanced labelling system for simultaneous tracking of three genomic loci were developed. Beside *S. meliloti* strains lacking one or both megaplasmids [2], strains with mono-, bi- and tripartite genome configurations were constructed. We are applying these strains to study the influence of genome organization on the spatial organization and dynamics of the main chromosome and secondary replicons, and to elucidate the molecular mechanisms governing replicon coordination.

#### References:

[1] Frage B. *et al.* (2016) Mol Microbiol 100(5): 808-23

[2] diCenzo GC. *et al.* (2014) PLoS Genet; 10(10): e1004742

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### MCBP386

#### Packaging of *Dinoroseobacter shibae* DNA into Gene Transfer Agent particles is not random

I. Wagner-Döbler<sup>\*1</sup>, J. Tomasch<sup>1</sup>, H. Wang<sup>1</sup>, S. Koppenhoefer<sup>1</sup>, M. Preusse<sup>1,2</sup>, J. Petersen<sup>3</sup>, H. Brinkmann<sup>3</sup>, B. Bunk<sup>3</sup>, S. Bhuju<sup>1</sup>, M. Jarek<sup>1</sup>, R. Geffers<sup>1</sup>, A. Lang<sup>1,4</sup>

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Gene transfer agents (GTAs) are phage-like particles which contain a fragment of genomic DNA of the bacterial or archaeal producer and deliver this to a recipient cell. GTA gene clusters are present in the genomes of almost all marine *Rhodobacteraceae* (Roseobacters) and might be important contributors to horizontal gene transfer (HGT) in the world's oceans. For all organisms studied so far, no obvious evidence of sequence specificity or other non-random process responsible for packaging genomic DNA into GTAs has been found. Here we show that knock-out of an autoinducer synthase gene of *Dinoroseobacter shibae* resulted in overproduction and release of functional GTA particles (DsGTA). Next-generation sequencing of the 4.2 kb DNA fragments isolated from DsGTAs revealed that packaging was not random. DNA from low-GC conjugative plasmids but not from high-GC chromids was excluded from packaging. Seven chromosomal regions were strongly overrepresented in DNA isolated from DsGTA. These packaging peaks lacked identifiable conserved sequence motifs that might represent packaging recognition sequences for the GTA terminase complex. Low-GC regions of the chromosome, including the origin and terminus of replication, were under-represented in DNA isolated from DsGTAs. DNA methylation reduced packaging frequency while the level of gene expression had no influence. Chromosomal regions found to be over- and under-represented in DsGTA DNA were regularly spaced. We propose that a "headful" type of packaging is initiated at the sites of coverage peaks and, after linearization of the chromosomal DNA, proceeds in both directions from the initiation site. GC-content, DNA-modifications and chromatin structure might influence at which sides GTA packaging can be initiated.

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### MCBP387

#### The hidden lipoproteome of *Staphylococcus aureus*

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**Introduction:** *Staphylococcus aureus* devotes about 2 - 3 % of its coding capacity to lipoproteins (LPP), which are anchored to the outer leaflet of the membrane via a di- or tri-acylglycerol moiety. About 30% of the conserved LPP function as substrate binding proteins of ABC-transporters and thus play important roles in nutrient uptake. However, the majority of the remaining LPP are functionally poorly characterized.

**Objectives:** The aim of the presented study is to provide starting points for the experimental evaluation of poorly or uncharacterized *S. aureus* LPP.

**Materials & methods:** To achieve this goal, an integrated bioinformatics approach was used to (i) predict the theoretical lipoproteome (e.g. LipoP, SignalP, Proteinortho, PROSITE pattern PS00013 and G+LPPv2) of 123 sequenced *S. aureus* strains and (ii) use available literature, sequence and structural data to improve annotation of uncharacterized lipoproteins (e.g. SMART, BLAST, Aureowiki, ConSurf and I-TASSER).

**Results:** 43 different orthologous groups of LPP were conserved in at least 95% of the 123 analyzed *S. aureus* strains. For 23 of these LPP groups, for which functional data is not available so far, we suggest protein regions, which might be of functional relevance based on sequence comparisons and structural predictions. In addition, the extraction of expression data from our literature analysis indicated a cell wall associated function for many of the so far uncharacterized LPP.

**Conclusion:** Our bioinformatics characterization of the *S. aureus* lipoproteome provides first structural and functional evidence for LPP of unknown function. This work represents the basis for further experimental investigation and the understanding of the role of LPP in *S. aureus* physiology and the infection process.

#### MCBP447

##### A novel sactipeptide produced by *Staphylococcus spec.*

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**Introduction:** The rapid spread of resistance mechanisms among bacteria necessitates the search for new antibiotics which is facilitated by high-quality sequencing and bioinformatic tools.

**Objective:** The aim of this study is the investigation of an antimicrobially active substance produced by a clinical *Staphylococcus* isolate.

**Materials and Methods:** The genome of the strain was sequenced and assembled. Remaining gaps were closed by PCR and sequencing. Bioinformatic tools were used for analysis.

**Results:** antiSMASH identified a precursor and a radical SAM enzyme as part of a sactipeptide gene cluster. Sactipeptides are ribosomally synthesized and posttranslationally modified bacteriocins with intramolecular thioether bridges. Their prominent feature is the crosslink between the  $\alpha$ -carbon atom of an amino acid and the sulphur of a cysteine residue which is catalyzed by radical SAM enzymes and essential for the maturation of the precursor. Additional data showed that the gene cluster also contains two proteases, a transcriptional regulator and an ABC transporter.

MALDI-TOF analysis affirmed the existence of a small peptide of approximately 3016 Da. A disruption of the radical SAM enzyme via either homologous recombination or antisense technique was attempted to investigate the effect of the sactipeptide loss on antimicrobial activity and MALDI-TOF analysis.

**Conclusion:** A new sactipeptide biosynthesis gene cluster and its components were identified. The size of the peptide is estimated to be about 3016 Da. Next steps include cloning of the peptide and radical SAM enzyme as well as the proteases. Future experiments will focus on the investigation of the sactipeptide regarding its structure, biosynthesis, and mode of action.

## ePoster Session 2

### Microbial Diversity and Evolution (MDEP)

17 April 2018 • 14:30–17:00

#### MDEP388

##### A new anti-fungus Sesquiterpenoid originating in a actinomycete from *Elephas maximus* feces

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**Introduction:** Actinomycetes, a class of microbes that produce the most antibiotics, have made great contributions to human health. Actinomycetes in animal feces are a rare field of study.

**Objectives:** The aim of this study is to find new bioactive substances from actinomycetes in animal feces.

**Materials & methods:** Isolation, identification and fermentation of test strain, isolation, purification and structure elucidation of metabolites in the strain, and genome analysis were carried out by using my laboratory's procedures.

**Results:** An actinomycete strain YIM 101047 was isolated in a fresh fecal sample collected from an elephant (*Elephas Maximus*) living in Xishuangbanna National Nature Reserve, Xishuangbanna, Yunnan Province, P. R. China. The strain was similar with *Streptomyces albolongus* based on 16S rRNA gene sequences. The BLAST result showed that the sequence similarity was 99.89%. But the similarity between YIM 101047 and *Streptomyces albolongus* was only 94% based on the comparative genomics. Therefore YIM 101047 should be a full new species of genus *Streptomyces*. The strain produces Bafilomycins and Odoriferous Sesquiterpenoids. A Sesquiterpenoid ( $1\beta$ ,  $4\beta$ ,  $4a\beta$ ,  $8a\alpha$ )-4,8a-dimethyloctahydronaphthalene-1,4a(2H)-diol, molecular formula  $C_{12}H_{22}O_2$ ) of them was identified as a new compound. The Sesquiterpenoid has high anti-fungus activities to many pathogenic fungi (*Candida albicans* ATCC MYA-2876, *Candida parapsilosis* ATCC 22019, and *Cryptococcus neoformans* ATCC 208821 etc.), and it is expected to be developed as a new anti-fungus drug. Genome of the YIM 101047 strain was analyzed. The genome consists of an 8,027,788 bp linear chromosome. Forty-six putative biosynthetic gene clusters of secondary metabolites were found. The sesquiterpenoid gene cluster was on the left arm (0.09 – 0.10 Mb), and the bafilomycin biosynthetic gene cluster was on the right arm (7.46 – 7.64 Mb) of the chromosome. Twenty-two putative gene clusters with high or moderate similarity to important antibiotic biosynthetic gene clusters were found, including the antitumor agents bafilomycin, epothilone and hedamycin; the antibacterial/antifungal agents clavulanic acid, collismycin A, frontalamides, kanamycin, streptomycin and streptothricin; the protein phosphatase inhibitor RK-682; and the acute iron poisoning medication desferrioxamine B. The genome

sequence reported here will enable us to study the biosynthetic mechanism of these important antibiotics and will facilitate the discovery of novel secondary metabolites with potential applications to human health.

**Conclusion:** The results indicate that animal fecal actinomycetes are a new important source for drug discovery.

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#### MDEP389

##### Spectrum of mutations in pathoadaptive loci of *P. aeruginosa*.

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Pathoadaptive loci are defined as those genes that acquire a high number of mutations during persistence in a chronic patient habitat like the CF lung. *P. aeruginosa* is a pathogen derived from aquatic habitats that contributes most to the shortened life times of patients with Cystic Fibrosis (CF). Twenty genes including *lasR*, *nuoL*, *algG*, *pelA* and *pelF* were identified as pathoadaptive loci in a set of 12 CF patient courses (6 mild and 6 severe) as well as in the literature. The aim of this project is to screen how mutations in the investigated loci are distributed in environmental, acute infection and cross-sectional chronic infection isolates. Using amplicon sequencing on a Illumina NextSeq we were able to sequence the pathoadaptive loci of more than 500 isolates (83 acute infections, 98 environmental, 350 chronic infection isolates) in a fast and cost effective manner. After filtering for decent coverage and read distribution we were able to detect a various number of mutations in the strain panel. The range of mutations in a loci varied between a dozen and more than 200 candidate positions whereby some rare positions seem to be specific for one habitat. Both synonymous and non-synonymous mutations are present in the same amount of affected positions within the genes with the limitation that neutral mutations tend to be present in more non-CF isolates and non-synonymous exchanges are overrepresented in yet undiscovered genome position with a significant overrepresentation in isolates of chronic infections. Early stop mutations were only present in five loci with those in *gacA* only found in non-CF isolates. Another example for a rare mutation is an early stop mutation in *algG* only found in two chronic infection isolates.

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#### MDEP390

##### Regulation of P4-type integrase expression in uropathogenic *Escherichia coli*

M. Chitto\*<sup>1</sup>, M. Berger<sup>1</sup>, U. Dobrindt<sup>1</sup>

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**Question:** Horizontal acquisition and loss of genomic islands (GEIs) play key roles for bacterial evolution and adaptation of pathogens during pathogen-host interaction. P4-type integrases are involved in the chromosomal insertion and excision of GEIs. They usually specifically recognize their encoding GEI. However, not much is known about the regulation of integrase expression. We therefore investigate (i) environmental conditions which modulate expression of integrase-encoding genes in *E. coli*, and (ii) the impact of nucleoid associated proteins on expression of integrase genes.

**Methods:** Reporter gene fusions were generated in the chromosome of uropathogenic *E. coli* strain 536 to monitor promoter strength and regulation of the P4 integrase-encoding genes of PAI I<sub>536</sub>-PAI VI<sub>536</sub>. The corresponding

promoter sequences were fused to a promoterless *yfp* gene plus a selectable marker (*cat*). We compared the integrase gene promoter activities under different growth conditions. The genes *fis*, *hns*, *hupA*, *hupB*, *ihfA*, *ihfB*, *lrp*, *stpA*, *dps* coding for the major nucleoid-associated proteins (NAPs) were inactivated in the reporter strains to investigate the impact of these proteins on the expression of PAI-encoded P4-like integrases.

**Results & Conclusion:** Our results demonstrate that the promoter of the integrase gene of PAI I<sub>536</sub> is the most active followed by the promoter of the integrase genes of PAI II<sub>536</sub> and PAI III<sub>536</sub>, while the activity of the promoters of the integrase genes of PAI IV<sub>536</sub>, PAI V<sub>536</sub> and PAI VI<sub>536</sub> are very weak under the conditions tested. The expression level of the integrase genes may correlate with the instability of their cognate GEIs. The impact of different NAPs on the integrase promoter activities is currently under investigation.

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#### MDEP391

##### Novel strategies for autotrophic carbon fixation in thermophilic prokaryotes

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The fixation of carbon dioxide into cellular carbon is a prerequisite for life. Today, six autotrophic pathways as well as many variants thereof have been described, whereas more pathways might exist in nature. Here we studied inorganic carbon fixation in two thermophilic bacteria, *Desulfurella acetivorans*, which does not possess key genes for any of the known carbon fixation pathways, and *Ammonifex degensii*, which possesses genes for two putative autotrophic pathways in its genome.

We found that *D. acetivorans* harbours a novel variant of the reductive tricarboxylic acid cycle, termed the reverse oxidative tricarboxylic acid cycle (roTCA). In this pathway, citrate cleavage proceeds not via ATP-citrate lyase reaction but is catalyzed by citrate synthase, a thermodynamically unfavourable reaction that is facilitated by a highly adapted central metabolism (1). *A. degensii* uses both the reductive acetyl-CoA pathway and a modified Calvin-Benson cycle in parallel. The latter differs from the canonical cycle in that ribulosephosphate is regenerated by an unusual transaldolase-dependent pathway, and that heat-stable archaeal enzymes are utilized (2).

The autotrophic strategies in *D. acetivorans* and *A. degensii* convey advantages and drawbacks that differ from the canonical pathways. The roTCA cycle is the most thermodynamically efficient of all known carbon fixation pathways but presumably depends on highly reducing conditions, while the use of two autotrophic pathways allows for higher metabolic versatility but requires the maintenance and expression of additional genes. The modified Calvin-Benson cycle in *A. degensii* is active under thermophilic conditions, at the cost of a higher oxygen sensitivity.

#### References:

[1] Mall *et al.*, *Science*, in press.

[2] Mall *et al.*, submitted

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#### MDEP392

### Marker free intraclonal competitive fitness experiments of sequential *P. aeruginosa* strains analysed by a multiplex PCR amplicon pipeline

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The colonization of CF airways with the common gram-negative bacterium *P. aeruginosa* is one of the few opportunities to observe the microevolution of a pathogen during chronic infection in real life. We wanted to explore if and to what extent the microevolution in the CF lungs had influenced the fitness of *P. aeruginosa* to grow in its natural inanimate aquatic habitat. To study robust and standardized aquatic model habitats, we examined the growth of the serial *P. aeruginosa* isolates from 12 CF-patients (six patients with a mild and six with a severe course of infection) in two widely-used liquid media, i.e. Luria broth (LB) as a model for an animate environment and a minimal mineral medium (MM) with succinate as sole carbon source as a model for an inanimate environment. Separate precultures of serial isolates of a patient course were mixed in equal amounts; samples were taken at 0 h, 48 h (with continuous culturing every 12 h) and 120 h (without continuous culturing). The composition of the bacterial communities at time points 48 h and 120 h was determined by sequencing of bacterial DNA-derived amplicons spanning strain-specific SNPs. In nearly all 12 courses one or two strains could outcompete all others. Furthermore, we could evidence that in all courses, expect 2, the first isolate displayed the later ones. Only in the two mildest courses, it could be shown that an intermediate or a late isolate belongs to the winner strains. For some of these winner strains we identified SNPs, stop-mutations, deletions or extra genes which could be linked to fitness advantage. It seems to be that competitive growth in MM is more variable than in LB. The outcome of the fitness experiments is already visible after 48h and the 120h time point only supports the results after 48h.

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### MDEP393

#### *Pseudomonas aeruginosa* microevolution in cystic fibrosis lungs

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In the airways of most cystic fibrosis (CF) patients chronic infections with *Pseudomonas aeruginosa* are established during childhood and typically determine the clinical course. Over the years the bacteria undergo microevolution presumably enhancing the adaptation to the lung habitat.

At our local clinic *P. aeruginosa* isolates have been collected semi-annually from 35 chronically infected patients since the 1980s. To monitor microevolution against the CF background we investigated sequential isolates from twelve patients, six with the mildest and six with the most severe clinical course. Isolates of a persisting clone were genome sequenced (approx. 170 isolates in total) and interrogated for variations manifested during the infection course. The isolates were also tested for mutation rates and phenotypic traits such as morphology, motility and virulence factor secretion.

The phylogenies deduced from the occurrence of genome variations displayed the whole spectrum of evolutionary modes ranging from the presence of a single adapted strain to long-term persistence of co-existing clades, with mixed types in between.

Overall, >4800 mutations occurred in the twelve courses, mostly nucleotide exchanges but also frame-shifting indels and accessory genome variations. Most "hotspots" of CF lung microevolution (genes mutated in several patient courses) were associated with either antimicrobial resistance or biosynthesis of surface components such as alginate.

Comparison of mild and severe clinical courses revealed hints for differences in the bacterial microevolution in the respective airways. Co-existence of persisting clades was more frequent in mildly affected patients while stop or frame-shifting mutations occurred more often in isolates from severe clinical courses.

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### MDEP394

#### Analysis of Bacterial Gene Neighborhood Conservation

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<sup>1</sup>Philipps-University Marburg, Marburg, Germany

Gene neighborhoods are groups of genes that remain in context with each other over multiple genomes. Early work has shown that because the prokaryotic genome is highly fluid, gene neighborhoods are not stable and mutate at a faster rate than protein sequences. Therefore, when gene neighborhoods are detected over long evolutionary distances, it poses the question, what are the evolutionary forces keeping them there? The release of thousands of previously un-sequenced genomes due to the advent of next-generation sequencing allows us to expand gene neighborhood analysis. We aim to develop a resource for researchers to be able to quantitatively access gene neighborhood information. In construction of such a resource goes a parallel analysis of the data looking for new gene clusters and trends in bacterial gene neighborhood conservation. To that end we have developed GECCO (GENomic Context CONservation), a web-tool that allows searching for the genomic neighborhoods for all bacterial ortholog groups covered by OrthoDB. Users can visualize the genes most conserved in their neighborhoods, at which position relative to the gene it is conserved and how the conservation profile changes when limiting the analyzed genomes to the four most sequenced phylum. Our initial analysis of this data revealed, alongside several canonical gene neighborhoods, many neighborhoods that combined very different cellular processes. The rate in which genes remained conserved to one another was shown to exponentially decrease as a function of the distance between them. Co-conserved genes were often maintained in the same orientation as one another. We believe GECCO will be a useful research for the bacterial community, both as a useful hypothesis generator and a source for supplementary evidence.

## MDEP395

### Next Generation Biodiversity Analysis: the Bacterial Metadatabase BacDive

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Microbial data and metadata are scattered throughout the scientific literature, databases and unpublished lab notes. This makes them difficult to access. BacDive mobilizes data from internal descriptions of culture collections and initial descriptions of novel taxa in the primary literature and currently offers data for 62,683 bacterial and archaeal strains. Here we describe exemplary mobilization projects like the Reichenbach collection of myxobacteria, where information on 12,535 typewritten index cards were digitized and a total of 37,156 data points were extracted by text mining. Another mobilization project targeted Analytical Profile Index (API®) tests on paper forms that were collected in culture collections over 30 years. Overall 8665 API® tests were digitized, which provide physiological data for 5059 microbial strains. Published at BacDive, this collection becomes the largest publicly available API® test data collection in the world. Species descriptions published in the *International Journal of Systematic and Evolutionary Microbiology* (IJSEM) are of particular interest as a source for metadata. For over 1000 new species descriptions up to 150 different metadata-types were extracted manually, yielding 72,618 data points. This collection was complemented by a set of metadata for over 4000 IJSEM species descriptions, integrated from a phenotypic trait database published by Barberán et al. By publishing these data in BacDive, the metadata not only became accessible and searchable but are also linked to strain taxonomy, isolation source, cultivation condition, and molecular biology data. Thereby, BacDive enables a broad potential for new analytical approaches in biodiversity research.

## MDEP396

### Phylogenetic distribution of RsbR orthologs and sequence conservation in stressosome proteins and their putative output modules

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**Introduction:** The stressosome is a 1.8 MDa multiprotein complex that senses and responds to stress ultimately controlling the activity of the alternative sigma factor, SigB, in *Bacillus subtilis*, the model organism for stressosome activity. The stressosome proteins, RsbR, RsbS and RsbT, are located within the genome as part of an operon with downstream encoded proteins, which act as a cognate output module to the stressosome's sensory input.

**Objective:** We will present a census of stressosome gene clusters and co-occurring output modules and highlight potential functionally relevant regions of stressosome proteins.

**Method:** *RsbRST* gene clusters and downstream encoded regulatory proteins were identified within microbial and archaeal genomes using BLAST searches. Sequence alignments and phylogenetic trees were constructed to identify signature amino acids for each group of Rsb proteins. Available structural information was used to investigate localization of conserved amino acid residues in Rsb proteins.

**Results:** The *rsbRST* module is found within many bacterial species. Cognate downstream genetic modules show a diverse range of potential output systems, which are often predicted to control the level of second messengers such as c-di-GMP. Signature residues, which might play important roles in protein-protein interaction during stressosome complex formation were identified in alpha helix 3 and adjacent regions of the STAS domain of RsbR, RsbR paralogs and RsbS.

**Conclusion:** We used a combination of different bioinformatic tools to gain insight into the occurrence of stressosome gene clusters within the bacteria and archaea and to identify regions within Rsb proteins likely important for structure and function of the stressosome complex.

## MDEP397

### New members of the family Eggerthellaceae isolated from a human fecal sample: *Rubneribacter badeniensis* gen. nov., sp. nov., *Enteroscipio rubneri*, gen. nov., sp. nov.

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**Introduction:** The *Eggerthellaceae* is an upcoming family of the human gut microbiome; members are known as normal dwellers and are often isolated from feces. However, some species are opportunistic pathogens. In this study, two unknown anaerobic strains were isolated from a human fecal sample.

**Materials & methods:** Identification and classification was performed using a polyphasic approach including phenotypic (Gram-staining, morphology, catalase, oxidase), biochemical (fatty acids, quinones, polar lipids and API tests) and molecular biological methods (16S rRNA, WGS).

**Results:** Analysis of 16S rRNA gene sequences indicated that both strains represent distinct lineages within the *Eggerthellaceae*. *R. badeniensis* showed 92.3% similarity to the type strains of *Eggerthella* and *Gordonibacter*, while *E. rubneri* clustered together with *Paraeggerthella hongkongensis* and *Arabia massiliensis* (94.8%). Quinone analysis revealed that MK-5 and MK-7 are present, which are described for the *Eggerthellaceae* for the first time. Polar lipids in both strains consist of eight and six glycolipids, respectively. Both strains possess phospholipids, phosphatidylglycerol and diphosphatidylglycerol. The percentage of total branched fatty acids is relatively high for *R. badeniensis* (42%) and *E. rubneri* (50%) but comparable to the value of *G. pamelaeeae*.

**Conclusion:** Our results indicate that the two unknown bacterial strains belong to the *Eggerthellaceae* and represent

both new genera and new species: *R. badeniensis* ResAG-85<sup>T</sup> (= DSM 105129<sup>T</sup> = JCM 32272<sup>T</sup>) and *E. rubneri* ResAG-96<sup>T</sup> (= DSM 105130<sup>T</sup> = JCM 32273<sup>T</sup>).

**Funding:** This work was part of the project "Importance and Bioactivity of the Microbial *trans*-Resveratrol Metabolites Dihydroresveratrol and Lunularin" funded by the "Deutsche Forschungsgemeinschaft".

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#### MDEP398

##### Diversity study of *Ochrobactrum* spp. isolated from medicinal leeches

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The *Alphaproteobacteria* genus *Ochrobactrum* shares a high phylogenetic relatedness with the genus *Brucella*. *Ochrobactrum* spp. occur in diverse habitats, free-living in water or associated with plants, animals, and humans. *Ochrobactrum* spp. are well known as endosymbionts in medicinal leeches specifically colonizing leech nephridia and bladders (Nelson & Graf 2011). The function of *Ochrobactrum* spp. in leeches is not known so far.

The aim of this study was to isolate and study the diversity of leech-associated *Ochrobactrum* spp. to address the question of host specificity. In total, of 318 *Ochrobactrum* spp. isolates were obtained from 68 separately cultured leeches. Isolates were phylogenetically identified by 16S rRNA gene sequencing. From 143 isolates, 41% were closest related to the type strain of *Ochrobactrum pseudogrignonense* (99.6%) and 30% closest related to *Ochrobactrum lupini/anthropitype* strains (99.8%). In order to identify if genetically different isolates were among those two determined phylotypes, isolates were differentiated at the strain level (genotypes) by genomic fingerprinting using BOX-, (GTG)<sub>5</sub>-, and RAPD-PCR. Many leeches carried genetically identical, but also genetically slightly different *Ochrobactrum* spp. isolates. To determine if these genotypes are leech specific or not, currently all the collected isolates are compared to isolates collected from other sources by a Multi Locus Sequence Typing (MLST) approach in order to address the hypothesis of host specificity.

#### References:

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#### MDEP399

##### Different diversity of abundant heterotrophic and methylophilic bacteria cultivated by dilution-to-extinction cultivation from the phyllosphere of *Arrhenatherum elatius* and *Galium album* plants, exposed to ambient and elevated atmospheric CO<sub>2</sub>

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Global climate changes increase atmospheric CO<sub>2</sub> concentrations. This affects plants, bacterial communities colonizing the aerial part of plants (phyllosphere) and the

interactions of plants and phyllosphere bacteria. Aim of this study was to determine the effect of elevated CO<sub>2</sub> on the concentration and diversity of heterotrophic and methylophilic phyllosphere bacterial communities. Leaves of two abundant grassland plant species of the Giessen Free Air Carbon Dioxide Enrichment system (GI-FACE) in Linden (Germany) were collected in August 2015 from three areas of three control (CC) and three rings exposed to +20% elevated CO<sub>2</sub> (CE). Leaf associated heterotrophs and methylophilic bacteria were enriched by dilution-to-extinction cultivation. The concentration of cultivated bacteria was determined by the most probable numbers method (MPN). Bacterial assemblages enriched in the three highest positive dilutions were compared by community fingerprinting using 16S rRNA gene targeted PCR- DGGE analysis. Bacteria from highest positive dilutions were furthermore isolated and identified by 16S rRNA gene sequencing. Concentrations of cultured heterotrophs and methylophilic bacteria were not affected by elevated CO<sub>2</sub>, but non-metric multidimensional scaling (NMDS) of community patterns showed significant differences of the phylogenetic compositions of cultivated bacteria. *Shingomonas* were isolated in a higher abundance from the phyllosphere of CC ring plants, while *Arthrobacter*, *Flavobacterium*, *Microbacterium*, and *Stenotrophomonas* mainly from the phyllosphere of CE ring plants. This study indicates an adaptation of specific phyllosphere taxa to elevated CO<sub>2</sub>. The high number of representative isolates now enables the investigation of the community shifts in distinct plant microbe interaction studies.

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#### MDEP400

##### The evolution of phage resistance in *Vibrio alginolyticus* K01M1 in response to two different filamentous bacteriophages

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**Experimental Evolution:** An evolution experiment was performed using *Vibrio alginolyticus* K01M1, which has been isolated from a healthy pipefish *Syngnathus typhle*, and two different temperate phages, that differ in the lytic activity using six replicate populations per treatment. After 48 h of being challenged by the phages, the amount of resistant clones per population was about 75 %. Follow-up analyses revealed that in both treatments, lysogens, which were dominating during the first generations, were rapidly outcompeted by phage-resistant strains. The spread of non lysogenic mutants was significantly faster in bacterial populations with a highly lytic phenotype. Furthermore, lysogens revealing a weak lytic activity remained at low densities during the entire experiment, whereas highly lytic phenotypes became extinct after 30 generations.

**Genomic Analysis:** Analysis showed shared SNPs and INDEL events correlating to the different treatments. Lysogens revealed the existence of either complete phages or with regional deletions. In contrast the genomes of the mutant strains exhibited different deletions in a gene cluster encoding extra cellular pili structures.

**Conclusion:** In our experiment the reaction of a growing *Vibrio alginolyticus* culture exposed to temperate phages can be considered as a time scheduled multiple response. Two strategies of bacterial defense were seen where the immediate response results in lysogenic evolution and a

dominance of resistance mutants in the following phase.

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## MDEP401

### On The Evolution of Earth and Circadian Clocks

N. Schmelling\*<sup>1</sup>

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Four billion years ago, shortly after the origin of the Earth-Moon System, it is predicted that the length of day was close to four hours. Life on Earth originated not long after that. Since then the length of day changed continuously to the 24 hour day as we know it today. In addition, environmental conditions such as the atmospheric composition changed dramatically. One group of prokaryotes that lived through all these changes are cyanobacteria, which are among the oldest organisms on Earth. Cyanobacteria are a unique group of bacteria as they are the only known prokaryotes to perform oxygenic photosynthesis as well as the only known prokaryotes to have a circadian clock. The circadian clock and the photosynthetic machinery of cyanobacteria evolved and adapted to these changing conditions. The origin of different factors have been dated back in time and elucidate parts of the evolutionary trajectory of these systems. Going in back in time and figuring out the environmental conditions at important events in the history of life can help us better understand the changes necessary for the evolution of such complex systems. Analyzing the evolution of such complex systems like a circadian clock is not something, which can easily be done in a laboratory setting. Even with directed evolutionary approaches, the reconstruction of the evolution of a circadian clock would probably take decades of hard work. However, the recent developments in machine learning, artificial intelligence, and artificial life as well as computer simulations, which have been used extensively for years to study evolutionary processes, can help to unravel the basic building blocks and environmental conditions necessary for the evolution of such complex systems.

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## MDEP402

### Insights into the genome and metabolism of *Clostridioides difficile* CD3022, a clade 5 representative with active expression solely of the binary toxin

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**Introduction:** *Clostridioides difficile* is a facultative nosocomial pathogen responsible for antibiotic-associated diarrhea worldwide. Its population structure comprises several major clades containing non-toxigenic and toxigenic isolates. Major virulence factors of toxigenic strains are the toxins A, B and the binary toxin CDT, typically organized within a so-called PaLoc or CdtLoc, respectively. Here, we present first insights into the genome and metabolome of *C. difficile* CD3022, an atypical clade 5 representative.

**Materials and Methods:** Genome sequencing was carried out on the PacBio RSII. Genome assembly yielded in the complete genome of strain CD3022. A final genome quality

score of QV60 was attained after Illumina short-read correction. Genome annotation was performed using Prokka. Production of toxins TcdA and TcdB was determined by ELISA. Extracted RNA was reversely transcribed into cDNA to determine the expression of the binary toxin CDT by qPCR. Metabolomic samples and fermentation products were analyzed by GC-MS.

**Results:** The genome of *C. difficile* CD3022 consists of one chromosome and one episomal bacteriophage. Analysis of the toxin repertoire revealed the presence of an atypical PaLoc and a complete CdtLoc. Whereas a lack of TcdA and TcdB production was confirmed by ELISA, a positive *cdtA* and *cdtB* expression was determined by qPCR. Metabolic profiles showed an individual profile of strain CD3022 in comparison to other *C. difficile* model strains.

**Conclusion:** The evolution and mobility of virulence factors is potentially important in the origin, evolution and spread of toxigenic and non-toxigenic *C. difficile* isolates. Unique genetic features are accompanied by metabolic properties different from typically investigated reference strains.

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## MDEP403

### Metagenomic reconstruction of the genetic potential of uncultivated prokaryotic key players in the redoxcline of the Black Sea

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Amplicon-sequencing of environmental samples from the redoxcline of the central Black Sea, encompassing suboxic to sulfidic waters, revealed that the microbial communities are strongly stratified along the redox gradient and dominated by several prokaryotic groups. Some of them, such as taxa belonging to the SUP05 cluster, sulfur-oxidizing *Epsilonproteobacteria*, ammonia-oxidizing *Archaea*, *Marinimicrobia* and *Chloroflexi*, are known to be widespread in globally distributed oxygen-depleted marine areas where they presumably mediate important biogeochemical transformations within the nitrogen, sulfur and carbon cycles. Other taxa seem to be characteristic for the Black Sea redoxcline, for example green sulfur bacteria (*Chlorobiales*) and several taxa of magnetotactic bacteria. For most of these taxa no cultured representatives are available and their physiology and functions in this stratified system are only poorly understood. These taxonomic groups were reconstructed by a reference sequence-based metagenomics approach. Thereby, we obtained partial and nearly complete genomes of these taxa in order to decipher their physiology and potential functions in this system. Genomic analysis of the *de novo* assembled contigs linked some of those key taxa to the biogeochemical cycling of nitrogen and sulfur, and identified their typical chemoautotrophic or heterotrophic lifestyles. A comparison of the genetic potentials of Black Sea taxa with related representatives of these groups from other oxygen-depleted systems revealed also substantial differences in the physiology of these taxa (e.g., for the *Marinimicrobia*).

## MDEP445

### Assembly of a thermophilic giant virus genome from Icelandic hot springs with an expanded set of translation system component genes

T. Luttermann<sup>\*1</sup>, D. Brandt<sup>1</sup>, C. Henke<sup>2</sup>, A. Sczyrba<sup>2</sup>, E. Gudmundsdottir<sup>3</sup>, A. Aevansson<sup>3</sup>, O. Fridjonsson<sup>3</sup>, B. T. Aðalsteinsson<sup>3</sup>, J. Vanhalst<sup>3</sup>, G. O. Hreggvidsson<sup>3</sup>, J. Kalinowski<sup>1</sup>

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Since in 2003 the first Mimivirus was described [1], the interest in giant viruses has increased sharply. Following the discovery of more giant viruses that all shared the unexpected feature of encoding central translation system components, it was proposed that these giant viruses are derived from a fourth domain of life [2]. In a recent study [3], a new group of giant viruses (Klosneuviruses, KNV) has been reported, and a phylogenetic analysis indicated that giant viruses instead originate from smaller viruses by gathering genes from their eukaryotic hosts. Here, we report the discovery of a new representative of the KNV group.

Analysis of a multiplexed metagenome from different hot springs (geysers) located on Iceland revealed a bin containing 56 contigs with many genes having the highest similarity to KNV genes. From this pooled dataset, we could assemble a 1.2 Mbp genomic sequence of a putative KNV that we named *Geyservirus*. Additionally, we performed a selective whole genome amplification using the phi29 polymerase followed by nanopore sequencing to reduce the number of contigs. After gene finding, the genes were compared to the Mimivirus protein motifs (COGs) and the KNV genes for functional annotation, resulting in 633 protein-coding (CDS) and 15 tRNA genes. Assessment of the annotated genes revealed that *Geyservirus* contained 32 central translation system components which is the second most in the KNV group. The core genome of *Geyservirus* and the KNV consisted of 26 CDS and subsequent phylogenetic analysis placed *Geyservirus* in the center of the KNV clade.

The poster will present the current state of the annotation of the new *Geyservirus* and its phylogeny.

#### References:

[1] La Scola, B., Audic, S., Robert, C., Jungang, L., de Lamballerie, X., Drancourt, M., ... & Raoult, D. (2003). A giant virus in amoebae. *Science*, 299(5615), 2033-2033.

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[3] Schulz, F., Yutin, N., Ivanova, N. N., Ortega, D. R., Lee, T. K., Vierheilig, J., ... & Kyrpides, N. C. (2017). Giant viruses with an expanded complement of translation system components. *Science*, 356(6333), 82-85.

## ePoster Session 2

### Systems Biology and Metabolomics (SBP, MetP)

17 April 2018 • 14:30–17:00

#### SBP435

##### What is flux balance analysis and how to simulate a gut microbiome

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With the advent of high-throughput omics techniques, the amount of data produced by metagenomic, metatranscriptomic, and metaproteomic approaches tremendously increased. A sophisticated way of making use of these data is the reconstruction of metabolic networks. Assuming steady-state conditions and a cellular optimization criterion, these networks can be used in flux balance analysis to predict internal metabolic fluxes, organismic growth rates and the production rates of interesting metabolites. Flux balance analysis for single species based on genome-scale networks with more than thousand reactions and metabolites are state-of-the-art. In contrast, analyzing complex microbial communities at the same level of detail is a substantially bigger challenge. Here, we present a community-wide and dynamic flux balance analysis simulator called *µbialSim*. Community-wide simulation is realized by connecting genome-scale networks of individual community members via pool metabolites which can be exchanged between members. Dynamic modelling is implemented by applying the steady-state assumption of flux balance analysis only to short time intervals and using predicted fluxes to update pool metabolite concentrations for the next time step. Preliminary simulations of a gut microbiome consisting of 773 species allowed for the prediction of metabolite exchange patterns as they evolve during a feeding pulse. The flux balance analysis simulator *µbialSim* enables deeper insights into the functioning of complex communities by exploiting easy to integrate data generated by next-generation sequencing and/or proteomics.

#### SBP436

##### Oligopermeases determine systems-level properties of extracellular peptide signaling in *Bacillus subtilis*

H. Babel<sup>1</sup>, P. Naranjo Meneses<sup>\*1</sup>, S. Trauth<sup>1</sup>, V. Soujik<sup>1</sup>, I. Bischofs<sup>1</sup>

<sup>1</sup>Max Planck Institute for Terrestrial Microbiology, Marburg, Germany

Signaling peptides control central physiological behaviors in bacterial populations. Peptides sensed by intracellular receptors from the RNPP-family are produced by an active export-import circuit. Here, we elucidate fundamental systems-level properties of this complex signaling circuit in *Bacillus subtilis* by monitoring the intra- and extracellular response dynamics to peptide stimulation. Using a FRET-reporter that utilizes the interaction of the RapA receptor with the response regulator Spo0F, acceptor photo-bleaching experiments and mathematical modeling we show that the receptor saturates within minutes as cells internalize the cognate PhrA-signal via the oligopermease Opp. Active signal uptake by Opp allows cells to amplify the extracellular signal concentration by several orders of magnitudes and can thus facilitate activation of a low affinity receptor. However, other peptides can compete with signal uptake and thereby interfere with signaling. Our data suggests that oligopermeases – a shared component of all RNPP-signaling circuits in Gram-positive bacteria – govern the sensitivity and dynamics of extracellular peptide signaling, while potentially also limiting its robustness.

### SBP437

#### Protein-protein interactions of the *Pseudomonas aeruginosa* aerobic respiratory chain supracomplex

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Biological energy generation is usually accomplished by membrane spanning electron transport chains composed of multiple protein complexes. Previously, we elucidated involved protein-protein interactions for the denitrification machinery of *Pseudomonas aeruginosa* using a proteomics based interactomics approach (1). A highly complex protein network was observed for the investigated supracomplex responsible for anaerobic energy recovery. Here, we are investigating the interactom of the corresponding protein complexes for aerobic respiration. For this approach 4 bait proteins were selected: NuoJ as part of the NADH dehydrogenase (Complex I), CoxA, CoxB as cytochrome c oxidase subunits (Complex IV) and CcoN2 as high affinity cytochrome c oxidase. Later functions under microaerophilic conditions during the transition to anaerobic conditions. Tagged versions of the bait proteins are expressed in the corresponding mutant background. After cultivation at the end of the exponential phase, para-formaldehyde mediated protein complex cross-linking is performed. Generated protein complexes are purified using Streptavidin-tagged column chromatography and bound prey proteins then identified via LC/MS/MS. Obtained data are used to reconstruct the protein network of the supracomplex involved in aerobic respiration. Future experiments include the dynamic rearrangement of these complexes during the shift from aerobic to anaerobic growth.

#### References:

[1] Borrero-de Acuña JM, et al. 2016. Protein Network of the *Pseudomonas aeruginosa* Denitrification Apparatus. J Bacteriol 198:1401–1413

### SBP438

#### Proteomic map of 3,3'-thiodipropionic acid utilization by *Variovorax paradoxus* TBEA6

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*Variovorax paradoxus* TBEA6 was isolated because of its ability to utilize the organic sulfur compound 3,3'-thiodipropionic acid (TDP), a feasible precursor substrate for the production of polythioesters, as sole source of carbon and energy. It was shown that 3-mercaptopropionic acid (3MP) is formed as an intermediate of TDP degradation. By transposon mutagenesis, genes involved in the catabolism of 3MP have been identified and subsequently studied in detail, but enzymes involved in the initial cleavage of this compound remained unknown.

This study presents a proteomic map of *V. paradoxus* TBEA6 during growth with TDP with special focus on the initial reactions of TDP catabolism. Aside from support for the already postulated metabolic pathway, interesting proteins putatively involved in TDP cleavage were identified. Four genes located in the same cluster were particularly striking: I) two enoyl-CoA hydratases (ECH-a, ratio TDP/Gluc 26.1 and ECH-b, ratio TDP/Gluc 4.1); II) a carnitiny-CoA dehydratase (ratio TDP/Gluc 26.1) and III) a putative acetolactate synthase (ratio TDP/Gluc 8.5). Deletion of the putative ECHs evoked restricted growth of the mutants *V.*

*paradoxus* Δech-a and *V. paradoxus* Δech-b with TDP, whereas growth with other carbon sources was not affected. Interestingly, the mutants were still able to utilize 3-sulfinothiopropionic acid, an intermediate of 3MP degradation, indicating that both ECHs participate in the initial reactions of TDP degradation. In general, enoyl-CoA hydratases convert CoA esters. Therefore, TDP-CoA was synthesized and used in thermal shift assays with purified ECH-a and ECH-b. A shift in the melting temperature of ECH-a in presence of TDP-CoA was observed, showing the direct interaction of the protein with the TDP-CoA molecule.

### SBP439

#### Adaption of *Clostridioides difficile* to osmotic stress

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Infections by the strict anaerobic, Gram-positive bacterium *Clostridioides difficile* are causing severe diarrheas which lead to several hundreds if not thousands deaths per year in Germany. Little is known about the gene regulatory, protein interaction and metabolic networks underlying the host associated life cycle of *C. difficile*. Here we are investigating the response of the bacterium to osmotic stress. Several osmoprotectant uptake transporters (Opu) including proline transporter (Put) play an important role in the protection of *Bacillus subtilis* against changing osmotic conditions [1]. Blast analyses identified gene homologues of *B. subtilis* opuC and putP in *C. difficile*. To determine their functional role mutants deficient in the production of functional clostridial OpuC (CD630\_09010) and PutP (CD630\_35750) were generated and grown in minimal medium with increasing salt and sugar concentrations. Different osmolytes (e.g. glycine betaine, carnitine, choline) will be tested for their contribution to osmoprotection. To obtain a holistic view on the osmotic protection process a systems biology approach encompassing DNA RNaseq, proteomics and metabolomics experiments will be performed.

#### References:

[1] Zapras A. et al. Osmoprotection of *Bacillus subtilis* through Import and Proteolysis of Proline-Containing Peptides. *Appl Environ Microbiol.* 2013 Jan; 79(2)

### SBP440

#### Understanding optimal resource allocation during cyanobacterial diurnal growth

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**Question:** We seek to understand the stoichiometric and energetic constraints that limit phototrophic life in complex environments.

**Method:** We developed a computational framework to investigate the optimal allocation of cellular resources during diurnal phototrophic growth based on a genome-scale metabolic reconstruction of the cyanobacterium *Synechococcus elongatus* PCC 7942. We formulate phototrophic growth as an autocatalytic process and solve the resulting time-dependent resource allocation problem using constraint-based analysis.

**Results:** Based only on a narrow and well-defined set of parameters, our approach results in an ab initio prediction of growth properties over a full diurnal cycle. The computational model allows us to study the optimality of metabolite partitioning during diurnal growth. The results obtained from the computational model are in good agreement recent time-series experiments of gene expression in cyanobacteria. – and provide insight into the time-dependent resource allocation problem of phototrophic diurnal growth.

**Conclusions:** Our approach may serve as a general framework to assess the optimality of metabolic strategies that evolved in phototrophic organisms under diurnal conditions.

#### References:

[1] Reimers AM, Knoop H, Bockmayr A, Steuer R (2017) Cellular trade-offs and optimal resource allocation during cyanobacterial diurnal growth. Proc Natl Acad Sci U S A. pii: 201617508

[2] Westermark S and Steuer R (2016) Toward Multiscale Models of Cyanobacterial Growth: A Modular Approach. Front. Bioeng. Biotechnol. 4:95

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#### SBP441

##### A metabolic labelling strategy for protein quantification in *Clostridium difficile*

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Caused by the worldwide rise in cases of drug-induced *Clostridium difficile*-associated diseases in hospitals, research on the proteome of the spore-forming anaerobic bacterium offers new targets for treatment options. Integrated omics techniques provides new starting points for drug development or therapy strategies.

For differential proteome analysis, quantification of complex protein mixtures is needed. Application of metabolic labeling would allow for accurate and bias free studies of e.g. membrane or surface proteome. Up to now, implementation of metabolic labeling strategies to *C. difficile* was hampered by the very specific metabolic requirements of this anaerobe pathogen.

To solve this problem, media were evaluated and the cultivation procedure with <sup>15</sup>N labeled media for the *C. difficile* 630Δ*erm* strain was optimized to gain a high incorporation rate. In a proof-of-principle experiment the cytosolic sub-proteome from three different cultivation media and two growth phases were mixed separately 1:1 with the <sup>15</sup>N standard and analyzed by high resolution mass spectrometry.

The optimization of the <sup>15</sup>N labeling results in an incorporation rate up to 98%. In this proof-of-principle experiment, the cytosolic sub-proteomes of *C. difficile* cells were analyzed and the following Census-based quantification offers reproducible data which are shown in detail.

These results describe the first metabolic labeling of *C. difficile* proteome which can be the new technique of choice for further quantification analysis such as membrane proteome, surface proteome or other challenging sub-proteomes of *C. difficile*.

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#### SBP442

##### Characterization of clinical isolates using the pan peptidome and SWATH-MS

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**Introduction:** The usage of data independent approaches (DIA) in modern proteomics permits the generation of comprehensive peptide identification and quantification data. As a consequence, missing values in large sample cohorts commonly inherent for classical shotgun proteomics can be avoided. Despite this paradigm shift in proteomics, analysis of DIA datasets is highly dependent on suitable spectral libraries working as a register that can be used for data mining of the DIA spectral inventories. Usage of DIA methods in high throughput studies of clinical isolates was precluded so far as these methods are restricted to previously measured data mostly performed for model organisms.

**Objectives:** To overcome this limitation, we seek to adapt the already widely accepted pan genome principle for registers of spectral libraries in order to develop a pan proteome approach allowing for thorough analyses of clinical strains.

**Methods+Results:** In frame of our project a pan spectral library of four *S. aureus* laboratory strains was built and innovatively combined with the DIA implementation OpenSWATH. This approach provides a faster and more precise method for peptide identification and quantification suitable for the characterization of clinical isolates and common strains.

For a 'proof of principle' we processed experimental samples from strains included in pan proteome and clinical *S. aureus* isolates (source: University Hospital, Greifswald).

**Conclusion:** The introduced approach not only uses the presence of proteins and peptides, but also its actual abundance representing the immediate state of the cell's growth and survival. Thereby it depicts a complementing tool for bacterial characterization in the fields of microbial proteomics and diagnostics.

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#### SBP443

##### Prediction and identification of small proteins in *Staphylococcus aureus* using a proteogenomics approach

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Due to automated annotation projects proteins smaller than 100 amino acids are underrepresented in databases used for proteomic analyses. However, in the last few years a variety of such small proteins with regulatory and virulence-associated functions have been detected in several bacteria.

Here we present a set of proteogenomics methods to predict and experimentally detect so far not considered small proteins in *S. aureus*.

Using all six reading frames from STOP codon to STOP codon a six frame translation database (6FRT-DB) was generated for strain Newman. For a second database we applied the sORF Finder algorithm and used sequences of stable RNAs as a negative training set to predict the coding potential of intergenic regions in strain Newman. The predicted new small ORFs (sORFs) were included in the *S. aureus* Newman protein database derived from NCBI (sORF-DB).

Proteins of cell lysates (OD540 of 1 and 7, Tryptone Soya Broth) were fractionated by a gel-based and a gel-free approach. U(H)PLC-MS/MS analyses (Orbitrap Velos pro or Orbitrap Fusion) were performed using a targeted and a non-targeted data dependent method.

The probability to detect more than one unique peptide for proteins with less than 100 aa is very low. Therefore, protein identifications obtained from the MaxQuant search engine were filtered by score and posterior error probability at peptide level. Using a custom-made bioinformatics analysis tool (*pepMapper*) peptides were mapped to the given reference genome sequence and additional information such as spectral counts of sufficient quality, potential open reading frames and ribosomal binding sites, support by different unique peptides, and phylogenetic conservation on both genus and species level was assessed.

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#### SBP444

##### Carbon Flux in Biotransformation of Glycerol to Ectoine Bethlehem L, Moritz KD, Sümmermann ML, Voß P, Galinski EA

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**Introduction and Objectives:** Ectoine is due to its remarkable properties marketed in a multitude of cosmetic and health care, as well as life science products, reaching an annual production scale of several tons [1]. To date ectoine is obtained by elaborate "bacterial milking" and "leaky mutant" bioprocesses, employing the moderate halophilic gram-negative  $\gamma$ -proteobacterium *Halomonas elongata* [2]-[3]. The optimization of this process for the production of ectoine under low salt conditions has been the objective of several research projects, but so far without great success [4]-[6].

**Results and Conclusion:** We constructed the ectoine biosynthetic pathway into *Escherichia coli* DH5 $\alpha$  using the ectoine genecluster of the non-halophilic *Acidiphilium cryptum* [7]. With a specific biomass-related productivity of up to 300 mg / gdcw x h in batch cultures, this value exceeds the maximum of the natural producer (50 mg / gdcw x h). Feeding of <sup>13</sup>C-isotope labelled substrates, followed by NMR spectroscopy of the conversion product (ectoine) allowed the reconstruction of fluxes in the main metabolic routes. This technique revealed a high flux through the anaplerotic branch from Phosphoenolpyruvate (PEP) towards Oxaloacetate (OxAc) and describes one potential bottle-neck in the development of an ectoine cell factory.

#### References:

[1] Lentzen, G. and Schwarz T. (2006), Appl. Microbiol Biotechnol. 72: 623–634

[2] Sauer T., Galinski E.A. (1998), Biotechnol Bioeng 59(1): 128

[3] Kunte H. J., Lentzen G., and Galinski E. A. (2014), Curr Biotechnol 3(1):10\_25

[4] Schubert T. et al. (2007) Appl Environ Microbiol 73: 3343-334

[5] Bestvater et al. (2008) Saline Systems 4: 12

[6] Ning Y. et al. (2016) Metab Eng 36:10-8

[7] Moritz et al. (2015) Extremophiles 19: 87-99

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#### MetP347

##### The impacts of Metabolomics and molecular profiling on preterm gut microbiota; a case of Probiotics supplementation.

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**Introduction:** Probiotics are live microbial supplements that colonize the gut and potentially exert health benefit to the host. We hypothesized that probiotics strains would successfully colonize the gut and protect the infants from developing gastrointestinal tract (GIT) disease.

**Objectives:** We used high-throughput techniques to analyses the probiotic functional diversity and study its impact on the bacterial community in gut of preterm infants.

**Material & Methods:** A total of 75 stool samples were classified into groups: before, during and after (probiotic intake), matched controls, and post discharge samples from a neonatal intensive care unit. Samples underwent analyses of their bacterial community composition, utilizing 16S RNA gene profiling, quantitative PCR (qPCR), and LCMS metabolomics approached.

**Results:** QPCR analysis showed significant difference of *B. bifidum* in infants who received probiotic treatment compared to controls ( $p < 0.01$ ), but no significance was observed *L. acidophilus* ( $p = 0.575$ ). The result from 16S profiling indicated greater *Bifidobacteria* during supplementation (15.1%) compared to the control group (4.0%) and greater *Lactobacillus* during supplementation (4.2%) compared to controls (0.0%). Metabolite profiling showed each group to cluster separately, with distinct metabolites associated with probiotic administration.

**Conclusion:** Probiotic strains found to colonize the gut of preterm infants with different level of abundance and they all increase with probiotic supplements. We suggest that probiotics have some systemic functions and play significant role in the gut microbial communities.

## MetP348

### Smuggling a Trojan horse with a deadly cargo into osmotically stressed *Escherichia coli* cells

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**Introduction:** As a stress response in a hyperosmotic environment, many bacteria take up compatible solutes such as glycine betaine (GB). In *E. coli*, GB is taken up by the salt-induced ProU and ProP transporters. Since pathogenic bacteria survive in hyperosmotic niches like the human urinary tract, toxic GB analogues have been proposed for the development of antibiotic substances [1]. In our analysis we focused on one of the toxic GB analogues, in which one methyl group is replaced by a 4-nitrobenzyl group.

**Objective:** Since the mechanism of the toxic activity of this derivate is still unknown, we studied its uptake and searched for resistant mutants in the zone of inhibition.

**Material and Methods:** The toxic activity in *E. coli* wild-type strain MC4100 was assessed by determination of the minimum inhibitory concentration (MIC). In agar diffusion tests we could visualize the toxic effect in MC4100 and in strains possessing either ProU or ProP.

**Results:** In a MIC assay we showed that ProU is the main transporter for the tested GB derivate, whereas ProP seems to be a minor importer. Resistant colonies, which occurred in the zone of growth inhibition of the wild-type strain, were isolated and will be analyzed further at the genomic level. A yellow color, which appears in the culture medium of the strains, could be ascribed to the degradation of the GB derivate to dimethylglycine and 4-nitrobenzaldehyde, probably the toxic warhead.

**Conclusion:** *E. coli* MC4100 is able to transport the toxic GB derivate with the 4-nitrobenzyl group primary via the GB transporter ProU. The mechanism of the toxic activity is not completely understood, but isolation and characterization of resistant mutants ought to shed light on this process.

## References:

[1] Cosquer *et al.* (2004) *Bioorg Med Chem Lett* **14**: 2061-65

## MetP349

### A semi-automatic metabolomics platform to investigate underexplored natural product producers

M. Marner<sup>\*1</sup>, F. Zubeil<sup>1</sup>, P. Hammann<sup>2</sup>, A. Vilcinskas<sup>1</sup>, A. Bauer<sup>2</sup>, J. Glaeser<sup>1</sup>

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Bacteria associated with freshwater plankton represent a surprisingly underexplored source for novel bioactive substances with potential pharmaceutical value. Low volume fermentations of this neglected bacterial community guarantee a high-throughput of unusual microbial extracts, which demand robust analytical methods to assess their chemical potential.

*Metabolomics*, the semi-automated analysis of large UPLC-HRMS datasets based on chemo-informatic algorithms, is considered the gold standard in dereplication of huge sets of

complex microbial extracts. This study focuses on the industrial application of metabolomic techniques like automatic annotation of compound mixtures followed by prioritization via PCA. Metabolic correlation heatmaps, based on cosine similarity, are generated to provide a visual way to investigate the cultured diversity on a metabolomic level.

Additionally, we set up a *molecular networking* platform, in order to analyze tandem MS data of bacterial extracts and quickly elucidate potential relationships to known compounds. *In silico* fragmented compounds from commercial databases as well as our in-house database are used for automatic annotation within each constructed network.

The presented set of analytical strategies might deepen our understanding of the metabolite output of target strains under specific conditions, including the low intensity metabolites, which might have been overseen in the past. This might increase chances to find new chemistry in underexplored biological niches, which could ultimately help to identify a novel lead structure for further pharmaceutical development.

## MetP350

### Characterization of CsrA-mediated modulation of bacterial metabolism and its effect on pathogenicity

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Most pathogenic bacteria are able to face different environmental conditions and survive in their corresponding host cells using different strategies to fight or evade the hosts' immune response to the infection. This ability requires a fast adaptation of the gene expression, translation and the metabolism on a molecular level. The *csr*-system (carbon storage regulator) is a global regulatory system which is conserved among different genera of bacteria, regulating virulence, metabolic pathways, motility and pathogenicity. The system consists of the dimeric mRNA binding protein CsrA and small regulatory RNAs which suppress CsrA activity. The regulatory function of CsrA is based on the binding of the protein to GGA motifs of the mRNA which are often located near the ribosomal binding site. Due to the binding of CsrA, the ribosomes cannot attach to the mRNA preventing protein translation and also causing a faster degradation of the target mRNA. Other regulatory mechanisms of the *csr*-system, like positive regulation of protein translation by stabilizing secondary structures of the mRNA are also described.

We analyse the global influence of CsrA on the bacterial metabolism using high-performance liquid chromatography and gas chromatography coupled to mass spectrometry for further metabolite identification. Combining different chromatography techniques will maximize the coverage of the metabolome giving insights in the regulatory function of CsrA and its effect on pathogenicity.

In addition transcriptome data illustrated the global regulatory function of the carbon storage regulator (*csr*) in EPEC. Lacking of CsrA leads to an imbalance of the central carbon flux and associated biosynthetic pathways like enterobactin biosynthesis, aromatic amino acid biosynthesis, lipid composition and biofilm formation. Together with the

transcriptome data, the global metabolomic approach reveals the strong effects of a *csrA* deletion in EPEC giving the overall picture of the *csrA* regulon new importance.

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## Short Lecture Biotechnology 3 18 April 2018 • 09:00–11:00

### BTV17

#### Metagenomic analysis of community response to fluctuating environments in anaerobic digestion

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Anaerobic digestion depends on highly diverse microbial communities which transform organic matter to methane and carbon dioxide. An understanding of microbial community dynamics is highly desirable, e.g. to optimize methane production in biogas plants. However, the high species richness as well as the fact that many species have not been cultured yet, make anaerobic digestion systems difficult to understand.

To understand community dynamics, two continuous stirred tank reactors digesting short chain fatty acids were operated under mesophilic conditions using various feeding regimes (continuous and discontinuous) and organic loading rates (1.55 gCOD/L/d and 4.65 gCOD/L/d). Sequencing of metagenomes with the Illumina MiSeq platform was performed to characterize reactor microbiomes and its differences induced by feeding regime and OLR on the taxonomical and functional level.

The community consisted of a mixture of bacterial and archaeal taxa. About 90% of the whole community was made up of ten genera, each having a relative abundance of over 1%. The bacterial community shows a higher variability between the different conditions, unlike the archaeal composition. Despite these differences, the microbiomes' functional repertoire was nearly identical.

Metagenomics revealed compositional changes of the reactor microbiome depending on feeding regime and OLR while the functional profile remained stable. This indicates that functional redundancies within the microbiome may allow anaerobic digestion to proceed even if microbial composition changes. Metagenomics only address the functional potential, whereas the actual activity of species should be determined by a metatranscriptomic approach to investigate these redundancies in more detail.

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### BTV18

#### Analysis of a thermophilic anaerobic microbial community and its potential for plant biomass degradation by using omic technologies

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**Introduction:** Next generation sequencing (NGS) techniques have not only been optimized during the last years, they have also accelerated the exploration of many non-cultivable microorganisms and their genetic information, which have led to new insights of diversity and physiology [1]. Additionally, this approach will lead to the discovery of novel enzymes that will be applied in several biotechnological approaches [2].

**Objectives:** In this study, a sample of an Azorean hot spring was cultivated anaerobically on plant biomass as substrate aiming at the performance of a multi-omic analysis. This approach will deliver detailed information on microbial composition as well as the identification of novel enzymes that act efficiently towards specific substrates.

**Materials & Methods:** Enrichment cultures, containing 0.75 % plant biomass as substrate and 1 % sample (Azorean hot spring) as inoculum, was incubated for 3 days at 60 °C. Afterwards extracted DNA and RNA from the enrichment cultures were used for a multi-omic approach.

**Results:** Metagenomic data revealed a diverse microbial composition, consisting of *Symbiobacterium thermophilum*, *Fervidobacterium nodosum* and *Dictyoglomus thermophilum* with highest abundance. Further investigation regarding gene expression indicated that a variety of genes encoding enzymes for biomass degradation such as beta-glucosidases or endoglucanases, compared to other EC class members, are highly expressed.

**Conclusion:** The usage of a multi-omic approach provides a detailed insight into the enrichment culture's diversity in combination with the discovery of a promising enzyme portfolio hydrolyzing plant biomass.

### References:

[1] Warnecke & Hess, J Biotechnol, 2009, 142;

[2] Elleuche et al., Curr Opin in Micobiol, 2015, 25

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### BTV19

#### Metabolic grafting of *Escherichia coli* for the biosynthesis of aromatic amines

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**Introduction:** Aromatic amines (AA) are an important group of industrial chemicals. They are widely used for technical and pharmaceutical applications as AA can be used as building blocks for anticancer drugs [1], antibiotics, plastics and aromatic polymers [2&4].

**Objectives:** The aim of this study was to generate genetically modified *E. coli* strains for the biosynthesis of three different AA, *para*-amino-L-phenylalanine (L-PAPA), *para*-amino-phenylethanol (PAPE) and *para*-amino-phenylacetate (4-APA).

**Methods:** We overexpressed the genes of *pabAB* from *C.glutamicum* and *papBC* from *S.venezuelae* to enable the biosynthesis of the intermediate *para*-aminophenylpyruvate (PAPP) from glucose or glycerol in *E.coli*. By additional overexpression of the *aro10* from *S.cerevisiae* *para*-aminophenylacetaldehyde is formed from PAPP which can be transformed either to PAPE or 4-APA. The recombinant *E. coli* strains were cultivated in shake-flasks and the formations of three AA were analyzed by HPLC and LC-MS.

**Results:** The overexpression of *pabAB* and *papBC* was sufficient to produce L-PAPA. A titer of 0.45 g l<sup>-1</sup> was achieved by using *E. coli* FUS4.7. The additional expression of *aro10* and *feaB*<sub>*E.coli*</sub> enabled the formation of 0.21 g l<sup>-1</sup> PAPE and 0.28 g l<sup>-1</sup> 4-APA from 5 g l<sup>-1</sup> glucose. By changing the cultivation condition to fed-batch condition a higher titer

of L-PAPA, PAPE and 4-APA of approximately 5.4 g l<sup>-1</sup>, 2.5 g l<sup>-1</sup> and 3.4 g l<sup>-1</sup> were achieved.

**Conclusions:** Here we demonstrated that three AA can be produced with promising yields and titers by genetically engineered *E. coli* strains from glucose and glycerol.

#### References:

- [1] Jobdevairakkam & Velladurai (2013)8,575,385.  
[2] He et al. Microbiology (2001)2817–2829.  
[3] Masuo et al. Sci Rep (2016)6,25764. [4] Gottlieb et al. Microb. Cell Fact (2014)13,96.

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#### BTV20

##### Metabolic engineering of *Oligotropha carboxidovorans* OM5 for the aerobic utilization of syngas

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**Introduction:** To reach independence from biotechnological substrates competing with human nutrition, the use of waste-based substrates is highly favorable. Syngas (CO, CO<sub>2</sub> and H<sub>2</sub>) from gasification of municipal or industrial waste leads to valorization of these resources and supports reduction of greenhouse and toxic gases. While anaerobic fermentation of C<sub>1</sub> gases with acetogenic bacteria is well-known, the energetically more advantageous aerobic utilization with carboxydophilic bacteria has not been well studied so far. Our studies focus on employment of the autotrophic *Oligotropha carboxidovorans* which is able to aerobically metabolize syngas. In this study, we established genetic tools for this organism and applied them to generate metabolically engineered strains.

**Methods and Results:** Using a broad-host-range plasmid, we established a transformation method for *O. carboxidovorans* OM5 via electroporation and obtained constitutive expression of the heterologous *gfp* and *mCherry* genes. An IPTG-dependent system was constructed, resulting in inducible expression of the methylglyoxal synthase and glycerol dehydrogenase genes (*mgsA* and *gldA*, respectively) from *Escherichia coli* and opening the possibility of producing 1,2-propanediol with *O. carboxidovorans*. Targeting the *lldD* gene and L-lactate metabolism as *proof of concept*, we also successfully established gene inactivation and gene deletion in the genome via homologous recombination.

**Conclusions:** We created a versatile genetic tool box for *O. carboxidovorans* OM5, including inducible expression of heterologous genes and gene inactivation, thus opening up the possibility to metabolically engineer *O. carboxidovorans* for utilization of syngas for biotechnological purposes under aerobic conditions.

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#### BTV21

##### Manipulation of the hydrocarbon biosynthesis pathway in *Micrococcus luteus*

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*Micrococcus luteus* naturally produces olefins and represents a promising host to produce hydrocarbons as constituents of

biofuels and lubricants. In this work, we alter the genes for key enzymes of the olefin biosynthesis pathway in *M. luteus* and demonstrate how these genes can be used to manipulate the production of specific olefins. Because the first metabolic steps of branched chain amino acid (BCAA) degradation lead also to the formation of primer molecules for olefin biosynthesis, targeted alterations in the BCAA metabolism can be used in order to produce olefins with a desired isomer composition. By generating deletion and up-mutants of several gene candidates, we could identify the gene cluster encoding the components of the branched chain  $\alpha$ -keto acid dehydrogenase (BCKD) complex, as well as the genes for the downstream steps in the degradation of valine, leucine and isoleucine. Overexpression of the BCKD gene cluster resulted in about threefold increased olefin production whereas deletion of the cluster led to a drastic reduction in branched chain fatty acid content and a complete loss of olefin production. The specificities of the acyl-CoA dehydrogenases of the BCAA degradation pathways were deduced from the fatty acid and olefin profiles of the respective deletion mutant strains. The olefin biosynthesis system of *M. luteus* seems to be relatively unspecific, allowing the incorporation of straight chain acyl-CoA primers and even of primers not found in nature. In conclusion, our experiments allow the unambiguous assignment of specific functions to the genes for key enzymes of the BCAA metabolism of *M. luteus*. We also show how this knowledge can be used to engineer the isomeric composition and the chain lengths of the olefins produced by this organism.

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#### BTV22

##### In vivo functionalization of bacterial magnetic nanoparticles by magnetosome expression of peptides and reporter enzymes

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Magnetosomes are magnetic core-shell nanoparticles biomineralized by magnetotactic bacteria, in which they serve as geomagnetic field sensors. Their unique properties make them ideal for many biomedical and nanotechnological applications. Furthermore, crystal morphologies and the composition of the enveloping membrane can be manipulated by genetic means, allowing a controlled functionalization of the particle surface [1].

For the generation of multifunctional magnetic nanoparticles with tailored properties a versatile genetic "toolkit" is being created. Using an optimized expression system, highly abundant magnetosome membrane (Mam) proteins are utilized for the surface display of enzymes and peptides.

Multicopy expression of arrayed enzymes resulted in magnetosomes with maximized protein-to-particle ratios. Arrays of up to five monomers of the model enzyme glucuronidase GusA plus the additional fluorophore eGFP were genetically fused as single large hybrid proteins to the highly abundant MamC as anchor. GusA activity followed Michaelis-Menten kinetics, and reaction rates were stepwise increased with the number of GusA monomers [2].

Furthermore, we explore the expression of molecular connectors like streptavidin or nanobodies, as well as various organic coatings for increased stability and controlled surface reactivity. Thereby we expect the generation of tailored particles with improved biocompatibility and tuneable characteristics.

## References:

- [1] Uebe R., Schüler D. *Nat. Rev. Microbiol.* 2016, 14:621-637
- [2] Mickoleit F., Schüler D. *Adv. Biosyst.* 2017, DOI: 10.1002/adbi.201700109

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### BTV23

#### Phage surface display derived short peptides for the development of new biosorbents for the recovery of valuable elements from primary and secondary sources

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In the course of evolution, nature has developed diverse strategies to avoid toxic effects of metals in the surrounding environment. Many organisms are able to bind metals to their surface via a variety of structurally diverse biomolecules carrying different functional groups. Because of the need for green and sustainable economic solutions, innovative biotechnological processes using biomolecules have become increasingly important. Biomolecules as sorbents are not only attractive for bioremediation, but also for the recovery of elements from recycling or mining. Currently, we are focusing on the development of new biosorbents based on short peptides for the selective recovery of valuable or toxic elements in complex process water streams such as Co, Ni, Ga or As and microparticles containing rare-earth elements from fluorescent lamp powder. To select and identify these peptides we recently established Phage Surface Display Technology (PSD) as novel biotechnological platform in our group. Different adapted experimental setups for the peptide selection of each respective target material could be established and will be presented in detail. Specific and selectively metal binding peptides for particulate materials as well as for ionic species could be identified from commercially available phage libraries. These libraries contain a pool of genetically engineered phage of up to 10<sup>9</sup> different peptide motifs presented at their surface, which will be reduced in variability in an iterative biopanning process to capture the best binders. This contribution will give an insight into current research activities in our group focusing on PSD, introducing suitable spectroscopic techniques to characterize the metal-peptide complexes and discuss strategies for technical applications.

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### BTV24

#### Bio-based $\alpha,\omega$ -functionalized hydrocarbons from multi-step reaction sequences with bio- and metallo-catalysts based on the fatty acid decarboxylase OleTJE

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<sup>2</sup>Institute of Biotechnology and Biochemical Engineering, Graz, Austria

**Introduction:** The constant depletion of fossil resources is creating new opportunities in the field of renewable chemistry. A very promising source of both fuels and chemicals are fatty acids, which can be converted into a myriad of different products by chemical and biocatalytic reactions. In this work, we present the development of different chemoenzymatic cascades to convert hydroxy fatty acids in bi-functionalized hydrocarbons.

**Objectives:** The objective of this project is to convert hydroxy fatty acids into high value hydrocarbons with different functional groups using the combination of enzymes and traditional chemical catalysts.

**Materials and methods:** Several enzymes from different organisms where produced using *E. coli* as a host. Then, by combining them in different cascade reactions and testing several cofactor regeneration methods the desired products were obtained, purified and characterized. Also, the combination of biocatalysis with a ruthenium catalyst was employed to obtain long chain diols.

**Results:** During the development of the cascades, the individual reactions were optimized gaining valuable insights in the performance of the enzymes. Also, the main hurdles for future applications, such as byproduct formation, differences in reaction conditions or poor enzymatic activity for the given substrate where identified.

**Conclusion:** With the combination of enzyme screening and reaction engineering, the established cascades could yield the desired products starting from renewable hydroxy fatty acids. This offers a clear proof of concept for the future use of this technology in synthetic chemistry and gives an idea of the existing challenges that should be overcome.

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## Short Lecture

### Environmental Microbiology and Ecology 3

18 April 2018 • 09:00–11:00

#### EMV17

##### Anaerobic degradation of phenanthrene by a novel sulfate-reducing bacterial enrichment culture

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Polycyclic aromatic hydrocarbons (PAHs) are widely distributed pollutants producing hazardous effects on human health. In PAH-contaminated sites, oxygen is rapidly depleted. Thus, microorganisms able to use these compounds as a carbon source in the absence of molecular oxygen are crucial for their consumption.

This work aims at elucidating the mechanisms for the anaerobic degradation of phenanthrene by a novel sulfate-reducing enrichment culture (TRIP) obtained from a natural asphalt lake.

The metagenome of TRIP was sequenced and annotated at the MicroScope platform. The analysis of the metagenome sequences included phylogenetic binning, gene prediction and metabolic network reconstruction. The predicted metabolic pathways were corroborated by transcriptomics, proteomics and metabolite analyses of TRIP culture.

Five bacterial draft genomes were reconstructed from the metagenome sequences. The key player of TRIP belongs to the Desulfobacteraceae family of deltaproteobacteria. Analysis of the metabolic capacity of this bacterium revealed the key enzymes for dissimilatory sulfate reduction, a

complete Embden-Meyerhof-Parnas pathway and a complete tricarboxylic acid cycle. This bacterium also presents the key genetic elements of the Wood-Ljungdahl pathway. Genes encoding enzymes potentially involved in the degradation of phenanthrene were identified. Particularly, two gene clusters encoding a carboxylase enzyme involved in the activation of phenanthrene, as well as genes encoding reductases potentially involved in subsequent ring dearomatization and reduction steps. These metabolic steps were also evidenced by metabolite analyses. This work provides first evidence of the anaerobic biodegradation of a higher molecular weight PAH with three aromatic rings.

#### EMV18

##### Two dedicated class C radical SAM methyltransferases synthesize the low-potential redox mediators 8-methylmenaquinone and 7,8-dimethylmenaquinone

S. Hein<sup>\*1</sup>, O. Klimmek<sup>1</sup>, M. Kern<sup>1</sup>, J. von Irmel<sup>2</sup>, M. Gallei<sup>2</sup>, R. Meusinger<sup>2</sup>, J. Simon<sup>1</sup>

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Many electron transport chains use isoprenoid quinones as membrane-bound redox mediators to transfer electrons between respiratory enzyme complexes. In anaerobic habitats, menaquinone (MK) as well as the MK derivatives methylmenaquinone (MMK) and dimethylmenaquinone (DMMK) are prevalent constituents of the membranous quinone/quinol pool in many microorganisms. The 8-MMK<sub>6</sub>-producing Epsilonproteobacterium *Wolinella succinogenes* was used to explore the biosynthesis pathway of MMK. Deletion of the gene encoding a phylogenetically widespread radical SAM methyltransferase (RSMT), MqnK, resulted in a *W. succinogenes* mutant unable to produce 8-MMK<sub>6</sub>. However, 8-MMK<sub>6</sub> formation was restored after genomic complementation using either the native *mqnK* gene or the homologous *menK* gene from *Adlercreutzia equolifaciens*, a human gut bacterium, which produces MMK<sub>6</sub> and DMMK<sub>6</sub>. An *in-vitro* assay demonstrated MK methylation using a purified maltose-binding protein/*A. equolifaciens* MenK fusion protein. Heterologous production in *W. succinogenes* or *E. coli* cells of another class C radical S-adenosylmethionine methyltransferase, MenK2, from *A. equolifaciens* resulted in MK methylation at position C-7. In combination with MqnK 7,8-DMMK<sub>6</sub> was produced in *W. succinogenes*. The results expand our knowledge on quinone synthesis and demonstrate an unprecedented function for two class C RSMTs in energy metabolism. The presence of *mqnK/menK/menK2* genes is considered a valuable biomarker to predict the capability of MMK/DMMK production.

#### EMV19

##### Functional and Regulatory Role of Rare Earth Metals (REEs) in *Pseudomonas putida* KT2440

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**Introduction:** To efficiently capture and detoxify biogenic volatile organic compounds (VOCs), Gram-negative organisms have evolved a periplasmic oxidation system based on pyrroloquinoline quinone-dependent alcohol

dehydrogenases (PQQ-ADHs) that is often functionally redundant. The present work reveals the role of rare earth metals (REEs) as cofactor for the corresponding enzymes in *P. putida* KT2440 and their function in transcriptional control.

**Methods:** Enzymatic assays with purified enzymes were used to define substrate scopes and metal dependencies. Growth experiment, lux-based reporter fusions, and peptide fingerprinting were used to determine the regulatory and functional impact of La<sup>3+</sup> in different strains.

**Results:** We recently reported the first description of a REE-dependent PQQ-ADH (PedH) in *P. putida* KT2440. PedH exhibits a similar substrate scope as its Ca<sup>2+</sup>-dependent counterpart PedE, but only in the presence of different REEs. We show that for efficient growth with various VOCs, the functional production of at least one of the PQQ-ADHs is essential. Transcriptional reporter assays further reveal that PedH and the two-component system PedS2/PedR2 are involved in the inverse regulation of *pedE* and *pedH* in response to La<sup>3+</sup> and that the underlying regulatory network is responsive to as little as 1-10 nM of La<sup>3+</sup>. Lastly, the global proteomic response to La<sup>3+</sup> was determined and compared in different strains.

**Conclusions:** We propose that the functional redundancy and inverse regulation of PedE and PedH represents an adaptive strategy of *P. putida* KT2440 to adapt and optimize growth with or in the presence of alcoholic VOCs in response to variable La<sup>3+</sup> availabilities in natural environments.

#### References:

[1] Wehrmann et al. (2017) mBIO:8e00570-17

#### EMV20

##### Heterotrophic <sup>13</sup>CO<sub>2</sub>-Fixation – A new indicator for microbial dissolved organic carbon utilisation?

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<sup>4</sup>Technical University of Munich, Chair of Analytical Chemistry and Water Chemistry, Garching, Germany

Virtually all heterotrophs incorporate CO<sub>2</sub>. So far no one made use of the fact that heterotrophic fixation of CO<sub>2</sub> depends on the organic substrate and thus this process has the potential to show which carbon source was utilised by the heterotroph.

*Bacillus subtilis* was grown in M9 minimal medium in the presence of H<sup>13</sup>CO<sub>3</sub><sup>-</sup> and various organic substrates. The <sup>13</sup>C-incorporation into bacterial biomass was determined by EA-IRMS and the <sup>13</sup>C-distribution in protein-derived amino acids was measured by GCMS.

The enzyme pyruvate carboxylase (PC) catalyses the conversion of pyruvate to oxaloacetate via the addition of CO<sub>2</sub>, thus replenishing the TCA-cycle. PC occupies a vital position in a metabolic hub of the central carbon metabolism, being responsible for the regulation of the cells' carbon flux. Depending on the carbon source, we expect different <sup>13</sup>C enrichment patterns of metabolic products, with an indicative pattern for each carbon source.

We found that growth on substrates that are funnelled through glycolysis led to enrichment in  $^{13}\text{C}$  of up to 12% in the amino acids directly derived from TCA-cycle metabolites. In case of growth on substrates that enter the central carbon metabolism "between" glycolysis and the TCA-cycle, the amino acids directly derived from TCA-cycle metabolites were enriched in  $^{13}\text{C}$  as well as the amino acid glycine, which was derived from gluconeogenic metabolites. In both cases, the replenishment of the TCA-cycle through PC was crucial and we found the expected distribution of  $^{13}\text{C}$  in the amino acids. In contrast, during growth on TCA-cycle metabolites the replenishment of the TCA-cycle through PC is negligible. This clearly shows that the  $\text{CO}_2$ -fixation patterns are indicative for different carbon sources and can be used to differentiate amongst them.

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## EMV21

### Identification of LaoABC as a novel system for the oxidation of long-chain alcohols derived from SDS- and alkane degradation in *Pseudomonas aeruginosa*

G. Panasia<sup>1</sup>, B. Philipp<sup>1</sup>

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The opportunistic pathogen *P. aeruginosa* is able to use a variety of toxic organic compounds as growth substrates like the toxic detergent sodium dodecyl sulfate (SDS) as well as alkanes. Regarding the metabolic pathways, only the initial enzymes in the SDS and alkane degradation have been analyzed, while the following enzymes are still unknown. Based on a transcriptome analysis of SDS- versus succinate-grown cells [1], several candidate genes were identified. Thereby, the ethanol oxidation system (*exa* system) and a so far unknown gene cluster, composed of a probable alcohol dehydrogenase (PA0364), a putative inner membrane protein (PA0365) and a presumable aldehyde dehydrogenase (PA0366), were induced. Respective deletion mutants were tested for growth with SDS, its degradation intermediates or alkanes. Remarkably, the  $\Delta\text{exaA}\Delta\text{PA0364}$  double deletion mutant was not able to grow with SDS, 1-dodecanol or dodecane. Complementation with either *exaA* or PA0364 could restore growth. Moreover, an activity staining of crude cell extracts indicated an interdependency of PA0364 and PA0365 for 1-dodecanol oxidation. Heterologous co-expression of both proteins enabled the purification of PA0364 and demonstration of its 1-dodecanol oxidation activity. To summarize, the gene cluster PA0364-PA0366 was elucidated as a new system to participate in the SDS- and alkane derived long-chain alcohol degradation and is designated as *laoABC* (long-chain alcohol/aldehyde oxidation). Detailed analysis of this system will give insight in how *P. aeruginosa* is able to degrade toxic substrates and, thus, to survive and grow in hostile anthropogenic environments.

#### References:

[1] Klebensberger J. *et al.* (2009). *Environ. Microbiol.* 11(12):3073-86.

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## EMV22

### Microbial biodegradation of natural rubber latex

W. Röther<sup>1</sup>, J. Birke<sup>1</sup>, D. Jendrossek<sup>1</sup>

<sup>1</sup>Institute of Microbiology University of Stuttgart, microbial cell biology and biopolymers, Stuttgart, Germany

Natural rubber is an item of daily use and vast amounts of waste, e.g. from tires, are permanently released into the environment. Research on its microbial degradation

investigates the mechanisms involved, aiming to turn waste into value added fine chemicals or biofuels.

In Gram-negative *Xanthomonas* sp. 35Y, rubber oxygenase A (RoxA) is responsible for the cleavage of polyisoprene into a C15 degradation product (ODTD). In the recently determined draft genome, the homologue rubber oxygenase RoxB was identified. We successfully overexpressed and purified RoxB featuring properties similar to RoxA (2 *c*-type hemes, comparable molecular mass) but did not produce ODTD but a mixture of oligo-isoprenoids that was described for latex clearing protein (Lcp) from Gram-positive bacteria.

*Xanthomonas* knockout strains  $\Delta\text{RoxA+B}$  and  $\Delta\text{RoxA}$  were unable to grow with latex as sole carbon source, whereas  $\Delta\text{RoxB}$  and the wildtype grew well. Agar containing a sole carbon source was cast into shaking flasks to prevent the latex (1%) from coagulation and adherence to the cells. Liquid medium was added and enabled the measurement of growth curves by monitoring of the OD600. Glucose (0.4%) or sodium-oktanoate (0.05%) served as controls.

Cells were harvested during the exponential growth phase. Fractionation of cell compartments was achieved and samples of the extracellular medium, periplasm, membrane fraction and cytoplasm were subjected to proteome analysis by MALDI-TOF MS and quantitative LC-MS.

#### References:

[1] Ilcu L. Röther W. *et al.* 2017. *Scientific Reports*. 7: 6179 DOI:10.1038/s41598-017-05268-2

[2] Birke J. Röther W. Jendrossek D. 2017. *AEM* 83: e00721-17 DOI:10.1128/AEM.00721-17

[3] Röther W. Birke J. *et al.* 2017. *Microbial Biotechnology* 10(6) 1426–1433 DOI:10.1111/1751-7915.12748

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## EMV23

### Microbiology of the terrestrial "plastisphere": enrichment and characterization of plastic-associated microbial communities

J. MacLean<sup>1</sup>, S. Mayanna<sup>2</sup>, S. Liebner<sup>1</sup>, D. Wagner<sup>1</sup>

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The overabundance and accumulation of plastic debris in land and waters creates new geological properties where soil profiles contain layers of fragmented plastics of various types and origins. The plastisphere was first suggested as a distinct microbial habitat by studies carried out on aquatic plastic debris and very few studies have proposed a similar term for terrestrial ecosystems. The objective of this study was to characterize the bacterial and archaeal communities in plastic-contaminated soil and test their biodegrading potential in vitro. Plastic-associated microbes were isolated on a medium that contained a synthetic polymer stemming from an abandoned landfill as only carbon source. Isolated strains were incubated with different polymer types, and their biodegrading features explored by SEM imaging, while  $\text{CO}_2$  production and weight-reduction of the incubated plastics was monitored. The structure and diversity of the bacterial and archaeal community was elucidated with Illumina paired-end sequencing directly from environmental samples. The high plastic content of the soil reduced the overall diversity of

both bacterial and archaeal communities. An ammonia-oxidizing archaeon *Nitrosocosmicus oleophilus* of the Soil Crenarchaeotic Group accounted for almost all archaeal OTU's. The predominant bacterial groups were *Pseudomonas* and *Streptomyces*, from which two isolated members *Pseudomonas koreensis* and *Streptomyces* sp. 5-22 were also shown to be the most efficiently degrading strains. A putative specialized and substrate-adapted microbial community was herewith firstly described as inhabitants of the terrestrial plastosphere.

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#### EMV24

##### Establishment of a promising electrochemical coculture for current production from glycerol

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**Introduction:** In microbial electrolysis cells (MEC) the chemical energy of organic compounds gets converted into electrical energy and hydrogen by using microbial catalysts. Glycerol is a promising electron donor for MECs. It is the main by-product of biodiesel production, hence a cost-effective feedstock. Moreover, it can be used electrochemically to produce current under anaerobic conditions.

**Objectives:** The aim of our studies is the establishment of a current-producing syntrophic relationship of the well-known, current-producing *Geobacter sulfurreducens* and a suitable partner. Within this relationship, current should be produced via a two-step process, in which the selected bacterium degrades glycerol to organic acids, such as acetate, which is then oxidised by *G. sulfurreducens*, unable to convert glycerol, finally resulting in the production of current.

**Methods:** Standard electrochemical setup: half-cell reactor, anaerobic conditions, graphite electrodes, 30 °C, 20 mM glycerol, 0.2 V vs. Ag/AgCl. Partial 16S rRNA sequencing, HPLC and flow cytometric analysis.

**Results:** The microbial consortium of an enriched biofilm was analysed by 16S rRNA sequencing and the Gram-negative bacterium *R. electrica* was chosen as suitable partner for the cocultivation as it was shown to produce acetate with glycerol as electron donor. Acetate has to act as a substrate for *G. sulfurreducens* in case of current production, which was proved by the electrochemical cocultivation of both bacteria. To quantify the two subpopulations flow cytometry was successfully used.

**Conclusion:** A current-producing cocultivation of *R. electrica* and *G. sulfurreducens* with glycerol as electron donor was established. Further attempts will be focused on a detailed characterisation of this new model system.

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## Short Lecture

### Infection Biology 2

18 April 2018 • 09:00–11:00

#### IBV09

##### Atypical Lymphocytes and Cellular Cannibalism: A Phenomenon, First of its Kind to be Discovered in Chronic Periapical Lesions

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Lymphocytes are often termed to be isomorphic, having a monotonous light microscopic appearance. Morphological aspects of lymphocytes in tissue sections thereby are not routinely taken notice of as their morphology seems to vary only in case of lymphoid malignancies, hematological malignancies apart from certain viral infections. Atypical lymphocytes are the lymphocytes with unusual shape, size or overall structure. These are more commonly known as reactive lymphocytes. The unusual histomorphological feature of these cells includes larger size than normal lymphocytes; in some cells the size exceeds even 30 microns. The large size is the result of antigenic stimulation of the cell. Alongwith these, the other rare feature which is recently coming under light is "Cellular Cannibalism" which is defined as a large cell enclosing a slightly smaller one within its cytoplasm. Previously, this feature was noted only in cases of malignant tumors. But the present section reveals the role of antigenic stimulus for such response. These cells are specifically responsive to bacteria such as those of *Haemophilus* family of the beta subgroup, *Streptococcus* family and *Neisseria meningitidis*. The continuous antigenic stimulus is responsible to evoke the TI challenges which are long lasting. These responses cause continuous production of plasmablast and they are seen in tissue sections.

The objectives of this study were to determine the proportion of atypical lymphocytes in chronic periapical granulomas and cysts; to determine the proportionate cellular cannibalism in these periapical lesions & also to isolate the bacteria's evoking such responses to administer combination antibiotics.

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#### IBV10

##### Trehalose, a temperature- and salt-induced solute with implications in pathobiology of *Acinetobacter baumannii*

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**Introduction:** The nosocomial pathogen *Acinetobacter baumannii* has extraordinary survival abilities. It can withstand desiccation as well as osmo- and thermostress, but the molecular basis for stress protection is obscure.

**Objectives:** We aimed to determine the pool of compatible solutes accumulated in *A. baumannii* in response to osmotic stress and to elucidate their role in stress protection.

**Materials & methods:** The solute pool was analyzed by NMR, HPLC and enzymatic assays. Mutant studies, reporter gene assays and qRT-PCR were used to analyze role and regulation of *otsB*, encoding a trehalose-6-phosphate phosphatase.

**Results:** Glutamate, mannitol and trehalose were accumulated in response to NaCl stress. Deletion of *otsB* completely disabled trehalose biosynthesis. Growth of the

deletion mutant was not impaired at 500 mM NaCl but completely at 45 °C. The *otsBA* promoter was activated by high salt and high temperature. Trehalose pools under high salt and high temperature were comparable.

**Conclusion:** *A. baumannii* has emerged as a notably stress tolerant nosocomial pathogen that accumulates glutamate, mannitol and trehalose as stress protectants. Although mannitol and glutamate relieve osmotic stress, trehalose is specifically required for survival at temperatures above 37 °C. The disaccharide trehalose plays a double role as osmo- and thermoprotectant in *A. baumannii* and is essential for infecting the eukaryotic host [1].

#### References:

[1] M. Gebhardt, L. Gallagher, R. Jacobson, E. Usacheva, L. Peterson, D. Zurawski and H. Shuman (2015), *mbio* **6**: e01660-15

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#### IBV11

##### The role of two *pemIK/mazEF* family toxin-antitoxin systems in the acquisition of vancomycin resistance by staphylococci

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**Introduction:** Staphylococci are one of the most frequent causes of nosocomial and community-associated infections. The problem is exacerbated by their growing antibiotic resistance. This might be exemplified by horizontal transfer of vancomycin resistance genes from enterococci to staphylococci in the course of co-colonisation. The phenomenon may be accelerated by toxin-antitoxin (TA) systems as they promote maintenance of mobile genetic elements.

**Objectives:** The aim of this study was to determine the role of *pemIK-Sa4* and *pemIK-Sa5* TA systems in acquisition and maintenance of vancomycin resistance determinants.

**Materials & methods:** Genome sequences of two vancomycin-resistant *Staphylococcus aureus* (VRSA) strains were examined for presence of *pemIK/mazEF* TA systems. The coding sequences of discovered toxins were cloned into expression vectors to assess their functionality.

**Results:** Operon sequences of *pemIK-Sa4* and *pemIK-Sa5* were found in putatively plasmid contigs in both strains together with vancomycin resistance determinants. Homologous loci were also found in known enterococcal plasmids. The expression of *PemK-Sa4* and *PemK-Sa5* led to severe growth inhibition of bacterial cultures, which clearly suggests that the TA systems are functional and may contribute to vancomycin resistance spreading.

**Conclusion:** The results suggest a potential role of the two newly discovered TA systems in dissemination of vancomycin resistance among different species of bacteria. Notably, these TA systems can potentially promote maintenance of resistance determinants even in the absence of selective pressure.

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#### IBV12

##### Take it with a pinch of salt! Zinc intoxication to control mycobacteria infection!

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Nutritional immunity describes the battle raging at the interface between a host and its intracellular pathogen for access to (micro)nutrients. In the case of transition metals, recent studies revealed a "double edge sword" problem. Indeed, phagocytes can restrict intravacuolar bacterial growth either by depleting essential ions, such as Fe<sup>2+</sup>, or accumulate them, for example Cu<sup>+</sup> and Zn<sup>2+</sup>, to intoxicating concentrations. Despite recent efforts, the roles of NRAMP transporters in Fe<sup>2+</sup> depletion, and ZnT transporters in Zn<sup>2+</sup> accumulation during *Mycobacterium tuberculosis* infection of macrophages are still unclear.

We use the *Dictyostelium/Mycobacterium marinum* system to study the role of iron, zinc and their transporters during mycobacterial infection. We visualised and quantitated the appearance of Zn<sup>2+</sup> in bead- or *M. marinum*-containing vacuoles (MCVs). In regular phagosomes, Zn<sup>2+</sup> is delivered immediately after particle uptake, mainly by fusion with "zincosomes" of endosomal origin. We localised the four *Dictyostelium* ZnT transporters in endosomes, the contractile vacuole and at the MCV membrane and study the impact of knockouts (KOs) on the homeostasis of Zn<sup>2+</sup>. We show that an *M. marinum* KO mutant in *CtpC*, a cation efflux transporter, is attenuated in intracellular growth. We also demonstrate that the virulence of this mutant is further reduced in a *Dictyostelium zntA* KO. Strikingly, in a *Dictyostelium zntB* KO that is depleted in endosomal Zn<sup>2+</sup> *M. marinum* growth is also attenuated.

Finally, we bring evidence that *nramp* KOs of *Dictyostelium* are more susceptible to infection, illustrating the opposite strategies of bacterial control exerted by the host via the homeostasis of different transition metals.

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#### IBV13

##### Biochemistry and bactericidal effect of porcine Calprotectin

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Calprotectin, the heterodimer of S100A8/A9, binds metals like Zn<sup>2+</sup> and Mn<sup>2+</sup> with high affinity. The prolonged c-terminus of S100A9 holds a particular role in the sequestration of metal ions. The human protein is able to bind two of them per dimer [1]. Interestingly, the porcine S100A9 possess a longer, histidine-rich c-terminus, which we here propose to be an enhanced or additional ion binding motif. Since the sequestration of those metals by calprotectin has bactericidal properties, we investigate the ion binding capacity and antimicrobial effect of porcine calprotectin on *Salmonella enterica* choleraesuis. Porcine Calprotectin might provide better bactericidal properties than the human protein due to enhanced sequestration of metals. The genes for porcine S100A8/A9 were expressed in *E. coli* cells. The heterodimer was purified by IMAC and SEC. The metal binding capacity was analysed by structural approaches in vitro and bacterial growth assays in vivo. We showed, that the porcine S100A9 can be purified by IMAC due to its histidine-rich c-terminus that functions as a natural his-tag. Furthermore, we showed the dose dependent antimicrobial properties and the ion binding capacity of porcine

calprotectin. Bacterial superoxide dismutases are dependent on cofactors like Zn<sup>2+</sup> and Mn<sup>2+</sup> and sequestration of these metals by calprotectin compromises their function resulting in increased susceptibility of the cell to oxidative damage [3]. Here we demonstrated the bactericidal activity of porcine S100A8/A9.

#### References:

[1] Damo et al., PNAS 2013

[2] Sansone et al., Microbiology 2002

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#### IBV14

##### Functionality, complex composition and sensitivity of ClpP proteins against acyldepsipeptide antibiotics in *Streptomyces lividans* cells

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Antibiotic acyldepsipeptides (ADEPs) derived from the natural product ADEP1 of *Streptomyces hawaiiensis* NRRL 15010 exhibit an unprecedented mode of action by targeting the proteolytic core (ClpP) of the bacterial caseinolytic protease, an ATP-dependent serine protease significantly involved in protein homeostasis.

ADEPs inhibit all natural functions of ClpP by outcompeting the associated Clp-ATPases for binding, while simultaneously inducing a conformational change in ClpP that leads to opening of the entrance pore to the proteolytic chamber. Thus, indispensable cell proteins are able to enter and fall prey to uncontrolled degradation with a lethal outcome.<sup>1</sup>

ClpP is highly conserved among prokaryotes and in the genomes of most bacteria usually one or two homologs are present. Intriguingly, streptomycetes encode multiple copies<sup>2</sup>, raising the question of interaction between their ClpPs in the physiological context, as well as in the presence of a deregulating antibiotic.

*S. lividans* encodes five ClpP homologs, organized in one monocistronic and two bicistronic operons. The expression of ClpP1/2 and ClpP3/4 is tightly regulated and either ClpP1/2 or ClpP3/4 are essential for viability.<sup>3</sup> Here we investigate ClpP functionality and complex composition in *S. lividans* as well as the sensitivity of different ClpP homologs against ADEP1 by Western Blotting, bioassay analyses and studying specific *clpP* knockout mutants. We show that the ClpP homologs differ in their sensitivities to ADEP1 and that interaction between them is necessary for the processing of individual ClpP monomers and functionality of the ClpP complex.

#### References:

[1] Brötz-Oesterhelt et al. (2005) *Nat. Med.*, 11(10):1082-1087

[2] Viala et al. (2000) *Mol. Microbiol.*, 38(3), 602-612

[3] Gominet et al. (2011) *Microbiology*, 157:2226-2234

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#### IBV15

##### Proteomic response of *Streptococcus pneumoniae* to iron limitation

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*Streptococcus pneumoniae* causes annually more than one million infections ranging from mild forms like otitis media to severe diseases such as septicemia worldwide. In the majority of cases, children under the age of five, elderly and immune-suppressed patients are affected. Iron as an essential trace element is involved in various key metabolic pathways in bacterial lifestyle. Within the human host iron is extremely limited. Hence, the acquisition of iron from the environment is essential for a successful infection of bacteria. Therefore, pneumococci have to adapt their metabolism and virulence factor repertoire to various host niches for survival and proliferation. In this study the response of *S. pneumoniae* to iron limitation as infection-relevant condition was investigated on the proteome level by mass spectrometry.

To induce iron limitation, the iron chelator 2,2"-bipyridine (BIP) was applied in two different media mimicking different physiological situations. Thus, the influence of the initial iron concentration on pneumococcal protein expression in response to restricted iron availability was investigated. Interestingly, one major difference between these two iron limitation experiments is the regulation of proteins involved in pneumococcal pathogenesis. In iron-poor medium several proteins of this group were downregulated whereas these proteins are upregulated in iron-rich medium. However, iron limitation in both environments led to a strong upregulation of the iron uptake protein PiuA and the significant downregulation of the non-heme iron-containing ferritin Dpr.

On the basis of these results, we demonstrated that the pneumococcal proteome response to iron limitation is strongly dependent on the initial iron concentration in the medium or the environment.

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#### IBV16

##### Combining multiple sequencing technologies to investigate mono- and multispecies communities in necrotizing soft tissue infections

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Necrotizing soft tissue infections (NSTI) are the most dramatic form of bacterial induced tissue pathology accompanied by extensive tissue inflammation and necrosis. NSTI is mainly caused by monomicrobial infections with the human pathogen *Streptococcus pyogenes*, but also associated with polymicrobial communities. The EU project INFECT was founded to unravel the complex host and pathogen interactions associated with NSTI development and progression.

Within this project we analyzed tissue biopsy samples of randomly selected NSTI patients from Scandinavian clinics.

16S rDNA amplicon sequencing identified mono-infections caused by streptococci and poly-infections to dominate the analyzed cohort. Poly-infections typically consisted of a defined set of commensal Clostridiales and Bacteroidales species in varying composition.

A subset of randomly selected samples was further characterized by *in vivo* metatranscriptomic host/pathogen analysis through RNA-Sequencing. This approach revealed distinct host responses potentially linked to differences in the metabolic capabilities and the expressed virulence factor profile of the identified bacterial agents.

In conclusion, this study will contribute to a better understanding of the pathophysiology of NSTI and enable the development of novel pathogen-specific treatment strategies.

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## Short Lecture

### Microbial Cell Biology 2 and Gene Regulation 2

18 April 2018 • 09:00–11:00

#### MCBV09

##### Disaggregation by AAA+ ATPase ClpC in *B. subtilis* cells is facilitated by adaptor protein McsB

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In *B. subtilis* the AAA+ ATPase and unfoldase ClpC is intricately involved in protein homeostasis and together with ClpP in regulatory proteolysis controlling stress response and developmental pathways such as competence development. ClpC relies predominantly on its set of adaptor proteins like MecA and McsB for its activation and substrate selection. MecA was discovered as the regulated adaptor protein responsible for the control of competence development by regulatory proteolysis of key regulator ComK, however MecA does not appear to be involved in heat stress response and protein homeostasis. In contrast, McsB is involved in the regulation of heat stress response by controlling the activity and stability of the heat shock repressor CtsR. ClpC was recently implicated as a new target for antibiotics, possibly because a misactivation and deregulated substrate selectivity caused by antibiotics interacting with ClpC resulted in cell death. Recently the identification and characterization of a hyperactivated and toxic ClpCF436A variant corroborated this hypothesis. Here we investigate the role of McsB in protein homeostasis with fluorescence microscopy using an aggregate specific reporter *in vivo* and model substrates *in vitro*, demonstrating that McsB but not MecA mediates the ClpC dependent disaggregation and/or degradation of aggregates. In a second set of experiments we demonstrate that expression of the hyperactivated ClpCF436A results in drastic increase of subcellular aggregates. Interestingly this toxic phenotype depends on the presence of McsB substantiating our finding that McsB is the adaptor enabling ClpC's role in protein homeostasis and suggesting that a deregulation and hyperactivation of the cellular protein quality control system can result in cell death.

#### MCBV10

##### Dynamics in Live Cells and Single-Molecule Tracking of DNA Polymerases from *Bacillus subtilis* reveals distinct functions for the three major polymerases

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<sup>1</sup>Philipps-University Marburg, SYNMIKRO (Centre for Synthetic Microbiology) and Department of Chemistry, Marburg, Germany

DNA replication is driven by two or three major DNA polymerases in all cells. In eukaryotes, two different polymerases mediate replication at leading or lagging strand, and polymerase I removes RNA primers at the lagging strand. In *Escherichia coli* cells, the same polymerase (PolC) acts at both strands, and polymerase I (PolA) removes RNA hybrids. Our work reveals that in *Bacillus subtilis*, PolA is not involved in DNA replication, but functions during DNA repair throughout the chromosome, while PolC and DnaE have different binding kinetics at the replication forks, in agreement with their involvement in leading or lagging strand synthesis. Our data suggest that *B. subtilis* forks represent a third scheme for DNA replication, and provide an explanation why PolA is dispensable for growth in *B. subtilis*, but not in *E. coli*. We are therefore able to study the binding events at replication forks (static fraction) and the number of dynamic (non-bound) polymerases, and we can see differences in population sizes dependent on damage induction in case of PolA. We are also able to see where static molecules are positioned within the cell, and relative to replication forks, which can be visualized using DnaX-CFP as marker. Our initial results suggest that PolA is generally absent from the forks, indicating that *B. subtilis* replication forks are more eukaryotic-like. We will systematically analyze all DNA polymerases and finally determine.

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#### MCBV11

##### Identification of the first complete *in vivo* interactome of a genome-reduced bacterium

C. Blötz\*<sup>1</sup>, J. Stülke\*<sup>1</sup>

<sup>1</sup>University of Goettingen, General Microbiology, Goettingen, Germany

Cellular life depends on the functional interactions between biological molecules, and in particular on the interactions between proteins. Indeed, most proteins are only active when present in complexes. For a long time, bacteria were thought to have poor internal organization. In the past decade, the discovery of pathway-specific protein complexes, so-called metabolons has contributed to our understanding of bacterial physiology. So far, high throughput analyses of protein-protein interactions have relied on artificial systems whereas high-throughput *in vivo* interactions screens have not been possible. Due to their small genomes, bacteria of the genus *Mycoplasma* have attracted much attention in systems and synthetic biology. We have developed an setup for *in vivo* cross-linking of proteins and subsequent mass spectrometric identification of the interacting peptides, and have applied this technique to detect *in situ* protein-protein interactions in *M. pneumoniae*. In this study, more than 90% of the proteins that are expressed in wild type cells were covered by cross-linked peptides. In total, we detected more than 9,000 intramolecular cross-links from which topological information can be deduced, and nearly 400 intermolecular cross-links that specify interacting proteins. Our data provide valuable insights into the proteome organization and allowed the identification of novel protein functions based on the interaction profiles. Moreover, we got important insights into the interacting machineries of RNA and protein synthesis. To the best of our knowledge, this unprecedented study is

allows for the first time the identification of the complete *in situ* interactome in a complete living organism.

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## MCBV12

### Deciphering the glycogen mobilization strategy that enables resuscitation of a dormant cyanobacterium from chlorosis

S. Doello<sup>\*1</sup>, A. Klotz<sup>1</sup>, K. Gutekunst<sup>2</sup>, K. Forchhammer<sup>1</sup>

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During the process of awakening from nitrogen-chlorosis, the cyanobacterium *Synechocystis* sp PCC 6803 passes through a heterotrophic and mixotrophic phase before re-entering photoautotrophic growth. In this study, we investigated the contribution of the different carbon catabolic routes in this process using knockout mutants on key enzymes in carbon catabolism. Addition of nitrate to nitrogen-starved cells immediately starts the awakening program. Metabolism switches from a maintenance phase, characterized by residual photosynthesis and low cellular ATP levels, to an initial heterotrophic metabolic phase, characterized by respiration and a rapid increase in ATP levels. Respiration relies on glycogen degradation, catalyzed by glycogen phosphorylase GlgP2. Cells enter then a transient phase of mixotrophy and re-start photosynthesis while glycogen is still being consumed. Successful resuscitation depends on the parallel operation of the oxidative pentose phosphate (OPP) cycle and the Entner-Doudoroff (ED) pathway while the glycolytic route via the Embden-Meyerhof-Parnas pathway seems of minor importance. Interestingly, the key enzymes for glycogen catabolism are already expressed during the preceding chlorotic phase, preparing for rapid resuscitation. Overall, our results show that the awakening of dormant *Synechocystis* cells from chlorosis requires a delicate combination of carbohydrate oxidation and photosynthesis.

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## MCBV13

### A glutamine riboswitch is a key element for the regulation of glutamine synthetase in cyanobacteria

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Glutamine synthetase (GS) is a key enzyme of bacterial nitrogen (N) assimilation. Accordingly, it is targeted by complex regulatory mechanisms at multiple levels. These include transcriptional and allosteric control and also covalent modifications impacting enzymatic activity. Cyanobacteria are unique among bacteria because they have evolved specific inactivating factors (IFs), which regulate GS activity linear with their abundance. Many cyanobacteria possess two different IFs, encoded by the genes *gifA* (IF7) and *gifB* (IF17). Their expression is controlled by NtcA, the main transcriptional regulator of N-assimilatory genes. Here we show that translation initiation of the IF17 mRNA is regulated by a riboswitch. Riboswitches

consist of RNA aptamers located in 5'UTRs of various mRNAs where they selectively bind metabolites that trigger changes in RNA structural conformation. In-line probing assays using the 5'UTR of *gifB* from *Synechocystis* sp. PCC 6803 confirmed the selective binding of L-glutamine, which is also the primary product of GS activity. *In vivo*, the glutamine-induced structural conformation strongly increased *gifB* translation. Mutagenesis of the riboswitch confirmed its biological function as a critical control element for IF17 production and the regulation of GS activity. Hence, this is the first detailed report on the biological function of a glutamine riboswitch. Glutamine riboswitches are frequently but exclusively found in cyanobacteria, where they are primarily associated with *gifB* gene homologs. The RNA-based sensing mechanism establishes glutamine as an additional signaling molecule in cyanobacterial N control.

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## MCBV14

### Posttranscriptional regulation of SAM and m6A modification of RNA in the alpha-proteobacterium *Sinorhizobium meliloti*

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The key bacterial RNases E and J co-exist in the nitrogen fixing plant symbiont *Sinorhizobium meliloti*. We found that in *S. meliloti*, both RNases are needed for homeostasis of the major methyl donor SAM. In *rne* and *rnj* mutants the SAM level was increased sevenfold, explaining many overlapping effects like the threefold increase in the N6-methyladenosine (m6A) content (Baumgardt, Melior et al. 2017). This suggests that RNase E and RNase J have one or very few common sRNA or mRNA targets responsible for SAM regulation. To identify them, mutant strains that were complemented by IPTG-induced ectopic expression of the respective RNase, were subjected to RNase depletion. Already 5 min after IPTG removal, the *metB* mRNA level was increased six-fold in each depleted strain. We considered transcript level changes upon this short-term depletion as primary effects and performed RNAseq analysis. This revealed an overlap of 19 transcripts (putative substrates of both RNases) with levels changed upon depletion of RNase E and RNase J. Several genes out of the 19 candidates are under investigation. Furthermore, we found that secondary effects of RNase depletion like changes in the amount of N-acyl homoserine lactones and in the m6A-level are observed 15 and 25 min after IPTG removal, respectively. To get insights into genome-wide m6A modification, m6A iCLIP was performed before and after 25 min of IPTG removal. The results suggest that for many sRNAs and mRNAs a small proportion is methylated at specific sites in the RNase-complemented state, and this proportion is increased upon depletion. In addition, methylation at new or alternative sites was observed. For example, increased methylation and new methylation was detected in single stranded regions of the transcription regulator 6S RNA.

## MCBV15

### The involvement of the antisense RNA RSaspufL in regulated formation of photosynthesis complexes in *Rhodobacter sphaeroides*

C. M. Reuscher<sup>\*1</sup>, G. Klug<sup>1</sup>

<sup>1</sup>Institute for Micro- und Molecularbiology, Gießen, Germany

The facultative phototrophic model bacterium *Rhodobacter sphaeroides* is known for its metabolic versatility. Under microaerobic conditions it synthesizes intracytoplasmic membranes harbouring the pigment protein complexes needed for anoxygenic photosynthesis. For fast adaption to varying environmental conditions the transcription of genes in the photosynthetic gene clusters is tightly regulated. The *puf* operon comprises genes which encode proteins of the light harvesting complex I (LHI) and of the reaction centre (RC). In the near relative *R. capsulatus* an RNase E cleavage is essential for differential *puf* mRNA processing and degradation which contributes to the stoichiometry of LHI and RC complexes. For *R. sphaeroides* RNAseq and Northern blot analysis of transcripts derived from the *puf* operon unveiled that also certain small RNAs are transcribed. Up to date two different *puf* operon associated sRNAs were identified. The Hfq-dependent sRNA RSaspufL was detected antisense to the 5' region of the *pufL* gene extending into the *pufA-pufL* intercistronic region. Northern blot results confirmed the presence of RSaspufL (~ 180 nt) under microaerobic and phototrophic conditions. An artificial increase in the amount of the RSaspufL by plasmid driven over-expression led to a reduction in the amount of LHI/RC-complex. Additionally, we could show that the over-expression also influences the half-life of the polycistronic *puf* mRNA. Because of the fact that RSaspufL overstretches an RNase E cleavage site and the Shine-Dalgarno sequence of *pufL* we hypothesize that the antisense RNA RSaspufL contributes to the regulated processing and degradation of the *puf* mRNA in *R. sphaeroides* which should be investigated via Northern blot analysis, Real-Time PCR and reporter gene fusion.

## MCBV16

### CRISPR RNA-guided target recognition by minimal Class I surveillance complexes

H. Müller-Esparza<sup>\*1</sup>, D. Gleditsch<sup>1</sup>, L. Randau<sup>1</sup>

<sup>1</sup>Max Planck Institute for Terrestrial Microbiology, Marburg, Germany

**Question:** Type I CRISPR-Cas systems employ Cascade surveillance complexes to facilitate duplex formation between CRISPR RNA (crRNA) and complementary target DNA. The targeted DNA contains a protospacer adjacent motif (PAM) that is usually recognized and bound by a large Cascade subunit. Small subunits bind the displaced non-target DNA strand. We have identified a minimal Type I CRISPR-Cas variant in *Shewanella putrefaciens* CN32 that lacks large and small subunits and investigated how target recognition is achieved.

**Methods & Results:** This CRISPR-Cas system was transferred into *Escherichia coli* and PAM-dependent interference activity against phages and plasmids was observed (1). Cas7fv was identified to bind the crRNA spacer sequence and modulation of the crRNA length resulted in varying numbers of Cas7fv Cascade subunits (2). Structures of Cascade were solved in the absence and presence of target DNA (3). The subtype-specific protein Cas5fv was shown to bind to the crRNA 5'-tag and to recognize a "GG" PAM sequence via a specific alpha-helical domain. In addition, Cas5fv and Cas7fv proteins were found to contain lysine-rich "wrist" loops, which guide and stabilize the non-target strand. These features compensate for the missing

large and small Cascade subunits and highlight a minimalistic solution for efficient DNA surveillance.

**Conclusions:** Cascade assemblies are vulnerable to inhibition by viral anti-CRISPR proteins and Cascade structure variations are proposed to represent anti-anti-CRISPR measures.

## References:

[1] Dwarakanath, S. et al. (2015) Nucl Acids Res, 43, 8913-8923.

[2] Gleditsch, D. et al. (2016) Nucl Acids Res, 44, 5872-82.

[3] Pausch, P. et al. (2017) Mol Cell, 67, 622-632.

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## Short Lecture

### Microbial Diversity – Drivers of Bacterial Evolution

18 April 2018 • 09:00–11:00

#### MDV01

##### Reconsidering microfossil morphologies via induced cellular plasticity of iron cycling bacteria

D. Kanaparthi<sup>\*1</sup>, A. Klingl<sup>2</sup>, B. Zhu<sup>1</sup>, T. Lueders<sup>1</sup>

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Although the theory of chemical evolution with an intermediate proto-cell stage is now widely considered to be the way biological life had evolved on earth, evidence for the actual existence of such proto-cells on earth is at lack. Here we compared the morphologies of Pre-Cambrian microfossils with that of active bacteria, after transforming them into liposome-like cells, which have lost all control over their morphology or cell division. Unlike the lengthy process of genetic modifications required for such a transformation, we achieve this by manipulating the osmolality of the media. In such a state, phototrophic Fe+2 oxidizing and Fe+3 reducing bacteria we tested exhibited morphologies that closely resemble the oldest known microfossils on earth found within banded iron formations (BIF), formed between 3.7-2Ga. Using this top down approach we were able to replicate the morphology of a wide span of described microfossils, many of which have been previously considered to be morphologically too complex for single bacteria. Our work suggests that the some of the most studied Pre-Cambrian microfossils, which were assumed to be Cyanobacteria or Eukaryotic algae are most likely liposome like proto-cells. Our work also contradicts earlier hypothesis about the evolution of Cyanobacteria during the Archaean eons and their role in the formation of BIFs. We propose that the microbial fossils observed in the Pre-Cambrian BIFs are protocells of Fe+2 oxidizing and Fe+3 reducing bacteria. Our results have profound impact on our understanding on the early evolution of cellular morphologies and the formation of banded iron formations.

## MDV02

### Diversity of timing systems in Bacteria

I. M. Axmann<sup>\*1</sup>, N. Schmelling<sup>1</sup>

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We know the time of day even without a wrist watch – an inner clock in our cells is ticking, providing this information. Although there is substantial evidence on how clocks are functioning in diverse model organisms, little is known on how such an internal timer evolved. Astonishingly, prokaryotic cyanobacteria, although dividing faster than once a day, also use an inner timing system to foresee the accompanying daily changes of light and temperature and regulate their physiology and behavior in 24-hour cycles. The underlying biochemical, post-translational oscillator made of solely three proteins is evolutionary not related to transcriptional-translational based circadian systems prevailing in eukaryotes. Reconstituted from the purified protein components KaiC, KaiB, and KaiA, it can tick autonomously in the presence of adenosine 5'-triphosphate (ATP). Over the past 10 years, we discovered how the biological clock in cyanobacteria works in detail providing a structural basis to understand periodic assembly of the protein oscillator. Our systematic analysis of 11,264 genomes clearly demonstrates that components of the "standard" KaiABC clock are present in Bacteria and Archaea. However, all components of the clock and its in- and output pathways are less abundant in other organisms than Cyanobacteria and some components are only present in the latter. Thus, only reduced KaiBC-based or even solely KaiC-based timing systems might exist in Bacteria and Archaea, which might be capable of driving simple timers like hourglasses.

## MDV03

### The pan-genome of *Geobacillus* and *Parageobacillus* species: an insight into protein repertoire and evolution of the geobacilli

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The genera *Geobacillus* and *Parageobacillus* comprise of metabolically and ecologically versatile aerobic thermophiles that have become prominent because of their use in a wide range of biotechnological applications. While extensive research has been done on the thermostable enzymes and biomolecules they produce, little is known about their evolutionary histories. Here we describe a comprehensive analysis of the protein repertoire encoded in the genomes of seventy-four "geobacilli" with a view to provide additional insight into their evolution. A total of 12,449 distinct gene families, including 10,747 and 7,561 for the genera *Geobacillus* and *Parageobacillus*, respectively, were predicted to constitute the open "geobacilli" pan-genome. Of these, 853 and 1,753 gene families represent the core genome of *Geobacillus* and *Parageobacillus*, respectively, with the core genome of the latter genus reaching a plateau. Reconstruction of the patterns of genome evolution showed that, since their diversification, the two genera have undergone pervasive gene loss events. However, the data pointed at initial genome expansion events in the ancestral genomes of both genera with the last common ancestor of the *Geobacillus* spp. showing significant gains in genes linked to DNA replication, recombination and repair, including

a large cache of mobilome and bacterial defense features. Analysis of microevolution revealed that individual genes that are associated with important metabolic features showed significant signatures of selection pressure. Overall, the data suggest that the diversification of the extant "geobacilli" was shaped by different evolutionary events that gave rise to variations in both the genome structure and functional capacity between the two genera.

## MDV04

### Evolution of marine surface-associated bacteria revealed by population genomics of *Phaeobacter*

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The broad functional capacity of microorganisms in the ocean is sustained by a high diversity of marine bacterial species. Especially bacteria associated with particles or eukaryotic organisms show a higher diversity probably due to the microscale heterogeneity of their environment. This raises questions about the extent of genomic diversity of species with this substantially understudied lifestyle, their evolution and potential adaptation to ecological niches especially in contrast to so far investigated generalists or free-living bacteria. To answer these questions complete genomes of 32 closely related strains of the disjunct surface-associated *Phaeobacter* from the *Roseobacter* group were generated and their population structure and evolution elucidated by population genomic comparisons in combination with phenotypic characteristics. Interestingly, all strains share an exceptional large and highly syntenic core genome as known for bacteria with specialized lifestyles not metabolic versatile ones like *Phaeobacter*. In contrast to pelagic bacteria like *Pelagibacter*, gene flow analysis revealed that *Phaeobacter* chromosomes were continuously but slightly expanding over evolutionary time. The strains form clearly delineated phylogenomic clades which correspond to phenotypic clusters based on substrate utilization and to clusters based on functional predictions of proteins but are independent of geographic distribution. These clade-specific differences indicate adaptive advantages to different niches existing on marine surfaces which likely drive ongoing diversification. Our study reveals that the evolutionary trajectories of surface-associated marine bacteria can differ notably from free-living marine bacteria or marine generalists.

## MDV05

### A new order of homoacetogenic *Deltaproteobacteria* from insect guts (*Acetispirales* ord. nov.)

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Reductive acetogenesis from H<sub>2</sub> and CO<sub>2</sub> is an important process in termite guts. It has been attributed to a termite-specific lineage of spirochetes. Recent studies documented that the enzymes required for the Wood-Ljungdahl pathway are present also in the genome of '*Candidatus* Aduitrix intracellularis', an endosymbiont of termite gut flagellates that represents a deep-branching clade of *Deltaproteobacteria*. The Rs-K70 group is widely distributed in intestinal tracts of termites and cockroaches but has so far no cultured representatives. We isolated the first representative of this group from cockroach guts. *Acetispira formosa* gen. nov. sp.

nov. grew by reduction of CO<sub>2</sub> with hydrogen or formate and by the homoacetogenic fermentation of glucose and *N*-acetylglucosamine. Comparative genome analysis including the endosymbiotic '*Ca. Adiatrix intracellularis*' and the draft genome of an uncultured strain from a higher termite reconstructed by metagenomic analysis revealed the same set of genes involved in reductive acetogenesis as described for the endosymbiont. The hydrogen-dependent CO<sub>2</sub> reductase, a key enzyme of the pathway, which was most likely acquired via lateral gene transfer, appears to be of clostridial origin. However, all genomes lack genes for cytochromes and genes encoding an Rnf complex, which are involved in energy conservation in homoacetogenic *Firmicutes*. Instead, they possess an 11-subunit complex, which has been implicated in energy conservation of methyl-reducing methanogens, suggesting a new mode of energy conservation in *Acetispirales*, the first order of *Deltaproteobacteria* with a homoacetogenic metabolism.

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## MDV06

### Underlying convection currents and cell diffusion drive the evolution of Wrinkly Spreaders in static microcosms

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Adaptive radiation is a central process in evolution and has been investigated extensively in experimental bacterial evolution studies. In static liquid microcosms, populations of *Pseudomonas fluorescens* SBW25 radiate to produce a range of lineages including the Wrinkly Spreaders. These mutants have a competitive fitness advantage over the ancestor as they colonise the air-liquid (A-L) interface by forming a robust biofilm which allows better access to O<sub>2</sub> diffusing into the liquid column. We postulate that the underlying factor driving the adaptive success of the Wrinkly Spreaders in these static microcosms are the small convection currents and random cell diffusion that constantly move cells out of the top O<sub>2</sub>-rich layer immediately below the A-L interface which cannot be effectively countered by O<sub>2</sub>-directed swimming motility (aerotaxis) towards the surface. In this work we investigate the impact of currents and diffusion on cell distributions using the protonophore CCCP to inhibit swimming, and demonstrate that increasing liquid viscosity using very low concentrations of agar or polyethylene glycol effectively reduces Wrinkly Spreader numbers in radiating populations as well as competitive fitness. These findings add to our growing understanding of how abiotic and biotic factors contribute to the adaptive success of the Wrinkly Spreaders: ecosystem engineering by the ancestral colonists generate an O<sub>2</sub> gradient producing the ecological opportunity for Wrinkly Spreaders, but it is the underlying need to efficiently maintain position in the O<sub>2</sub>-rich layer by resisting currents and diffusion by biofilm-formation that ultimately explains the adaptive success in these static microcosms.

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## MDV07

### Global warming shifted the composition of the bacterial phyllosphere microbiota of *Galium album* and *Arrhenatherum elatius* plants grown in a long-term warming field-experiment

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Global warming affects plants and directly or indirectly microbes colonizing the phyllosphere (areal part of plants). Aim of this study was to determine the effects of increased surface temperature on phyllosphere inhabiting bacteria diversity and community composition in a seven-year running IR-lamp field-experiment. A cultivation-dependent approach was performed focused on abundant methylotrophic and heterotrophic bacteria and correlated with cultivation-dependent data with cultivation-independent analysis based on 16S rRNA gene amplicon studies. Leaves of two abundant plant species, *Galium album* and *Arrhenatherum elatius*, were collected from four control (C) and four warmed (T) plots, after continuous exposure to +2°C surface temperature. Warming significantly changed stomata aperture size and leaf metabolites of both plants. Concentrations of culturable bacteria was significantly lower on *G. album* leaves grown at +2°C. Non metric multidimensional scaling (NMDS) based on phylogenetic composition of leaf-associated bacteria showed, that the community composition was effected by plant species but stronger by elevated surface temperature. Clear differences in the composition of abundant cultured heterotrophs and methylotrophs were observed for both plants, which correlates with cultivation-independent data. Among 124 identified isolates several phylotypes occurred specifically in T plots. Genomic fingerprints and *Methylobacterium mxaF* gene analyses showed a correlation of specific genotypes and warming. We showed for the first time that global warming negatively affects the abundance of antagonists (*Sphingomonas*, *Methylobacterium*) and leads to an adaptation of specific ecotypes including potential pathogens in the phyllosphere of the two plant species.

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## MDV08

### Apple roots recruit different microorganisms from the same soil type but with different Apple Replant Disease history

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Apple Replant Disease (ARD) is a known world-wide problem that occurs when apple trees are planted repeatedly at the same site leading to a general growth reduction with consequent losses in fruit yield and quality. Although in the last two decades many studies have been performed, its etiology remains unclear.

Our study aims at unraveling the role of bacteria in ARD. A split root experiment was performed where apple seedlings were grown in rhizoboxes with different combinations of ARD soil (+ARD), gamma treated ARD soil (-ARD) and Grass soil (Control), taken from the same site in Germany (Ellerhoop). Total community DNA was extracted from rhizoplane (RP),

rhizosphere (RS) and bulk soil (BK) to study the bacterial communities.

ARD soil led to significant decrease in root growth in comparison to -ARD and Control soils. Fingerprinting analysis of 16S rRNA gene amplicons (Denaturing gradient gel electrophoresis, Illumina MiSeq) revealed significant differences in the bacterial community structure between +ARD vs. control in the rhizosphere and +ARD vs. -ARD soils in RP, RS, BK. Interestingly, a significantly lower evenness in RP of plants grown in +ARD soils in comparison to control soils was found, in contrast to RS and BK where the trend is the opposite. Moreover, an increase of unique taxa was observed in BK compared to RS and RP in +ARD soils. *Streptomyces*, *Variovorax*, *Niastella* and *Methylophilaceae* were highly enriched in the RP and RS in +ARD soils in comparison to control soils.

In summary our results showed an effect of the apple planting history on the soil bacterial community composition. A shift of the RP microbiome towards a dysbiosis after continuous apple planting is suggested that may be involved in causing ARD.

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## Short Lecture

### Environmental Microbiology and Ecology 4

18 April 2018 • 09:00–11:00

#### EMV25

##### Important late stage symbiotic role of *Sinorhizobium meliloti* exopolysaccharides

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**Introduction:** *Sinorhizobium meliloti* enters into a beneficial symbiotic interaction with species of *Medicago* legumes. Bacterial exopolysaccharides play critical signaling roles in infection thread initiation and growth during the early stages of root nodule formation. After endocytosis of *S. meliloti* by plant cells in the developing nodule, plant-derived nodule-specific cysteine-rich (NCR) peptides, which resemble mammalian defensins, mediate terminal differentiation of the bacteria into N<sub>2</sub>-fixing bacteroids. The legume symbiosis is an excellent model system to study molecular mechanisms of persistent bacterial infections.

**Objective:** We investigated if and how *S. meliloti*'s two symbiotically active bacterial exopolysaccharides, succinoglycan and galactoglucan, can protect *S. meliloti* against the antimicrobial activity of NCR247.

**Methods:** In addition to bacterial sensitivity and resistance studies, we used gel filtration and bio-layer interferometry to assess molecular interactions between succinoglycan and NCR247.

**Results and Conclusion:** We discovered that high molecular weight (HMW) forms of both exopolysaccharides have the ability to protect from the antimicrobial activity of the NCR247 peptide. The protective function of HMW succinoglycan occurs via direct molecular interaction between anionic succinoglycan and the cationic NCR247 peptide but this interaction is not chiral. Taken together, our

observations suggest that *S. meliloti* exopolysaccharides are not only critical during early stage nodule invasion, but are also up-regulated at late stage symbiosis to protect against the bactericidal action of cationic NCR peptides. Our findings are an important step forward to fully understand the complete set of exopolysaccharide functions during the legume symbiosis.

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#### EMV26

##### High diversity of soil microbiome reduces survival of *Salmonella* in the phytosphere

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Foodborne diseases are increasingly associated with fresh fruits and vegetables and *Salmonella* was their second most frequent cause in 2015. The biological diversity of soil plays a major role in the establishment of *Salmonella* in plant environment. Different members of the microbiome can be direct antagonist or can induce plant immune system. Here, we analyzed the tripartite interactions between tomato, lettuce and corn salad plants grown under greenhouse conditions, the human pathogen *Salmonella enterica* and the soil microbial community. We observed that *Salmonella* persisted in the rhizosphere of lettuce and tomato. In contrast, its numbers declined in the rhizosphere of corn salad. Very important was the observation that reduction of microbial diversity in soil increased the ability of *Salmonella* to persist in this environment. These results clearly show a dependency between the potential of *Salmonella* to colonize the rhizosphere and bacterial richness as well as the high physiological plasticity of *Salmonella*. In the following, we focused on the impact of induced resistance. In greenhouse experiments we primed crop plants by inoculation of *Ensifer meliloti* to the soil close to the roots. This bacterium produces the signaling molecule *N*-acyl-homoserine-lactone which might induce resistance against *Salmonella enterica*. Our results show that priming has a negative effect on the persistence of *Salmonella*. Primed plants are able to express defense related genes earlier than unprimed plants and are able to keep stomata closed. These results indicate the potential of priming to enhance resistance against *S. enterica*.

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#### EMV27

##### Understanding the chemical ecology of freshwater sponge-bacteria interspecies interaction

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Marine sponges are known as rich source of novel small molecules, which are mainly produced by their associated (symbiotic) microbiota. In contrast, sponges from freshwater habitats are barely studied and little is known about their associated (symbiotic) bacteria. In this proof of our concept study, we applied cultivation independent 16S rRNA amplicon sequencing and metagenomics along with two different cultivation approaches from freshwater sponges

sampled at different German lakes. First, we aimed for novel potential producers of bioactive molecules from the phylum Planctomycetes in a targeted cultivation approach. Second, we performed unbiased high-throughput cultivation to aim for novel bacteria from less-studied phyla to reveal their secondary metabolite potential. We isolated the novel planctomycetal strain spb1 from *Ephydatia fluviatilis* (targeted approach) and 11 strains belonging to a novel genus of the rarely cultivated phylum Gemmatimonadetes obtained from *Spongilla lacustris* (unbiased cultivation). The genome sequence of spb1 revealed the potential to produce small molecules while its cell biology points towards the utilization of complex carbon substrates through a novel fiber mediated uptake mechanism. Whether this strain is symbiotic, commensal or even pathogenic for its sponge host remains enigmatic. The same is true for the novel genus from the barely studied Gemmatimonadetes. They show an unusual filamentous cell morphology, while most free-living Gemmatimonadetes are rather rod shaped sometimes elongated or branched. First HPLC-MS2 analysis point towards production of small molecules as well. However, our ultimate goal is to reveal the entire microbiota of freshwater sponges and to unearth the secrets of their chemical interaction with the sponge.

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#### EMV28

##### **N-acyl-homoserine lactones are required for root colonization and beneficial activities of the Alphaproteobacterium *Rhizobium radiobacter* RrF4**

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The Alphaproteobacterium *Rhizobium radiobacter* F4 (*RrF4*) was originally isolated from the plant growth-promoting basidiomycete fungus *Piriformospora indica* (syn. *Serendipita indica*) that forms a tripartite Sebacinalean symbiosis with a broad range of host plants. Interestingly, the isolated bacterium showed biological activities widely comparable to those exhibited by *P. indica*, through the mechanism by which these are achieved is not fully understood. Chemical analysis showed that *RrF4* produces a spectrum of different N-acyl-homoserine lactones (AHLs) with acyl chains of C8, C10, and C12 and hydroxyl- or oxo-substitutions at the C3 position. To assess the impact of *RrF4*-produced AHLs on its beneficial activities, the AHL-depleted lactonase overexpressing strain *RrF4*NM13 was generated and assessed for colonization efficiency and beneficial activity. Quantitative PCR analysis showed that the colonization of wheat roots by *RrF4*NM13 was reduced at different time points compared with *RrF4*. Consistent with this, wheat biomass in *RrF4*NM13-treated plants was reduced compared to plants treated with *RrF4* wild-type. Furthermore, systemic resistance induced in wheat and *Arabidopsis* to the bacterial leaf pathogens *Xanthomonas translucens* pv. *translucens* and *Pseudomonas syringae* pv. *tomato* DC3000 was reduced in *RrF4*NM13-colonized plants compared to control plants colonized by the *RrF4* wild-type. Moreover and consistent with the above findings, growth promotion mediated by *RrF4* was greatly reduced in *RrF4*NM13-colonized *Arabidopsis*. In conclusion, our results show that AHLs are essential for biological activities of *RrF4*.

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#### EMV29

##### **Diversity, specificity, co-occurrence and hub taxa of the bacterial–fungal pollen microbiome**

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Flower pollen represents a unique micro-habitat, but the factors driving microbial assemblages and the microbe–microbe interactions remain largely unexplored. The aim of this work was to compare the structure and diversity of the bacterial–fungal microbiome associated to eight different pollen species (four wind–pollinated and four insect–pollinated) collected from close geographical locations. We used high-throughput sequencing of 16S rRNA gene (bacteria) and ITS2 (fungi) to characterize the pollen microbiome. Correlation of co-occurrence patterns was used to investigate the potential microbe–microbe interactions. *Proteobacteria* and *Ascomycota* were the most abundant bacterial and fungal phyla, respectively. The family *Enterobacteriaceae* and the genus *Cladosporium* were the most abundant taxa for bacteria and fungi, respectively. Both bacterial and fungal microbiota were significantly influenced by plant species and pollination type, but showed a core microbiome consisting of 12 bacterial and 33 fungal genera. Co-occurrence analysis highlighted significant inter- and intra-kingdom interactions, and positive correlations prevailed over the negatives. The interaction network was shaped by four hub taxa (all bacteria): *Methylobacterium* (two OTUs), *Friedmanniella* and *Rosenbergiella*. *Rosenbergiella* was specifically enriched in the insect-pollinated pollen, and was negatively correlated with the other hubs, thus indicating habitat preference of the microbial clusters. However, interkingdom correlations showed a prevalent effect of fungal on bacterial taxa. This study enhances our basic knowledge of pollen microbiota, and poses the basis for further inter- and intra-kingdom interaction studies in the plant reproductive organs.

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#### EMV30

##### **The quorum-sensing molecule**

##### **N-hexanoyl-L-homoserine lactone applied in seed priming effects phytosphere microflora, chlorophyll levels and crop yield of winter wheat.**

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Quorum sensing (QS) is a mechanism allowing bacterial communication within a population and the outside world. Bacterial QS molecules should not be considered xenogenic to niches associated only with bacteria, as many eukaryotes are able to sense and respond to these signals or related molecules and a range of eukaryotic-associate bacteria are also able to utilise N-acyl homoserine lactone (AHL) signals. This raises the interesting possibility that plants may respond to bacterial AHLs directly, and that exogenous AHLs applied in seed priming might be used to

improve crop yields. Investigation of the C6-HSL priming effects on the wheat phytosphere, chlorophyll levels and crop yield are objectives of this project. Microbiological and agricultural methods were applied. C6-HSL applied in seed priming influenced seed microflora including general bacteria, N-fixators and yeasts. However, this effect was absent in the early stages of rhizosphere development (7-14 d) but appeared latter (56 d) when N-fixators numbers declined. Such a relatively late effect might be a result of plant physiology changes which could also be seen in the morphometry of 2 d seedlings and 56 d dry plant weights. In a field trial, chlorophyll production was influenced by seed priming and at every stage of wheat ontogenesis chlorophyll levels were enhanced suggesting better photosynthetic ability. The seed priming treatment also influenced crop yield. Specifically, productive tillering and grain content per spikelet were substantially enhanced in primed plants. These yield increases were seen in field tests of two wheat varieties Jatran 60 and Volodarka. C6-HSL revealed phyto-stimulative activity as a seed priming compound for winter wheat.

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### EMV31

#### **A lichens' symbiotic community elucidated by a metaproteomics approach**

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Lichens are recognized by macroscopic structures formed by a heterotrophic fungus (mycobiont), which hosts internal autotrophic photosynthetic algal and/or cyanobacterial partners (photobiont). We analyzed the structure and functionality of the entire lichen *Lobaria pulmonaria* collected from two different sites by state-of-the-art metaproteomics. In addition to the green algae and the ascomycetous fungus, a lichenicolous fungus as well as a complex prokaryotic community (different from the cyanobacteria) was found, the latter dominated by methanotrophic Rhizobiales. Various partner-specific proteins could be assigned to the different lichen symbionts, for example, fungal proteins involved in vesicle transport, algal proteins functioning in photosynthesis, cyanobacterial nitrogenase and GOGAT involved in nitrogen fixation, and bacterial enzymes responsible for methanol/C1-compound metabolism as well as CO-detoxification. Structural and functional information on proteins expressed by the lichen community complemented and extended our recent symbiosis model depicting the functional multiplayer network of single holobiont partners. Our new metaproteome analysis strongly supports the hypothesis (i) that interactions within the self-supporting association are multifaceted and (ii) that the strategy of functional diversification within the single lichen partners may support the longevity of *L. pulmonaria* under certain ecological conditions. This study will be complemented by analyses of further samples to reveal strategies of the lichens' adaptation to different geographical environments.

### EMV32

#### **Real-time monitoring of a synthetic bacterial community under variable selection and increasingly strong dispersal**

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**Introduction:** Environmental selection and dispersal are among the most important assembly mechanisms for microbial communities. Their quantification could help us to predict how these communities will change in response to climate change. Microbial communities tend to diversify when selection is variable and to homogenize when dispersal is strong. However, the interplay between these two contrasting mechanisms is largely unknown and targeted experiments, rather than static observations, are required to understand it. For example we still do not know under which conditions dispersal homogenizes microbial communities.

**Objectives:** Here we quantified the interplay between selection and dispersal in a synthetic bacterial community to find under which conditions dispersal homogenizes the community.

**Materials & methods:** We designed a controlled experiment where we imposed variable selection and increasingly strong dispersal to the synthetic community. We measured the absolute abundances of each strain using a real-time, sequencing-free, approach based on flow cytometry. We then deployed mathematical modeling to simulate how the community develops under a fine gradient of increasing passive dispersal.

**Results:** We found that dispersal homogenizes the communities not only at high immigration rates, as theoretically expected, but also at low immigration rates, i.e., even when immigration is almost five times slower than growth. Communities become homogeneous due to the immigration of cells rather than due to the relaxation of selection caused by mixing.

**Conclusion:** Our results suggest that bacterial communities can be homogenized even when dispersal is much weaker than selection and can explain why bacterial communities are often homogeneous at fine spatial scales.

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### Short Lecture

#### **Membranes and Transport 18 April 2018 • 09:00–11:00**

##### MTV01

#### **The dynamic adaptation of the type III secretion injectisome to external cues**

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Bacteria that live in contact to eukaryotic cells greatly benefit from being able to manipulate host cell behaviour. One of the most direct and elegant ways to reach this aim is the type III secretion system (T3SS), a molecular syringe also known as "injectisome", used by bacteria to inject effector proteins into host cells.

Recently, we found that parts of the injectisome are dynamic: the cytosolic components shuttle between the cytosol and the injectisome in a secretion-dependent manner.

To determine the role of these dynamic components in effector secretion by the T3SS, we used fluorescence and superresolution microscopy to analyze the localization and dynamics of fluorescently labeled T3SS components in live *Yersinia enterocolitica* under different external conditions.

Our results show that the cytosolic components form defined complexes, and that their composition and mobility changes when the T3SS gets activated by external cues. Even more strikingly, the complete cytosolic interface is transiently released from the injectisome in response to low pH values, as likely encountered during the passage of the bacteria through the gastrointestinal system.

Our results provide a striking example how bacteria can use protein dynamics to adapt the function of protein complexes, such as the T3SS injectisome, to their needs in ever-changing external conditions.

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## MTV02

### Deletion of phospholipase PlaF affects membrane lipid composition and expression of virulence-related proteins in *Pseudomonas aeruginosa*

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A *Pseudomonas aeruginosa* infection is one of the leading causes of hospital-acquired pneumonia, preferentially in immunocompromised patients [1]. The Gram-negative bacteria produce many virulence factors, among them are several phospholipases contributing to phospholipid homeostasis, damage of host cell membranes and modulation of lipid signalling in eukaryotic cells [2]. The physiological roles of intracellular phospholipases A for bacterial virulence are largely unknown.

We have identified PlaF, a novel phospholipase A of *P. aeruginosa* which hydrolyses *in vitro* bacterial phospholipids resulting in release of fatty acids. Due to its subcellular localization in the cytoplasmic membrane, we assume that PlaF has access to an array of phospholipids as natural substrates. Comparison of membrane lipids of the *P. aeruginosa* wild-type and a  $\Delta plaF$  mutant by quantitative mass spectrometry revealed more than 10% of total lipids being different. Additionally, proteomic analysis of *P. aeruginosa* wild-type and  $\Delta plaF$  by quantitative mass spectrometry revealed 422 proteins differentially expressed among these two strains. Between them, many cytoplasmic membrane proteins linked to biofilm formation, swimming motility and iron acquisition were identified. Using biological assays, we confirmed that PlaF is an important determinant of the biofilm lifestyle, swimming and pyoverdine mediated iron acquisition. Our data suggest a PlaF-mediated modulation of membrane phospholipid composition being related to virulence of *P. aeruginosa*.

## References:

[1] Gellatly, S.L. and R.E.W. Hancock, Pathog Dis, 2013. 67(3): p. 159-73.

[2] Flores-Diaz, M., et al., Microbiol Mol Biol Rev, 2016. 80(3): p. 597-628.

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## MTV03

### The Rnf complex of *Acetobacterium woodii* is a ferredoxin:NAD<sup>+</sup>-oxidoreductase essential for energy conservation and redox balancing

A. Wiechmann<sup>\*1</sup>, L. Westphal<sup>1</sup>, J. Baker<sup>2</sup>, N. P. Minton<sup>2</sup>, V. Müller<sup>1</sup>

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**Introduction:** Many bacteria and archaea possess reversible, Na<sup>+</sup>-translocating ferredoxin:NAD<sup>+</sup>-oxidoreductase activity, suggested to be catalysed by a novel respiratory enzyme and encoded by the *rnf* genes. However, biochemical evidence for this notion is still missing.

**Objectives:** To address the physiological role of the Rnf complex in the model acetogen *Acetobacterium woodii*.

**Materials & methods:** A deletion mutant of the *rnf*-operon was generated and analysed. Fno-activity and Na<sup>+</sup>-translocation were tested on inverted membrane vesicles.

**Results:** Deletion of the *rnf*-operon in *A. woodii* led to a loss of ferredoxin-dependent NAD<sup>+</sup> reduction and concomitant Na<sup>+</sup> transport. The deletion mutant did not grow on H<sub>2</sub> + CO<sub>2</sub>. Interestingly, the mutant also did not grow on either ethanol or other substrates that are oxidised ferredoxin-independent. However, growth could be restored by addition of hydrogen that leads to the reduction of ferredoxin by the electron-bifurcating hydrogenase.

**Conclusion:** The Rnf complex is the respiratory enzyme that links acetogenesis from H<sub>2</sub> + CO<sub>2</sub> to energy conservation in *A. woodii*. It is also essential to provide reduced ferredoxin, important for acetogenesis, from NADH by the reverse reaction. This function is indispensable during heterotrophic acetogenesis with substrates whose redox potential is too high to reduce ferredoxin directly. These data put a new perspective on the physiological role of the Rnf complex in anaerobes in general.

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## MTV04

### Biochemical characterization of a MobH-family relaxase

J. H. Heilers<sup>\*1</sup>, E. M. Heller<sup>2</sup>, J. Koch<sup>2</sup>, S. H. J. Smits<sup>3</sup>,

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**Introduction:** Relaxases are essential proteins of conjugative Type-IV-Secretion Systems (T4SS). The relaxase TraI of the unique T4SS of *Neisseria gonorrhoeae* belongs to the biochemically uncharacterized MobH-family of relaxases, a large family of relaxases whose members are mostly found on integrative conjugative elements and genetic islands. Relaxases site-specifically cleave within the *origin of transfer (oriT)* thereby forming a covalent-intermediate with the DNA which is targeted to the secretion apparatus for transfer. MobH-relaxases comprise an N-terminal relaxase domain (TraI\_2), followed by a disordered middle part and a C-terminal domain of unknown function (TraI\_2\_C). In

contrast to other known relaxases, MobH-relaxases comprise two motifs suggested to be involved in the coordination of divalent metal-ions.

**Results:** Here, we show that Tral cleaves *oriT* ssDNA in a site-specific and Mn<sup>2+</sup>-dependent manner. The cleavage site was determined as well as several essential nucleotides and the minimal *oriT* sequence required for cleavage. Preliminary experiments indicate cleavage of *oriT* dsDNA in the absence of accessory proteins. Unlike other relaxases, we show that Tral does not form a stable covalent intermediate with the DNA. This enabled us to determine the turn-over numbers for Tral which have, to our knowledge, not been determined for any known relaxase yet. Moreover, we show that both metal-binding motifs are necessary for the cleavage reaction. Small-Angle X-ray Scattering of the N-terminal relaxase domain reveals a bean-like shape with its active site predicted to locate in the inner bean-like curvature.

**Conclusion:** Here, we biochemically characterize Tral, the prototype of the MobH family of relaxases. Our results suggest a novel cleavage mechanism.

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### MTV05

#### **In vivo validation of the "charge zipper" model for toxin TisB in *Escherichia coli***

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Bacterial chromosomes contain many toxin-antitoxin (TA) loci that have been linked to the formation of persister cells. Persister cells are stress-tolerant phenotypic variants and considered as a bet-hedging strategy for survival. In type I TA systems, the RNA antitoxin inhibits translation of the toxin mRNA in most cells. However, in individual cells the inhibitory action of the RNA antitoxin might be overcome by increased levels of the toxin mRNA, which finally results in toxin production. Among the toxins of type I TA systems, small hydrophobic proteins with a length of less than 50 amino acids are predominant. They are often targeted towards the inner membrane and supposedly break down the proton motive force, i.e. depolarization of the inner membrane. The subsequent depletion of ATP is believed to slow down growth and induce persistence.

A well-studied type I TA system in *E. coli* is TisB/IstR-1. Transcription of the toxin gene *tisB* is induced upon DNA damage. Under these conditions TisB contributes to formation of persister cells by depolarizing the inner membrane [1,2]. According to the "charge zipper" model, translocation of protons across the inner membrane is mediated by salt bridges formed between two TisB molecules aligned in antiparallel orientation [3]. In total, up to four salt bridges might be formed between positively charged lysine and negatively charged aspartic acid residues. Here, it was tested *in vivo* to which extent the charged amino acid residues contribute to TisB functionality and persister formation.

#### **References:**

[1] Dörr *et al.* (2010) *PLoS Biol* 8: 29–35.

[2] Berghoff *et al.* (2017) *Mol Microbiol* 103: 1020–33.

[3] Steinbrecher *et al.* (2012) *Biophys J* 103: 1460–9.

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### MTV06

#### **Partitioning of *Escherichia coli* F<sub>0</sub>F<sub>1</sub>-ATP-synthase into disordered lipid domains in the cytoplasmic membrane**

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Fluorescently labelled F<sub>0</sub>F<sub>1</sub>-ATP-synthase, carrying mNeonGreen at the C terminus of the membrane-integral subunit F<sub>0</sub>-a, was used to investigate the influence of the fatty acid environment on the localization and mobility of a membrane protein complex. The inner membrane of *E. coli* consists of glycerophospholipids with mainly three different fatty acid chains, C16:0, C16:1 and C18:1. A depletion of phospholipids with unsaturated fatty acids (UFA) due to a thermosensitive *fabA* mutation altered the distribution of the membrane protein complexes severely and leads to formation of membrane patches enriched in ATP-synthase complexes at 40°C as shown by time-lapse microscopy. However, these changes had no effect on the activity of F<sub>0</sub>F<sub>1</sub> measured in inverted membrane vesicles. Use of the membrane fluidity marker Laurdan and the fluorescently labelled trans-membrane model peptide WALP23 revealed that *in vivo* depletion of UFA resulted in separation of the membrane into domains of different fluidity. The phase separation leads to a distinct distribution of the F<sub>0</sub>F<sub>1</sub>-ATP-synthase into the remaining liquid-disordered membrane areas. However, phase partitioning can be completely reversed by addition of oleate to the growth medium. After developing appropriate protocols, single-particle tracking experiments using total internal reflection fluorescence-microscopy revealed that the F<sub>0</sub>F<sub>1</sub>-ATP-synthase is freely diffusible in wildtype membranes. However, a gradual depletion of UFA by distinct temperature shifts during cell growth showed that the mobility of the F<sub>0</sub>F<sub>1</sub>-ATP-synthase is strictly dependent on the amount of UFA present in the membrane. In cells grown at the restrictive temperature for *fabA-ts*, the diffusion coefficient of F<sub>0</sub>F<sub>1</sub>-ATP-synthase complexes is reduced by a factor of 50.

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### MTV07

#### **The application of an alternative uptake system for fructose / mannose utilization in *Corynebacterium glutamicum***

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**Introduction:** The analysis of *Corynebacterium glutamicum* carbon source metabolism is pivotal as *C. glutamicum* is an important working horse for the white biotechnology industry. Sugars like fructose, glucose and mannose are transported and phosphorylated by the phosphotransferase system (PTS) in *C. glutamicum* [1, 2]. The use of alternative PTS independent uptake system is of special interest because equimolar amounts of phosphoenolpyruvate are used for the sugar uptake and its phosphorylation by the PTS [3]. Here we analyzed the impact of a combined sugar uptake and phosphorylation system in *C. glutamicum*.

**Results:** The heterologous overexpression of the alternative uptake system consistent of the *glf* *z. mob.* (glucose facilitator) and *frk* *z. mob.* (fructokinase) from *Zymomonas mobilis* in *C. glutamicum*  $\Delta$ *ptsF* (EII<sub>Frc</sub>) enabled growth in minimal media (CGXII) with fructose as carbon source. Previous studies showed that Frk phosphorylates fructose and also mannose to mannose-6-phosphate [4]. *C. glutamicum*  $\Delta$ *ptsG* (EII<sub>Glc</sub>)

cannot use mannose for growth. By expression of *glfz*, *mob*, *frkz*, *mob* and *manA*<sub>C. glu</sub> (mannose-6-phosphate isomerase) growth was restored of *C. glutamicum*  $\Delta$ *ptsG* in CGXII with mannose as carbon source.

**Conclusion:** The presented alternative uptake system is attractive as it can replace the PTS-dependent fructose and mannose uptake and utilization in *C. glutamicum*. Further studies will be presented to analyze its impact for the biosynthesis of aromatic compounds.

References:

[1] Parche S, Burkovski A, Sprenger GA, Weil B, Kramer R, Titgemeyer F 2001 JMMB 3:423-428.

[2] Sasaki M, Teramoto H, Inui M, Yukawa H 2011 AMB 89:1905-1916.

[3] Lindner SN, Seibold GM, Henrich A, Kramer R, Wendisch VF 2011 AEM 77:3571-3581.

[4] Weisser P, Kramer R, Sprenger GA 1996 AEM 62:4155-4161.

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**MTV08**

**Copper pump and metallochaperone in a single gene: programmed translational frameshifting and cytoplasmic copper resistance in *Escherichia coli***

S. L. Drees<sup>\*1</sup>, M. Lübben<sup>1</sup>

<sup>1</sup>Ruhr University Bochum, Biophysics, Bochum, Germany

**Question:** In *E. coli*, cytoplasmic resistance to copper is mediated by CopA, an ATP-dependent exporter. Membrane-bound copper pumps such as CopA require loading with Cu(I) ions by metallochaperones, single domain proteins capable of binding and delivering a certain metal ion species. Unlike most other bacterial genomes, the *E. coli* genome does not contain a gene coding for a metallochaperone. How does *E. coli* overcome the lack of a designated copper chaperone?

**Objectives:** Characterization of the transcriptional and translational anomalies leading to the expression of a bi-functional metal binding domain (CopA-Z). Assessment of the physiological impact of CopA-Z in vitro and in vivo.

**Methods:** Elucidation of the programmed ribosomal frameshift leading to production of CopA-Z. Analysis of CopA and CopA-Z gene expression on transcript and protein level. Determination of copper binding affinities and copper-dependent ATPase activity. Characterization of the CopA-Z phenotype.

**Results:** A metal binding domain of CopA is repurposed as a copper chaperone in *E. coli*. While being translated as the very N-terminal domain of the ATPase, at the same time it is synthesized as a free chaperone by a mechanism called programmed ribosomal frameshifting, employing a highly abundant short fragment of the *copA* mRNA. As chaperone, CopA-Z directly delivers copper to the CopA transmembrane domain and thereby activates the ATPase. Expression of CopA-Z improves resistance of *E. coli* against copper shock.

**Conclusion:** The mechanism by which CopA-Z is produced constitutes a new dogma in protein biosynthesis. The overexpression of metallochaperones may be a general rapid response to heavy metal exposure.

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**Plenary Lecture**  
**Genome Evolution**  
**18 April 2018 • 12:00–13:00**

**IL13**

**Plasmids as turtles in an evolutionary race**

T. Dagan<sup>\*1</sup>

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No abstract has been submitted.

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**IL14**

**Population genomics of bacterial pathogens and novel antibiotic producers**

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In recent years, abundant bacterial genome sequence data have enabled studies of bacterial evolution at unprecedented detail. Most data is available for pathogenic bacteria, showing that bacterial pathogens constitute measurably evolving populations, which continuously accumulate genetic change over timescales that are directly observable and epidemiologically relevant. Accordingly, analyses of temporally structured samples of genome sequences have revealed the temporal dynamics of bacterial evolution and spatial spread, often in direct relation to human activities.

Short-term evolutionary rates vary among bacterial species, for reasons that are currently not understood. Our recent investigation of *Staphylococcus aureus* during human nasal colonization suggests that its evolutionary rate may be directly correlated with cellular replication rates *in situ*, i. e. in the bacterium's natural niche. A yet deeper zoom into bacterial diversity within individual hosts revealed the dynamics of bacterial metapopulations over time, including the occasional migration of bacterial subpopulations among colonized individuals, which is relevant for understanding both, the recruitment of microbiome constituents and the dynamics of infectious disease outbreaks.

Another exciting route of development is the integration of large genomic datasets with experimental data to identify statistical associations between genomic traits and specific microbial phenotypes. This was successfully applied to the identification of genetic determinants of antibiotic resistance, and beyond. We have recently used a similar approach to identify links between specific biosynthetic gene clusters in the genomes from myxobacteria and their production of biotechnologically interesting secondary metabolites.

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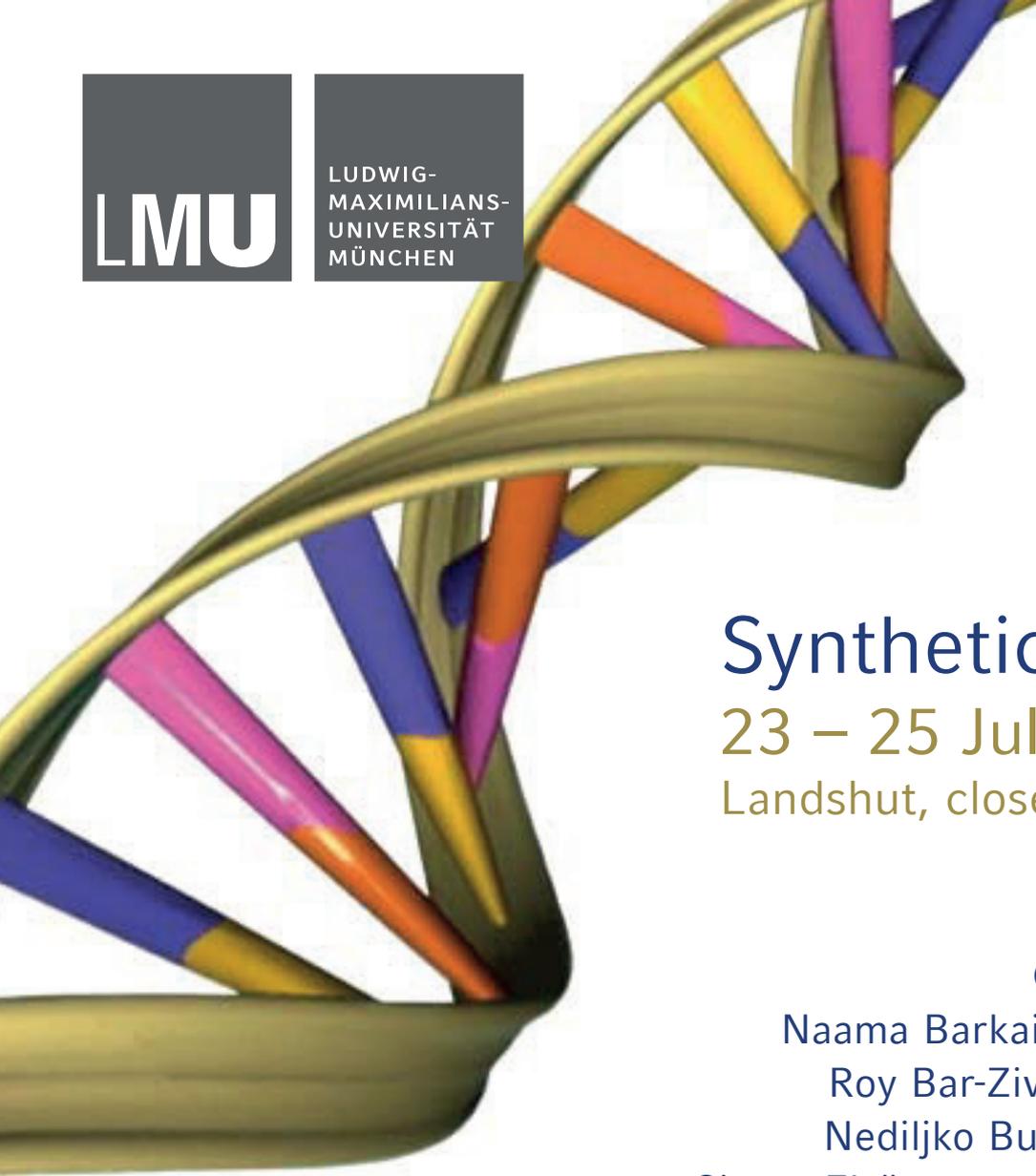
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## Abstract Submission Deadline

4 May 2018

## Early Bird Registration Deadline

4 May 2018

## Registration Deadline

15 June 2018

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