Final Symposium on “bacterial survival strategies”

October 07th-09th

Tübingen, Germany

Contents

Welcome adresses

The organizing committee welcomes all participants to the final Symposium of the GRK 1708.2 „Molecular principles of bacterial survival strategies“ on the topic of BACTERIAL SURVIVAL STRATEGIES.

Our special thanks go to the invited speakers

Dr. Megan Bergkessel (University of Dundee, UK)
Konstantin Eckel (Technical University of Munich, Germany)
Prof. Dr. Michael Gray (The University of Alabama at Birmingham, United States)
Dr. Alexander Klotz (Biozentrum, University of Basel, Switzerland)
Prof. Dr. Katherine P. Lemon (Baylor College of Medicine, United States)
Dr. Lisa Maier (University of Tübingen, Germany)
Prof. Dr. Thorsten Mascher (Technical University of Dresden, Germany)
Dr. Alicia María Muro Pastor (Centro de Investigaciones Científicas Isla de la Cartuja, Spain)
Prof. Dr. Simon Ringgaard (LMU Munich, Germany) and
Dr. David Whitworth (Aberystwyth University, UK)

Additionally, we thank the invited Alumni speaker of the GRK Dr. Nermin Akduman (University of Tübingen, Germany) and Dr. Sergii Krysenko (University of Tübingen, Germany).

The organizing committee

Dr. Louisa Wessels Perelo
Antje Bauer
Markus Burkardt
Yu-Ming Huang
Carina Rohmer

Welcome from Karl Forchhammer, spokesman of the GRK 1708

The present international symposium on bacterial survival strategies is the final symposium of this PhD research training group (GRK) after almost 9 years of funding by the German research foundation. We are looking back at an exciting research period, which witnessed remarkable discoveries in the research field of bacterial survival strategies. Together with our international renowned guests, the GRK students and their PIs will use the present occasion to address questions, how bacteria adapt to and survive in dynamic environmental situations that challenges survival of microbes.

We are looking forward to hear reflections on the diverse topic on bacterial survival strategies covering a wide range of topics, such as cellular differentiation strategies, specific metabolic acclimation strategies, the role of storage compounds and the underlying regulatory mechanisms in stress sensing and signaling. We want to shed light on the struggle of bacteria with stressful environmental cues, on strategies to cope with predation and the mechanisms how they protect their niches against competing members of the biota, employing the use of secondary metabolites.

We wish all participants an interesting and successful scientific event!

Tübingen, October 2020

Acknowledgement

We thank the GERMAN RESEARCH FOUNDATION (DFG) for financial support.

Scientific Program

Day 1 (Wednesday 07.10.2020)

12.30-12.45	**Opening session**

Session I – Stress signaling and metabolism I

UTC+2	Presenter	Affiliation
12.45-13.15	**Alicia María Muro Pastor** Bacterial adaptation through cellular differentiation. The case of heterocysts	**IBVF** **University of Sevilla**
13.15-13.20	**Short discussion**	
13.20-13.35	**Ritu Garg** The fully developed envelope with the glycolipid layer is prerequisite for stress tolerance in filamentous cyanobacteria	**AG Maldener GRK** **University of Tübingen**
13.35-13.45	**Extended discussion**	
13.45-14.05	**Alexander Klotz (Alumni)** Filling the gaps: Role of the central carbon metabolism in *Pseudomonas aeruginosa* virulence and survival	**Biozentrum** **University of Basel**
14.05-14.10	**Short discussion**	
14.10-14.25	**Niels Neumann** On the Role of a Specific Survival Glycogen in Cyanobacteria	**AG Forchhammer GRK** **University of Tübingen**
14.25-14.35	**Extended discussion**	
14.35-15.10	**Coffee break**	

Day 1 (Wednesday 07.10.2020)

Session II – Stress signaling and metabolism I

UTC+2	Presenter	Affiliation
15.10-15.40	**Simon Ringgaard** Awakening of the sleeping σ factor by threonine phosphorylation	**Department of Microbiology** **L.M.-University of Munich**
15.40-15.45	**Short discussion**	
15.45-16.05	**Sergii Krysenko (Alumni)** The key role of GlnA3 for survival of *Streptomyces coelicolor* and *Mycobacterium tuberculosis* under polyamine excess	**IMIT** **University of Tübingen**
16.05-16.15	**Extended discussion**	
16.15-16.45	**Michael Gray** Disentangling inorganic polyphosphate from the general stress response of *Escherichia coli*	**Department of Microbiology** **The University of Alabama in Birmingham**
16.45-16.50	**Short discussion**	
16.50-17.05	**Hanna Rosigkeit** The Role of the Biopolymer Polyphosphate (PolyP) for Survival of *Ralstonia eutropha*	**AG Jendrossek GRK** **University of Stuttgart**
17.05-17.15	**Extended discussion**	
17.15-18.00	**Poster Session**	

Day 2 (Thursday 08.10.2020)

Session III – Predators and Bacteria

UTC+2	Presenter	Affiliation
09.30-10.00	**David Whitworth** Surviving the Microbial Predator-Prey Arms Race.	**Institute of Biological, Environmental and Rural Sciences** **Aberystwyth University**
10.00-10.05	**Short discussion**	
10.05-10.20	**Antje Bauer** Interactions of cyanobacteria with predators	**AG Forchhammer** **GRK** **University of Tübingen**
10.20-10.30	**Extended discussion**	
10.30-11.00	**Coffee break**	
11.00-11.20	**Nermin Akduman (Alumni)** Bacterial derived vitamin B_{12} enhances predatory behaviors in nematodes	**AG Maier** **IMIT** **University of Tübingen**
11.20-11.25	**Short discussion**	
11.25-11.40	**Carina Rohmer** The bacterial host background determines the life-cycle of Sa3-bacteriophages	**AG Wolz** **GRK** **University of Tübingen**
11.40-11.50	**Extended discussion**	
11.50-13.00	**Lunch**	

Day 2 (Thursday 08.10.2020)

Session IV – Microbiome and Infection

UTC+2	Presenter	Affiliation
13.00-13.30	**Eric Kemen** Microbial survival in the plant phyllosphere	**Microbial Interactions in Plant Ecosystems** **University of Tübingen**
13.30-13.35	**Short discussion**	
13.35-13.50	**Yu-Ming Huang** Meta-omics reveal the activity and microbial interaction of autotrophic nitrate-reducing iron(II)-oxidizing bacteria from freshwater ecosystems	**AG Kleindienst GRK** **University of Tübingen**
13.50-14.00	**Extended discussion**	
14.00-14.30	**Lisa Maier** Dissecting the impact of commonly used drugs on the human microbiome	**IMIT** **University of Tübingen**
14.30-14.35	**Short discussion**	
14.35-14.50	**Thomas Hagemann** Fitness genes of E. coli that allows survival in the inflamed gut	**AG Frick GRK** **University of Tübingen**
14.50-15.00	**Extended discussion**	
15.00-15.40	**Coffee break**	
15.40-16.10	**Katherine P. Lemon** Microbe-microbe interactions in human nasal microbiota	**Department of Molecular Virology & Microbiology** **Baylor College of Medicine**
16.10-16.15	**Short discussion**	
16.15-16.30	**Benjamin Torres Salazar** Inhibitory Activity of Nasal Staphylococcal Products	**AG Peschel GRK** **University of Tübingen**
16.30-16.40	**Extended discussion**	

Day 3 (Friday 09.10.2020)

Session V – Stress signaling and metabolism II

UTC+2	Presenter	Affiliation
09.00-09.30	**Thorsten Mascher** Cannibalism Stress in *Bacillus subtilis*: from peptide toxin production to coordinated population response	**Institute of Microbiology** **Technical University of Dresden**
09.30-09.35	**Short discussion**	
09.35-09.50	**Robert Kluj** Salvage of the amino sugar and amino acid components of the peptidoglycan cell wall provides a survival benefit to *Bacillus subtilis*	**AG Mayer GRK** **University of Tübingen**
09.50-10.00	**Extended discussion**	
10.00-10.30	**Coffee break**	
10.30-11.00	**Megan Bergkessel** Regulation during growth arrest in *Pseudomonas aeruginosa*	**Division of Molecular Microbiology** **University of Dundee**
11.00-11.05	**Short discussion**	
11.05-11.20	**Sofía Doello** Energy homeostasis in the developmental transitions of a cyanobacterium	**AG Forchhammer GRK** **University of Tübingen**
11.20-11.30	**Extended discussion**	
11.30-12.30	**Lunch**	

Day 3 (Friday 09.10.2020)

Session VI – Bioactive compounds

UTC+2	Presenter	Affiliation
12.30-13.00	**Heike Brötz-Oesterhelt** Contribution of bacterial Clp protease to bacterial survival	**IMIT- Microbial Bioactive Compounds** **University of Tübingen**
13.00-13.05	**Short discussion**	
13.05-13.25	**Konstantin Eckel** Chemical Inhibition of Caseinolytic Protease P Attenuates Virulence and Disrupts its Oligomeric State	**Department of chemistry** **Technical University of Munich**
13.25-13.35	**Extended discussion**	
13.35-13.50	**Alicia Engelbrecht** Biosynthesis of the cyclopropyl moiety of belactosin A	**AG Kaysser GRK** **University of Tübingen**
13.50-14.00	**Short discussion**	
14.00-14.15	**Johanna Rapp** Biosynthesis and Mode of action of 7-deoxysedoheptulose – an inhibitor of the shikimate pathway	**AG Forchhammer GRK** **University of Tübingen**
14.15-14.25	**Extended discussion**	
14.25-14.45	**Karl Forchhammer** Final thoughts on bacterial survival strategies & closing remarks	**IMIT Microbiology / Organismic Interactions** **University of Tübingen**

Abstracts – oral presentations

Bacterial adaptation through cellular differentiation. The case of heterocysts.

Alicia M. Muro-Pastor, Manuel Brenes-Álvarez, Isidro Álvarez-Escribano, Elvira Olmedo-Verd and Agustín Vioque

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Nitrogen is an essential nutrient for all organisms. In the absence of a source of combined nitrogen some bacteria can fix atmospheric N_2. Nitrogen fixation is sensitive to the presence of oxygen and therefore requires an anaerobic or microaerobic environment. Heterocystous cyanobacteria are multicellular bacteria able to fix N_2. Because of their photosynthetic metabolism, nitrogen fixation can´t take place in the vegetative cells that produce oxygen. Instead, upon nitrogen deprivation, a specific cell type called heterocyst differentiates along filaments in a semi-regular pattern. Mature heterocysts can fix N_2 and provide vegetative cells with fixed nitrogen, while the photosynthetic vegetative cells provide the heterocysts with carbon skeletons for nitrogen assimilation. Thus, nitrogen-fixing cyanobacterial filaments are multicellular organisms with two cell types that have complementary metabolic capabilities and cooperate to achieve growth of the filament as a whole.

Heterocyst differentiation relies on gene expression programs that are not only cell-specific but also changing with time as the morphological and metabolic maturation of the heterocysts progresses. At the transcriptional level, the process is under control of the global nitrogen regulator NtcA, but also requires a second regulator, HetR, that is specifically involved in cellular differentiation. Our work deals with the transcriptional and post-transcriptional events involved in the successful differentiation of heterocysts. Specifically, we are focusing on the possible roles of several nitrogen-regulated non-coding RNAs, both trans and cis-acting (antisense) RNAs. Based on our previous RNA-Seq analysis of the responses to nitrogen deprivation in the wild type strain and a heterocyst-deficient mutant (1), we have constructed a co-expression network (2) that allows the identification of heterocyst-specific transcripts, including non-coding RNAs that might contribute to the specific regulation of the gene expression programs leading to heterocyst differentiation and function. Some examples will be discussed.

(1) Mitschke J, et al. (2011). Dynamics of transcriptional start site selection during nitrogen stress-induced cell differentiation in *Anabaena* sp. PCC7120. *Proc. Natl. Acad. Sci. USA* 108, 20130-20135. doi: 10.1073/pnas.1112724108.

(2) Brenes-Álvarez M, et al. (2019) Elements of the heterocyst-specific transcriptome unravelled by co-expression analysis in *Nostoc* sp. PCC 7120. *Environ. Microbiol.* 21: 2544-2558. doi: 10.1111/1462-2920.14647

The fully developed envelope with the glycolipid layer is prerequisite for stress tolerance in filamentous cyanobacteria

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The filamentous cyanobacterium *Anabaena variabilis* ATCC 29413 can differentiate heterocysts and akinetes to survive under different stress conditions. Under nitrogen limited condition, heterocysts form in a semi regular pattern and provide the filament with nitrogen by fixing N_2 [1]. Akinetes are spore-like dormant cells, which allow survival during adverse growth conditions. They can germinate to produce new filaments under suitable conditions and are resistant to cold and desiccation [2].
Heterocysts and akinetes are characterized by the presence of a thick multilayered envelope partially composed from specific glycolipids [3]. While the function of this glycolipid layer in heterocysts was known as oxygen barrier in an oxic environment, nothing was known about the function of such layer in dormant akinetes.
A putative polyketide synthase, HglB, was discovered to be involved in synthesis of the heterocyst glycolipids in *Nostoc* sp. PCC 7120, which does not form akinetes. To investigate the function of the glycolipids in akinete envelopes, we created a mutant in the homologue gene from *A. variabilis*, and investigated its role in each cell type. The *hglB* mutant strain showed delayed heterocyst and akinete differentiation compared to the wild type (WT). The heterocyst ultrastructure was aberrant and the mutant was not able to grow without combined nitrogen source. Chemical analysis, fluorescence and electron microscopy showed the absence of a glycolipid layer in heterocysts and akinetes of the mutant. By performing survival experiments comparing germination efficiency of the WT with the *hglB* mutant, we revealed the role of the glycolipids in stress tolerance and protection of the dormant cells against severe unfavorable conditions such as freezing, desiccation, oxidative stress and lysozyme. Whilst the WT akinetes could cope with these conditions, the mutant was not able to germinate again. Remarkably, the same structure fulfills such different functions in the envelopes of two different cell types in multicellular cyanobacteria.

References

1. Maldener I, Summers ML, and Sukenik A (2014) Cellular differentiation in filamentous cyanobacteria. The Cell Biology of Cyanobacteria: 263-291.
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3. Perez R, Wörmer L, Sass P, Maldener I (2018) A highly asynchronous developmental program triggered during germination of dormant akinetes of filamentous diazotrophic cyanobacteria. FEMS Microbiol Ecol, 94:1–11.

Filling the gaps:
Role of the central carbon metabolism in *Pseudomonas aeruginosa* virulence and survival

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Pseudomonas aeruginosa is an opportunistic human pathogen causing a range of life-threatening acute and chronic infections. In the face of rapidly increasing antibiotic resistance, *P. aeruginosa* was recently listed as critical priority pathogen by the WHO[1]. *P. aeruginosa* is acknowledged for its highly versatile metabolism that promotes growth under various environmental conditions and fosters colonization of different host tissues. However, little is known about the metabolic properties and regulation required for host colonization. Here, we analysed distinct physiological functions of three paralogous glyceraldehyde 3-phosphate dehydrogenases (GAPDH), key enzymes of the central carbon metabolism. To dissect the role and regulation of individual GAPDHs, we investigated deletion mutants and strains carrying transcriptional *gap* reporters. We show that GapA is bi-functional, while GapB and GapC primarily catalyse the glycolytic and gluconeogenic reactions, respectively. The expression profiles of the transcriptional reporters support the specific function of the three GAPDHs in defined media. Nonetheless, *P. aeruginosa* displays a striking heterogeneous GAPDH expression on single cell level when facing starving conditions. In contrast to GapA and GapB, GapC was strictly required for virulence in a *Galleria mellonella* model, indicating that gluconeogenesis is of key importance for host colonization. Surprisingly, GapC specifically binds the global bacterial second messenger c-di-GMP, indicating that the activity of this protein is tightly controlled during growth. While the role of c-di-GMP in bacterial behaviour and virulence control is well-established, its interference with bacterial metabolism is unexplored.
This study will provide the basis for the physiological role of GapC and its control by c-di-GMP during *P. aeruginosa* growth in surrogate host systems. Our long-term goal is to use this information to assess important metabolic processes of *P. aeruginosa* in the human patient.

1. Tacconelli, E. *et al.* Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *Lancet Infect. Dis.* **18**, 318–327 (2018).

On the Role of a Specific Survival Glycogen in Cyanobacteria

Niels Neumann[1], Karl Forchhammer[1]

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To cope with nitrogen deplete conditions, the unicellular cyanobacterium Synechocystis sp. PCC 6803 undergoes by a genetically determined program, a process called chlorosis (Klotz, Georg et al. 2016). Chlorosis is characterized by depigmentation and an arrest of cell cycle while at the same time storage polymers in the form of glycogen and polyhydroxybutyarate (PHB) are accumulated. This results in a dormant like state, where long term survival is provided due to low level photosynthesis. (Görl, Sauer et al. 1998) . Recent studies showed that resuscitation from the chlorotic state is fueled by degradation of the previously accumulated glycogen. Several isofunctional enzymes are involved in glycogen synthesis and degradation in Synechocystis.(Doello, Klotz et al. 2018) The phenotype of respective knockout mutants revealed that despite catalyzing the same reactions and showing comparable activity, chlorosis and resuscitation proceeds successfully only with a specific set of these enzymes. Furthermore, we can show that different forms of glycogen are present in Synechocystis and reveal a sophisticated regulatory network of glycogen synthesis and degradation.

Doello, S., A. Klotz, A. Makowka, K. Gutekunst and K. Forchhammer (2018). "A Specific Glycogen Mobilization Strategy Enables Rapid Awakening of Dormant Cyanobacteria from Chlorosis." Plant Physiol 177(2): 594-603.

Görl, M., J. Sauer, T. Baier and K. Forchhammer (1998). "Nitrogen-starvation-induced chlorosis in Synechococcus PCC 7942: adaptation to long-term survival." Microbiology-Uk 144: 2449-2458.

Klotz, A., J. Georg, L. Bucinska, S. Watanabe, V. Reimann, W. Januszewski, R. Sobotka, D. Jendrossek, W. R. Hess and K. Forchhammer (2016). "Awakening of a Dormant Cyanobacterium from Nitrogen Chlorosis Reveals a Genetically Determined Program." Curr Biol 26(21): 2862-2872.

Awakening of the sleeping σ factor by threonine phosphorylation

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Bacteria constantly experience changes to their environment and have to cope with external stresses. They usually respond by adapting their transcriptional expression profile accordingly, in a response that is tailored specifically to cope with the stress that they encounter. Often during stress conditions, the primary σ factor of the RNA polymerase is replaced by an alternative extracytoplasmic function (ECF) σ factor, which drive expression of specific stress related genes. ECF σ factors are intrinsically active and are generally sequestered by an anti-σ factor in an inactive state until their action is warranted. Then, only upon a specific stimulus is the inhibitory effect of the anti-σ factor alleviated and the σ factor released for interaction with the RNAP.
We have discovered a novel mechanism of transcriptional regulation in bacteria, which is based on intrinsically inactive sigma factors that rely on protein phosphorylation for their activation. We have identified an ECF σ factor / threonine-kinase pair (named EcfP / PknT) that is responsible for sensing polymyxin antibiotics stress and mediating bacterial polymyxin resistance in the human pathogen *Vibrio parahaemolyticus.* We show that upon treatment of V. parahaemolyticus with polymyxin antibiotics, PknT is activated and phosphorylates the σ factor EcfP. This results in EcfP activation and expression of an essential polymyxin resistance determinant. The intrinsic inactive state of EcfP is due to the absence of a negatively charged DAED motif in the region between σ2.1 and σ2.2, which usually mediates binding to the RNAP core enzyme. Instead, the DAED motif has been replaced by non-charged amino acids, which renders EcfP inactive and unable to bind the RNAP. Strikingly, phosphorylation of EcfP at residue Thr63 mimics the negative charge, usually provided by the DAED motif, and permits interaction of EcfP with RNAP in formation of the holyenzyme and the consequential expression of EcfP target genes. This constitutes a widespread mechanism of signal transduction and transcriptional regulation in bacteria, where the input and output cues are modular based on the sensing domain fused to the kinase and the promoter sequence recognized by the σ factor, respectively, while the signal transduction pathway of σ phosphorylation is conserved.

The key role of GlnA3 for survival of *Streptomyces coelicolor* and *Mycobacterium tuberculosis* under polyamine excess

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Human intracellular pathogenic actinobacterium *Mycobacterium tuberculosis* has developed strategies to access nutrients from the host and to exploit the host to synthesize more resources for its growth and propagation. *Mycobacterium tuberculosis* can induce the polyamine biosynthesis during the shift in metabolic state of macrophages. The pathogen is able to utilize polyamines as a sole *N*- and *C*-source to support its own intracellular growth in macrophages. In our previous studies in a model actinobacterium *Streptomyces coelicolor* M145, which possesses three glutamine synthetase (GS)-like enzymes, we demonstrated that only one GS-like protein GlnA3$_{St}$ (SCO6962), is involved in the first step of polyamine utilization pathway[1,2]. GlnA3$_{St}$ is a gamma-glutamylpolyamine synthetase (GPS) that ensures both nutrients availability (*C*- and *N*-source) and resistance against high polyamine concentrations in *Streptomyces coelicolor*[1]. Since there is a homologue of GlnA3$_{Mt}$ (Rv1878) in *Mycobacterium tuberculosis,* this GPS enzyme is a particularly interesting target for drug development. In our current studies we were able to show that GlnA3$_{Mt}$ can glutamylate polyamines, demonstrating GPS activity[3]. Thus, GlnA3$_{Mt}$ is a novel specific drug target in a relevant human pathogen.

1. Krysenko S., Okoniewski N., Kulik A., Matthews A., Grimpo J., Wohlleben W. and Bera A. (2017) Gamma-Glutamylpolyamine Synthetase GlnA3 Is involved in the first step of polyamine degradation pathway in *Streptomyces coelicolor* M145. *Front Microbiol* 8: 726.

2. Krysenko S., Matthews A., Okoniewski N., Kulik A., Girbas M. G ., Tsypik O., Meyners C. S., Hausch F., Wohlleben W., Bera A. (2019) Initial metabolic step of a novel ethanolamine utilization pathway and its regulation in *Streptomyces coelicolor* M145. mBio 10(3).

3. Krysenko S., Oswald M., Meyners C., Kulik A., Reiling N., Hilemann D., Paudyal B., Purder P., Hausch F. and Brötz-Oesterhelt H., Wohlleben W., Bera A. (2020) The γ-glutamylspermine synthetase GlnA3$_{Mt}$ is involved in the polyamine glutamylation in *Mycobacterium tuberculosis* (in preparation).

Disentangling inorganic polyphosphate from the general stress response of *Escherichia coli*

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Inorganic polyphosphate (polyP) is an evolutionarily ancient, universally conserved biopolymer with a wide range of functions in different kinds of cells. In bacteria, polyP is required for stress response, motility, biofilm formation, and virulence, and production of polyP is stimulated by multiple different environmental stress conditions. However, very little is known about the mechanisms by which polyP production is regulated or how polyP regulation is connected to the other, better-understood pathways of general stress response in bacteria. In this work, we have begun to clarify the roles of the ppGpp-dependent stringent response, the RNA-polymerase binding transcription factor DksA, and the alternative sigma factors RpoN, RpoE, and FliA in regulating polyP synthesis in *Escherichia coli*. While transcription is necessary for induction of polyP synthesis, transcriptomic experiments show that the gene encoding the polyP-synthesizing enzyme PPK is not upregulated in response to stress. Instead, a very complex transcriptional signature and subsequent mutational analyses have led us to identify interactions between RNA polymerase, DksA (classically involved in the response to amino acid starvation), RpoE (involved in the response to cell envelope stress), FliA (controlling flagellar biosynthesis), and RpoN (involved in the response to nitrogen starvation) that all interact to control polyP synthesis. Current work in our lab is focused on identifying the gene(s) that are regulated by these factors that directly control the activity of PPK.

Gray, M.J. Interactions between DksA and stress-responsive alternative sigma factors control inorganic polyphosphate accumulation in *Escherichia coli*. J Bacteriol 2020; 202(14): e00133-20.

Gray, M.J. Inorganic polyphosphate accumulation in *Escherichia coli* is regulated by DksA but not by (p)ppGpp. J Bacteriol 2019; 201(9): e00664-18.
Rudat, A.K., Pokhrel, A., Green, T.J., and Gray, M.J. Mutations in *Escherichia coli* polyphosphate kinase that lead to dramatically increased *in vivo* polyphosphate levels. J Bacteriol 2018; 200(6): e00697-17.

The Role of the Biopolymer Polyphosphate (PolyP) for Survival of *Ralstonia eutropha*

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PolyP in prokaryotes is present in form of insoluble polyP granules that are localized in the nucleoid region e. g. in *Caulobacter crescentus*, *Pseudomonas aeruginosa* and *Ralstonia eutropha* ((1, 2), unpublished data). PolyP obviously has the function of a reservoir for phosphorus but can also have several other functions: it is important for cell cycle control (2, 3) and is necessary to efficiently cope with various forms of stresses (nutrient deprivation, presence of reactive oxygen species or heavy metals, high temperature) (4). Furthermore, polyP-deficient mutants of pathogenic bacteria (e. g. *P. aeruginosa, Mycobacterium tuberculosis*) showed a decreased level of virulence (5, 6).
The β-proteobacterium *R. eutropha* H16 (*Cupriavidus necator*) is well-known for accumulating high amounts of poly(3-hydroxybutyrate) (PHB) when grown under unbalanced conditions. Our interest in other biopolymers than PHB revealed that *R. eutropha* also forms polyP in form of 50 to 200 nm DAPI-stainable polyP granules.
The genome of *R. eutropha* codes for seven individual polyP kinases (*ppk*): two PPKs (PPK1a and PPK1b) are members of the PPK1 family while the other five PPKs belong to the PPK2 family (PPK2a, PPK2b, PPK2c, PPK2d, PPK2e) (7). We deleted all seven *ppk* genes and the resulting Δ*ppk-7* strain was unable to form polyP (8). Growth experiments with *R. eutropha* showed that previously accumulated polyP served as a reservoir for phosphorous and even allowed growth in the absence of phosphate in the medium whereas strongly diminished growth was detected for the polyP-free Δ*ppk-7* strain in phosphate-free medium. Surprisingly, consumption of polyP was incomplete leading to residual polyP granules in a minority of cells. However, evidence for a function in stress tolerance (heavy metals, oxidative stress, heat, motility) was not obtained.
In conclusion, our data clearly support the function of polyP as a storage compound for phosphorous in *R. eutropha* but do not confirm the frequently published multiple functions of polyP as a stress mediator against heat or oxidants in other prokaryotic species.

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3. Rao NN, et al. (2009) Inorganic Polyphosphate: Essential for Growth and Survival. *Annu Rev Biochem,* 78:605-47.
4. Nikel PI, et al. (2013) Accumulation of inorganic polyphosphate enables stress endurance and catalytic vigour in *Pseudomonas putida* KT2440. *Microb Cell Fact*, 12:50.
5. Rashid MH, Kornberg A (2000) Inorganic polyphosphate is needed for swimming, swarming, and twitching motilities of Pseudomonas aeruginosa. *Proc Natl Acad Sci,* 97:4885–90.
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7. Tumlirsch T, et al. (2015) Formation of Polyphosphate by Polyphosphate Kinases and Its Relationship to Poly(3-Hydroxybutyrate) Accumulation in *Ralstonia eutropha* Strain H16. *AEM,* 81:8277–93.
8. Tumlirsch T, Jendrossek D (2017) Proteins with CHADs (Conserved Histidine α-Helical Domains) Are Attached to Polyphosphate Granules *In Vivo* and Constitute a Novel Family of Polyphosphate-Associated Proteins (Phosins). *AEM*, 83:e03399–16–14.

Surviving the Microbial Predator-Prey Arms Race.

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Predatory bacteria are found in several discrete taxa, but their predatory mechanisms can broadly be divided into epibiotic, endobiotic, or 'group attack'. The best characterised group attackers are the myxobacteria, virtually ubiquitous soil-dwelling members of the Deltaproteobacteria. Myxobacteria glide backwards and forwards over surfaces, secreting digestive enzymes and secondary metabolites, many of which have antibiotic activity. Sensitive prey are lysed by the myxobacterial secretions, releasing nutrients into the 'public commons'.

This mode of predation typically has a very broad prey range – including Gram-negative bacteria, Gram-positive bacteria and fungi. However prey susceptibility is patchy, with individual prey strains able to resist predation by some predator strains. In some instances, prey strains have been shown to resist attack by producing biofilms, or counter-attacking by secreting antibiotics of their own.

Myxobacterial genomes are typically huge, often exceeding 10 Mbp, however pan-genome analysis has shown they possess large accessory pan-genomes, conferring considerable individuality on strains even from within a single species. Undertaking a genome-wide association study on 29 myxobacterial isolates preying upon ten prey organisms, allowed identification of 139 candidate 'predation genes' whose presence/absence correlated with predation efficiency. Following up one candidate gene, we found that predation against *Pseudomonas aeruginosa* was affected by the ability of predatory strains to detoxify formaldehyde, implying that formaldehyde secretion confers predation-resistance to the prey.

Tens of 'predation genes' were typically identified per prey organism, suggesting that prey susceptibility is generally not dictated by the presence/absence of a key toxin, but rather is due the cumulative contribution of multiple genes. Our current model is that myxobacteria are under low selective pressure to streamline their genomes, allowing accumulation of genes which might only occasionally provide an advantage during predation. This seems to be consistent with their predatory mechanism: unregulated secretion of large numbers of diverse digestive enyzmes and toxic metabolites.

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Interactions of cyanobacteria with predators

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Cyanobacteria live as primary producers in ecosystems together with many predators. Their successful propagation in these environments implies efficient defense mechanisms against predation by amoeba, fungi or predatory bacteria. Literature shows only few examples for predatory bacteria of cyanobacteria.
A newly discovered spiral shaped bacterium, which we term Candidatus *Venatospira tubingensis* lyses the model organism *Anabaena variabilis* in a gliding movement along the cyanobacterial filament. As shown by life cell imaging, the vegetative cells of a filament are lysed within 90 minutes, whereas the spore-like akinetes are resistant to lysis, representing a survival mechanism of *Anabaena*. Preliminary results indicate that the attack of the predator induces an emergency akinete formation.
Further experiments will focus on the occurrence and prey specificity of *Venatospira*, and the response of non-akinete forming cyanobacteria. Furthermore, since the intercellular communication in filamentous cyanobacteria extends throughout the whole filament, the response of the entire filament during an attack will be addressed.

Bacterial derived vitamin B_{12} enhances predatory behaviors in nematodes

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The microbiome is known to affect host development, metabolism and immunity, however, its impact on behaviors is only beginning to be understood. Here, we investigate how bacteria modulate complex behaviors in the nematode model organism *Pristionchus pacificus. P. pacificus* is a predator feeding on the larvae of other nematodes including *Caenorhabditis elegans*. Growing *P. pacificus* on different bacteria and testing their ability to kill *C. elegans* reveals drastic differences in killing efficiencies with a *Novosphingobium* species showing the strongest enhancement. Strikingly, increased killing was not accompanied by an increase in feeding, a phenomenon known as surplus-killing whereby predators kill more prey than necessary for sustenance. RNA-seq revealed widespread metabolic rewiring upon exposure to *Novosphingobium*, which facilitated the screening for bacterial mutants leading to an altered transcriptional response. This identified bacterial derived vitamin B12 as a major micronutrient enhancing predatory behaviors. Vitamin B12 is an essential cofactor for detoxification and metabolite biosynthesis and has previously been shown to accelerate development in *C. elegans*. In *P. pacificus* vitamin B12 supplementation amplified, whereas mutants in vitamin B12-dependent pathways reduced surplus-killing. This demonstrates that bacterial vitamin B12 affects complex behaviors and thus establishes a connection between microbial diet and the nervous system.

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The bacterial host background determines the life-cycle of Sa3-bacteriophages

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Temperate bacteriophages can modulate the virulence of the opportunistic bacterial pathogen *Staphylococcus aureus* by carrying accessory virulence factors, by interrupting chromosomal virulence genes, and by mediating the horizontal gene transfer of the phage-encoded virulence factors. Phage phi13 belonging to the group of Sa3-phages[1] is able to specifically integrate into the chromosomal *hlb*-gene thereby leading to a loss of β-hemolysin (hlb) production. However, these *hlb*-converting phages carry additional genes that encode for human-specific immune modulatory factors[2], thereby providing the bacterial host with new virulence traits underlining their impact on bacterial survival strategy of *S. aureus* within the human host. During the lytic cycle, the newly produced Sa3 virions can infect naïve bacterial cells and spread the phage-encoded virulence factors in the population. How frequently this happens may depend on environmental as well as host genetic factors.

We were particularly interested to understand the contribution of the bacterial genetic background on the life cycle of the Sa3-phage Φ13. Therefore, we lysogenized the *S. aureus* strains 8325-4, USA300-c, Newman-c (clonal complex 8) and MW2-c (clonal complex 1) with a Φ13 phage carrying a kanamycin resistance cassette (Φ13Kana). In co-culture experiments we observed that phage transfer and phage induction occurred significantly more frequently in the MW2 and Newman background than in the 8325 and USA300 background. This is most likely due to the fact that phage-encoded genes as well as several staphylococcal genes involved in the SOS-response or yet unknown function are differentially regulated in distinct host backgrounds, as indicated by our preliminary Northern blot, RNASeq and phage-promoter activity analyses.

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Microbial survival in the plant phyllosphere

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All terrestrial plants are inhabited by diverse, complex and interactive communities of microorganisms. The leaf's physical and chemical properties, combined with fluctuating and often challenging environmental factors, create surfaces that require a high degree of adaptation for microbial colonization. As a consequence, specific interactive processes among microbes and with their host have evolved to establish a plant leaf niche. In natural habitats, microbe-microbe interactions are important for shaping leaf communities. To protect resources, plant colonizers have developed direct antagonistic or host manipulation strategies to fight competitors (Chaudhry et. al. 2020).
Leaf-colonizing microbes strongly respond to biotic fluctuations (Agler et al. 2016) and are therefore an important resource for adaptive and protective traits. Understanding the complex regulatory host-microbe-microbe networks is required to transfer current knowledge to biotechnological applications.
A key player in the phyllosphere, responsible for ~9% of bacterial community variation, is the White Rust causing agent of the genus *Albugo*, a microbial Eukaryote and protist. We have shown that *A. laibachii* significantly supresses bacterial diversity and we could identify a direct suppressive effect to bacterial growth looking into Albugo exudates. Isolating *in planta* exudates, called apoplastic fluid, and performing proteomics analyses, we could identify numerous predicted amyloid and antimicrobial proteins. We further confirmed *in vivo* fibril formation by Thioflavin T, a stain to quantitatively and qualitatively measure amyloid fibrils. To characterize the varying antimicrobial repertoire of different *Albugo* sp., we screened for sensitivity of plant associated bacteria to apoplastic fluid of infected and uninfected plants. We could identify a broad range of sensitivity by different bacteria to the apoplastic fluid of individual *Albugo* sp. To infer mechanisms of antagonism and resilience, we have cloned a broad range of candidates and tested for their antimicrobial properties and potential functions.
Our results help to unravel survival strategies in complex communities and will therefore give insights into potential mechanisms of microbial control via new antimicrobial compounds and their function in mixed communities.

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Meta-omics reveal the activity and microbial interaction of autotrophic nitrate-reducing iron(II)-oxidizing bacteria from freshwater ecosystems

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Nitrate-dependent ferrous iron [Fe(II)] oxidation (NDFO) is widely found in the environment and is known as a vital biochemical process in neutrophilic Fe(II)-oxidizing bacteria (FeOB). Yet the examples of autotrophic nitrate-dependent Fe(II)-oxidizing bacteria (NDFOB) are rarely cultivated, and the metabolic mechanisms remain unclear. A novel NDFO enrichment culture BP was isolated from a freshwater sediment in Bremen, Germany. We investigated the *in situ* microbial relative abundance of the sampling sites and applied metagenomic, metatranscriptomic and metaproteomic analyses to culture BP under autotrophic and heterotrophic conditions to investigate the microbial interaction and the metabolic mechanisms of the unidentified species related to NDFO. Seven metagenome-assembled genomes were constructed, including a potential key player of NDFO affiliated with *Gallionellaceae*. Metatranscriptomic and metaproteomic data indicated 10503 transcripts and 126 proteins, respectively, to be significantly more abundant under autotrophic conditions than under heterotrophic conditions. The genes and transcripts associated to the potential FeOB in culture BP, *Gallionellaceae* sp., were involved in metal oxidation (e.g. *cyc2*), denitrification (e.g. *nirS/K*, *norB/C*), carbon fixation (e.g. *rbcL*) and oxidative phosphorylation (e.g. respiratory chain complexes I-V). The putative Fe(II)-oxidizing proteins, Cyc2 in *Gallionellaceae* sp. and MtoB in *Noviherbaspirillum* sp., were detected under autotrophic conditions. We predict that *Gallionellaceae* sp. performs Fe(II) oxidation and carbon fixation to provide energy and organic carbons for the heterotrophic community members to survive and further complete denitrification. Overall, these results demonstrate the survival strategy of microbes from organic carbon and oxygen limited freshwater environments and further deepen our knowledge of NDFO.

Dissecting the impact of commonly used drugs on the human microbiome

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Over the last two decades, the fundamental role of the intestinal microbiome on host physiology and pathology has been widely recognized and shifts in the microbiome composition have been associated with a range of different diseases. More recently, medication has proven to be one of the factors that most strongly influence the composition of the intestinal microbiome. This observation goes beyond classical antibiotics, and also applies to many commonly used non-antibiotics drugs. Most of what we know today about the impact of drugs on the intestinal microbiome originates from metagenomics-based cohort studies, but we know little about the direct effects of both antibiotics and non-antibiotic drugs on intestinal microbes, microbiomes and their consequences on human health. To fill this knowledge gap, we are investigating drug-microbiome-host interactions from a microbiology-driven perspective using systematic approaches.
Here, I will present our recent findings on how non-antibiotic drugs target intestinal microbes and the consequences for the human host. I will also share our newest insights on how antibiotics cause collateral damage to the intestinal microbiome by globally mapping target spectra and by dissecting bacterial drug responses. Our ultimate goal is to reveal strategies to mitigate drug-mediated adverse effects on the intestinal microbiome and to repurpose non-antibiotic drugs as targeted microbiome therapeutics.

Fitness genes of E. coli that allows survival in the inflamed gut

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The human microbiome accounts for the concerted group of microorganism that live together with each individual (1). Our focus of interest is the understanding of *Escherichia coli* survival in inflammatory bowel disease (IBD).
IBD include Crohn's disease and ulcerative colitis (2). Its exact etiology is unknown but has been linked to different factors such as genetics, immunology and nutrition among others. It is characterized by mucosal damage, resulting in increased permeability of the intestinal barrier and infiltration of polymorph nuclear neutrophils (3). Altogether, this inflamed environment represents a challenge for the survival of *E. coli*, which is a Gram-negative facultative anaerobic bacterium normally present in low abundance in the gut. Interestingly, an increased abundance of *E. coli* has been widely reported in patients of IBD (4). Previously, it was shown that metabolic pathways such as those able to convert non fermentable nutrients/nitrate to fermentable nitrates operate as fitness factors under inflammatory conditions.
Preliminary data from our group shows that in a chemically induced colitis, *E. coli* primarily prefer ethanolamine as substrate over propanediol.
To evaluate if this was reflected on the genetic content of commensal *E. coli*, we did whole genome sequencing of *E. coli* strains isolated from fecal samples of 27 healthy patients and 24 IBD patients. After assembly and annotation of our strains, we found that the ethanolamine pathway was represented in 87.5 percent of our IBD isolates while in healthy isolates it was 85%. For this reason we believe that the ethanolamine pathway might contribute to the survival of *E. coli*. This might not only explain the colonization success of *E. coli* under this harsh condition comprising an environment with high presence of immune cells but also might work as a diagnostic tool for IBD assessment. Used as a biomarker, over representation of ethanolamine pathway genes might be helpful for predicting the onset of inflammation while still being in IBD remission phase.

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Microbe-microbe interactions in human nasal microbiota

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Microbial communities include a network of microbe-microbe interactions. To discover causal factors that shape human nasal microbiota, my lab elucidates molecular mechanisms of bacterial interactions that underlie associations observed in compositional microbiome data. Five advantages allow us to recapitulate human nasal microbiota *in vitro* with consortia of small numbers of cultivated species. 1) In most adults, 2 to 10 species make up 90% of the nasal microbiota. 2) At a population level, there are 19 common species of nasal bacteria; 3) of which, 18 are cultivated. 4) Most common nasal bacteria are genetically tractable and 5) sampling human nasal passages is simple and safe. We focus on characterizing interactions between harmless bacterial members of the nasal microbiota and the pathogenic members *Staphylococcus aureus* and *Streptococcus pneumoniae.* to identify strains and molecules that contribute to colonization resistance against these pathobionts.

K Lemon Lab website: https://www.bcm.edu/research/labs-and-centers/faculty-labs/katherine-lemon-lab

Inhibitory Activity of Nasal Staphylococcal Products

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Staphylococci are frequent colonizers of epithelial surfaces like the skin or nasal cavities of humans (and animals) and represent important members of the human microbiome. The most extensively investigated species is the opportunistic pathogen *Staphylococcus aureus*, which colonizes the nose of 30% of the human population and can cause a variety of infections. In contrast, the vast majority of staphylococci are considered to be commensals and less harmful, although they occasionally also can cause infections. Together with other bacterial species that share the same habitat, staphylococci form bacterial communities whose composition is, in part, influenced by interbacterial interactions. These interactions often involve secondary metabolites like bacteriocins, antimicrobial compounds that inhibit the growth of other bacteria. This strongly impacts the competition for colonization sites and/or nutrients. Thus, the production of bacteriocins represents an important bacterial strategy to ensure the survival of the producer and to influence that of other bacteria.

In order to investigate the frequency of bacteriocin producing staphylococci we isolated staphylococci from the nares of volunteers and observed that the majority of those isolates (84%) produce bacteriocins active against other members of the nasal microbiome [1]. Among them, two strains have shown an inhibitory activity against *S. aureus*. The first strain, *Staphylococcus lugdunensis* IVK28, produces the antibiotic lugdunin [2]. Lugdunin is the founding member of the new antibiotic class of fibupeptides, comprising a characteristic thiazolidine moiety. Most importantly, it is the first described human microbiome-derived antibiotic and was shown to have an impact on bacterial community composition, as nasal *S. aureus* carriage was 5- and 6-fold reduced in healthy and hospitalized individuals, respectively, that were also colonized with *S. lugdunensis*. The second strain is *Staphylococcus epidermidis* IVK83, which produces a novel bacteriocin with a particular heptaene scaffold. It is the first product of a NRPS/PKS hybrid system produced by staphylococci. Both strains have shown to outcompete *S. aureus* in co-cultivation and co-colonization assays and hence, they represent two examples of how bacteriocin production affects bacterial survival.

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Cannibalism Stress in *Bacillus subtilis*:
from peptide toxin production to coordinated population response

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Upon starvation, the Gram-positive model organism *Bacillus subtilis* initiates a complex differentiation program to adjust and prepare the population for deteriorating growth conditions. This leads to the diversification into distinct sub-populations of specialized cell types, before *B. subtilis* ultimately forms dormant endospores. One such sub-population produces antimicrobial peptides (AMPs) specifically targeting non-producing sibling cells, in order to feed on released nutrients and delay commitment to spore formation (Popp & Mascher 2019). Next to the well-described cannibalism toxins, the sporulation delay protein (SDP) and the sporulation killing factor (SKF), we recently characterized a third toxin, YydF, that is produced by *B. subtilis* and specifically and intrinsically targets its own sibling cells. A comprehensive mode of action analysis with extrinsically applied YydF demonstrated a narrow and highly specific cell envelope stress response (CESR) of *B. subtilis* predominantly launching the phage-shock protein (PSP) response mediated by LiaH. Subsequent mutational studies demonstrate that only the unique combination of membrane permeabilization and rigidification caused by YydF leads to this CESR profile and that LiaH represents the most efficient resistance determinant (Popp *et al*. 2020). Physiological studies on the *yydF-J* locus revealed that production of this AMP is surfactin-dependent. This lipopeptide is expressed in the sub-population of *B. subtilis* pursuing biofilm formation, thus indicating involvement of YydF in re-organization or targeted killing of sibling cells within this highly structured and organized environment. For the first time, a novel class of AMPs produced by *B. subtilis* was therefore linked to a physiological trait beyond delaying commitment to sporulation. This comprehensive analysis of the AMP YydF expands our understanding of multicellularity in isogenic bacterial populations.

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Salvage of the amino sugar and amino acid components of the peptidoglycan cell wall provides a survival benefit to *Bacillus subtilis*

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Bacteria usually are able to synthesize all their cellular constituents from simple nutrients via *de novo* biosynthetic pathways. As these pathways are energy demanding, they represent a burden for the cells when nutrients become scant. A survival benefit is provided by salvage pathways in which cellular constituents are produced from intermediates of the degradative pathway of its own or a similar substance. An intriguing example is the salvage (i.e. recycling) of the peptidoglycan cell wall, an exoskeleton-like polymer composed of glycan strands cross-linked by short peptides that accounts for more than 20% of the weight of a Gram-positive cell [1,2]. As bacteria grow and divide, peptidoglycan remodelling results in the continuous release of turnover products from the cell wall. Moreover, during autolysis of subpopulations of cells, which is accompanied with cell differentiation (e.g. during cannibalism and sporulation in *Bacillus subtilis*), massive amounts of cell wall degradation products are provided to the survivors [2,3]. We recently showed that recovery of the amino sugar part of the peptidoglycan provides a survival benefit to bacteria in stationary phase [3, 4].
So far, the recycling of the peptide part of the peptidoglycan has not been addressed. We show here that *B. subtilis* is able to salvage cell wall-derived peptides and degrades them intracellularly. We suggest a pathway, in which non-crosslinked and crosslinked peptides are sequentially degraded by an Glu-DAP endopeptidase, an L-Ala-D/L-iso-Glu epimerase, an L,D-aminopeptidase and by a unique D,D-amino-peptidase/D-amidase. These enzymes are encoded on the genome next to an annotated peptide ABC transporter, which was shown to be expressed in early stationary phase. Presumably, the salvage of cell wall-derived peptides occurs during nutrient limitation in stationary phase and provides a survival benefit to the cell population.

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Regulation during growth arrest in *Pseudomonas aeruginosa*

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Bacteria in natural environments spend very little time dividing exponentially at their maximum rates before they have depleted the locally available nutrients. That a single *E. coli* cell dividing at its maximum laboratory growth rate would yield a colony larger than the volume of the known universe in less than 3 days' time powerfully illustrates this idea. Instead, most bacteria spend most of their time in dormant or slow-growing states, where cell division and cell death are roughly in balance to maintain population sizes. Mounting evidence suggests that bacteria in dormant states are intrinsically tolerant to antibiotics, and the ability of opportunistic pathogens to maintain ubiquitous reservoirs of organisms in many low-nutrient environments can contribute to the spread of genetic determinants of antibiotic resistance. Because most of our knowledge of transcription, translation, and DNA replication comes from studies in a handful of model organisms doubling rapidly under nutrient-replete conditions, understanding how bacterial cells regulate their activities under conditions of no net population growth represents an understudied area in microbiology. Under these conditions, some new proteins must still be made, but the regulatory strategy must mitigate the challenges and risks of activating biosynthetic pathways when energy and substrates are severely limiting. We are exploring this topic using the opportunistic pathogen *Pseudomonas aeruginosa* as a model organism. We have identified a novel transcriptional regulator specific to slow-growing states, and we are working toward understanding how its activities are integrated with those of other regulators expressed during growth arrest and with cellular metabolism as a whole. Preliminary data suggests that per-cell activity levels in a growth-arrested population are heterogeneous and dynamic over time, motivating further exploration of the regulation that controls these dynamics.

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Energy homeostasis in the developmental transitions of a cyanobacterium

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The ability of restoring growth after a period of dormancy is an important survival strategy for the maintenance of bacterial biodiversity, the spread of pathogens and the development of antibiotic resistances. Energy homeostasis is critical in the transition into and out of a quiescent state. *Synechocystis* sp. PCC 6803, a non-diazotrophic cyanobacterium, enters metabolic dormancy as a response to nitrogen deprivation. We investigated how dormant *Synechocystis* cells produce sufficient ATP to ensure survival, and how they obtain the necessary energy to awaken from dormancy. During nitrogen starvation, cells reduce their ATP levels to facilitate metabolic quiescence and engage sodium bioenergetics to maintain the minimum ATP content required for viability. When nitrogen becomes available to dormant cells, they start the awakening process and their energy requirements rise. Cells immediately respond to this higher energy demand by increasing ATP production. Surprisingly, we identified a yet unknown, cryptic sodium-motif force as primary source for ATP synthesis. Furthermore, glycogen catabolism is required to constantly support ATP synthesis in the resuscitating cells. Although most glycogen degrading enzymes are already present in dormant cells, glycogen degradation only starts when cells are provided with a combined nitrogen source. In the complex regulatory network that controls glycogen metabolism, the phosphoglucomutase (Pgm) plays a key role. Pgm activity is controlled via phosphorylation on a peripheral seryl-residue, thereby strongly affecting glycogen metabolism. Altogether, this study reveals a precise regulation of the energy metabolism essential for bacterial survival during periods of nutrient deprivation.

Contribution of bacterial Clp protease to bacterial survival

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The bacterial Clp protease is a multi-component molecular machine, ubiquitous in bacteria and essential for coping with certain environmental stress conditions in many species. For instance, in *Bacillus subtilis*, the Clp protease is required for growth at elevated temperature and stationary phase survival. It is also essential for the survival programme of sporulation. In many pathogenic species, such as *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Listeria monocytogenes* and others, the Clp protease functions as a global virulence regulator and Clp deletion mutants are impaired in intracellular survival or failed to colonize or infect the host. In Actinobacteria including *Mycobacterium tuberculosis* and *Streptomyces sp.* the Clp protease system is even essential for growth under all conditions. An exceptionally complex Clp system distinguishes streptomycetes with many more components than usual, and it is almost unstudied. Here, we present the first functional reconstitution of a *Streptomyces* Clp protease machine *in vitro*, and we characterized its composition, interactions and activities in degrading natural and non-native substrates. *In vitro* data are corroborated by parallel whole-cell studies that prove the physiological relevance of the observed results. We also report on a new Clp protease inhibitor produced by an Actinomycetales producer capable of inhibiting *S. aureus* ClpP and accordingly interfering with virulence. When Actinobacteria synthesize a Clp protease inhibitor or deregulator, the producer strains have to develop a survival concept to defend themselves from their otherwise toxic secondary metabolites. We discovered three ClpP proteins which function as self-resistance factors, and for one of them, we could already unravel the unprecedented resistance mechanism on a molecular level.

Chemical Inhibition of Caseinolytic Protease P Attenuates Virulence and Disrupts its Oligomeric State

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Multiresistant bacterial pathogens such as Methicillin-resistant *Staphylococcus aureus* (MRSA) are responsible for a variety of severe infections that pose a significant threat to global health. To approach this challenge new chemical entities with an unprecedented mode of action are desperately needed. This presentation will cover our latest efforts to identify new anti-bacterial targets and corresponding chemical inhibitors with the main emphasis on caseinolytic protease P (ClpP).

The barrel-shaped ClpXP protease, comprised of the chaperone ClpX and the ClpP protease, is a highly conserved virulence regulator in bacterial pathogens. Genetic ClpP knockouts in *S. aureus* revealed a reduction in virulence, i.e. the expression of bacterial toxins, resulting in attenuated infections in murine abscess models.[1] The same phenotype was observed by inhibiting ClpP with aliphatic or aromatic beta-lactones, the first specific inhibitors reported for ClpP.[2] However, their limited selectivity, stability and potency urged the need for novel inhibitory scaffolds. An unbiased high-throughput screen revealed a new class of covalent inhibitors: phenyl esters.[3] These modifiers of ClpP allow for an investigation of its proteolytic mechanism and can disrupt the oligomeric state of ClpP by a stereogenic methyl switch. A subsequent SAR study resulted in a second generation of peptide-based phenyl esters which retain their inhibitory potential also for the full ClpXP complex and can arrest proteolysis by altering the stoichiometry of ClpXP to an unprecedented hexamer-heptamer assembly.[4]

This talk will focus on proteomic profiling to identify targets of these compounds, their structural optimization and dissection of their mechanism of action.

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Biosynthesis of the cyclopropyl moiety of belactosin A

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Bacteria produce a plethora of secondary metabolites, and such natural products can serve as intermediary in survival strategies for example as siderophores, quorum-sensing molecules in microbial interactions or virulence factors. Small molecule proteasome inhibitors have also been described to act as virulence factors. Streptomyces sp. UCK14 produces the proteasome inhibitor molecule belactosin. Belactosin A, which harbours a unique 3-(trans-2-aminocyclopropyl) alanine (AcpAla) moiety, shows comparable inhibitory activity against the proteasome to belactosin C, raising the question why UCK14 is investigating in the biosynthesis of such a complex moiety. In this project we aim to investigate in the elucidation of the biosynthesis of the AcpaAla moiety and further understand its role in the survival strategy of UCK14.

Recently, we identified the biosynthetic gene cluster of belactosin by genome mining and gene deletion studies.[1] Gene cluster analysis showed a putative operon *belKLMN* with homology to genes from the hormaomycin biosynthetic pathway that were postulated to be involved in the formation of 3-(trans-2-nitrocyclopropyl) alanine (NcpA).[2] To gain first insight into a possible biosynthesis of the AcpAla moiety and potential precursor molecules, feeding experiments with stable-isotope labeled precursor molecules were conducted in the producer strain *S. sp*. UCK14. Thereby it could be shown, that the precursor molecule of the AcpAla moiety in belactosin A is lysine, supporting a common biosynthetic route to AcpA and NcpA. Heterologous pathway expression, gene deletion studies and chemical complementation confirmed this hypothesis. Furthermore we could confirm, that BelN, a molybdopterin-dependent reductase, is involved in the conversion from NcpA to AcpA.

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Biosynthesis and Mode of action of 7-deoxysedoheptulose – an inhibitor of the shikimate pathway

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Cyanobacteria are known as producers of a wide range of bioactive compounds. Most of them were isolated from filamentous species. Only little is known about bioactive compounds which are formed by unicellular cyanobacteria. The unicellular model-organism *Synechococcus elongatus* PCC 7942 has a small and stream-lined genome and no bioactive compound was identified so far. In our work we isolated and characterised this compound as a rare deoxy-sugar, namely 7-deoxysedoheptulose (7dSh) which inhibits the growth of other cyanobacteria (Brilisauer et al. 2019). We further identified 7dSh as a competitive inhibitor of the dehydroquinate synthase which is the second enzyme of the shikimate pathway. Therefore, 7dSh also showed bioactivity towards plants (Brilisauer et al. 2019). Furthermore, we identified the biosynthetic pathway of 7dSh production in *S. elongatus*. In this pathway 5-deoxyadensine, a byproduct of radical SAM enzymes, is processed by moonlighting activity of enzymes of the primary metabolism which results in the excretion of 5-deoxyribose (5dR) and 7dSh. This example impressively showed that the presence of specific gene clusters for the synthesis of secondary metabolites is not necessary.

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Abstracts – poster presentations

Requirement for Sodium during vegetative growth and chlorosis in the Cyanobacterium *Synechocystis* sp. PCC 6803

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Salts play an important role in life. They serve as buffer substances, cofactors, enhancers, inhibitors, and much more. Sodium is the most abundant cation in the environment and is thus expected to influence all living cells. In cyanobacteria, sodium is reported to be required for the regulation of osmotic pressure, pH, bicarbonate uptake and photosynthesis (1, 2).
Synechocystis sp. PCC 6803, hereafter *Synechocystis*, is a non-diazotroph unicellular cyanobacterium. If faced with nitrogen starvation, it degrades its photosynthetic pigments, a process termed chlorosis and finally enters a state of metabolic quiescence (3).When a source of combined nitrogen becomes available to the dormant, chlorotic cells, they rapidly start a tightly regulated and highly reproducible process of resuscitation (4).
We have found the primary requirement for sodium in *Synechocystis* during vegetative growth to be carbon uptake through growth experiments. This extends to early chlorosis, during which the typical accumulation of large amounts glycogen is only possible in the presence of sodium. When resuscitation of chlorotic cells is initiated in the absence of soidum, the cells are still able to import and reduce nitrate and to switch on respiration supported by catabolism of glycogen. However, the cells are not able to fully recover pigmentation, indicating an early requirement for CO_2 fixation during resuscitation.

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Acidocalcisomes and Polyphosphate Granules Are Different Subcellular Structures in *Agrobacterium tumefaciens*

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Acidocalcisomes are membrane-enclosed, polyphosphate- (polyP) and calcium ions-containing acidic organelles in lower eukaryotes but have been described likewise also for *Agrobacterium tumefaciens* [1]. In our study we re-examined the subcellular localization and composition of polyphosphate-containing compartments in this α-proteobacterium. Surprisingly, - by fusion analysis of key proteins of acidocalcisomes (vacuolar H^+-translocating pyrophosphatase, HppA) and of polyphosphate granules (polyP kinases, PPKs) with fluorescent proteins (eYFP, mCherry) - we found that polyP granules and acidocalcisomes are different subcellular structures [2] and that polyP granules are not surrounded by a phospholipid membrane. Furthermore, we characterized the biochemical properties of the two polyP kinases of *A. tumefaciens*, PPK1 and PPK2 [3] and the effect of the absence of polyP in Δ*ppk1* and/or Δ*ppk2* mutants on survival in free-living and in plant-infection life styles of the bacteria.

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Amyloid proteins in microbe-microbe interactions

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The plant leaf microbiome is composed of microbes in constant competition for nutrients and protection. The plant provides these as limited resources, which places a high selective pressure on microbial mechanisms to fight off competitors. One of these microbes is *Albugo*, a highly adapted eukaryotic parasite. It relies on the plant for its nutrient assimilation and therefore relaxed selection has led to the loss of most of its metabolic pathways. Small protein effectors are secreted by *Albugo* to the extracellular space for modulation of the host immune response and, as described recently, also microbiome control agents (Snelders et al. 2020). Our group has shown previously that *Albugo* significantly modifies the microbiome of infected plants (Agler et al. 2016). However, the mechanisms behind this effect remain largely unexplored. Increasing evidence links functional amyloids, protein fibrils with highly ordered structure, with essential biological processes relating to survival. Such are: adhesion, structural support and defense in a variety of prokaryotes and eukaryotes. To test the role of amyloid proteins in the interaction of *Albugo* with the leaf microbiome we have selected candidate proteins based on *in silico* predictions for their amyloid formation and antimicrobial activity. Consequently, we have heterologously over-expressed the candidates in procaryotic and eucaryotic expression systems to test for these characteristics. We have found empirical evidence for the formation of extracellular amyloid fibrils by several candidate proteins that were in parallel checked for affinity to amyloid-specific dyes. Additionally, bacteria were screened for their sensitivity to the amyloid candidate proteins as well as related small *Albugo* effector proteins. We detected a specific antibacterial effect for an *Albugo* protein on two plant associated bacteria. Our results help to understand important components of the microbe-microbe dialogue in complex communities and will identify new antimicrobial proteins that regulate the interorganismic cross-talk.

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Biosynthesis of [*S,S*]-EDDS, a chelator required for *Amycolatopsis japonicum* to survive under Zn limitation

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Divalent metals (Mn, Fe, Co, Ni, Cu and Zn) are essential micronutrients for all life forms, and acquisition of these metals is therefore vital. In conditions of limited availability of an essential metal, various enzymes necessary for bacterial metabolism are inactive compromising cell survival. The survival of microorganisms in the soil depends on their ability to absorb metal ions. Since many bacteria cannot produce complexing agents themselves, but can use those from other bacteria or fungi to survive metal deficiency, complexing agents play an important role in the ecology of microorganisms.

Following iron, Zinc is the most abundant transition metal in bacteria. Given the important structural and catalytic role that Zn (II) plays in proteins, it is not surprising that bacteria have developed Zn specific complexing agents to counteract Zinc deficiency such as the production of zincophores. One example of such a zincophore is [*S,S*]-Ethylenediamine disuccinate (EDDS), a chelating agent synthesized by the strain *Amycolatopsis japonicum* (*A. japonicum*) under zinc deficient conditions, indicating that EDDS contributes to zinc uptake, as a zincophore. In contrast to its isomer EDTA, which is used for many applications, EDDS combine excellent chelate properties with the accessibility to biodegradation. Therefore, EDDS is considered as a sustainable chelating agent, possesing the potential to replace EDTA and other environmentally threatening chelating agents for Zn fertilization. However, no studies have been carried out in order to evaluate the suitability of using *A. japonicum* as biofertilizer.

Inhibition of *Staphylococcus aureus* ClpP by natural product β-lactones

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Bacterial survival strongly depends on the efficiently timed adaption to environmental changes. Proteolytic machineries, which keep proteostasis, mediate protein turnover, detoxify defective proteins and contribute significantly to survival of bacteria in adverse environments including the human host[1]. The Clp proteolytic system, composed of the proteolytic core ClpP and its partner ATPases, is involved in intracellular protein turnover and regulatory proteolysis. ClpP in its active state forms a tetradecameric barrel structure, shielding 14 active sites within the barrel. On its own, ClpP is only capable of degrading small peptides. However, in concert with its cognate Clp-ATPases, the protease complex is formed, allowing tightly regulated and specific protein degradation [2, 3].
During the last decade, ClpP attracted substantial interest as promising bacterial target and various modulators of ClpP were described. Besides the acyldepsipeptides, which exhibit a dual mode of action by deregulation and overactivation of ClpP, several inhibitors of ClpP were reported [4, 5, 6, 9]. Synthetic beta-lactones, for example, inhibit the key virulence regulator ClpP in *S. aureus* and by targeting virulence rather than viability, resistance development can be reduced [7]. Recently, the natural β-lactones cystargolide A and B were described to be produced by the rare actinomycete *Kitasatospora cystarginea* [8]. Since natural products represent promising lead-structures for antibiotic development, we investigated if cystargolide A and B represent novel inhibitors of ClpP. Applying biochemical and microbiological methods, such as kinetic measurements, size exclusion chromatography and an hemolysis assay, we characterized cystargolide A and B as novel and potent inhibitors of ClpP.

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Gated septal junctions allow cell-cell communication in filamentous cyanobacteria

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To survive in a nitrogen-poor environment, several filamentous cyanobacteria, e.g. the species *Anabaena* sp. PCC 7120 (hereafter *Anabaena*), differentiate N_2-fixing heterocysts. These specialized cells develop in a semi-regular spacing from photosynthetic vegetative cells upon depletion of combined nitrogen. To allow such a multicellular lifestyle, a cell-cell communication machinery is essential, enabling exchange of metabolites and signalling molecules[1]. The molecule exchange can be measured with fluorescent markers by fluorescence recovery after photobleaching (FRAP) experiments[2] and was supposed to occur thru direct cell-cell connections. Such proteinaceous septal junctions (SJs) traverse the shared peptidoglycan (PG) between adjacent cells of the filament[3]. The outer membrane of the Gram negative cell wall does not enter the septa. The septal PG is perforated by 80-150 nanopores, forming the nanopore array as framework for incorporation of the SJs[4].
Recently, we revealed the *in situ* architecture of the SJs via cryo-focused ion beam milling and imaging with electron cryotomography (ECT)[5]. The SJs comprise a cytoplasmic 5-fold symmetric cap, a cytoplasmic membrane-embedded plug module at both ends and a connecting tube which traverses the PG. Upon disruption of the proton motive force, a conformational change in the cap structure leads to closure of the SJs and inhibition of molecular exchange[5]. Closed SJs reopen in favourable conditions, which renders them a gated and dynamic channel in analogy to metazoan gap junctions. Furthermore, we identified the first known structural SJ component, the FraD protein[5]. To identify other components of the multimeric SJ complexes, we performed pulldown experiments using FraD as bait. Currently, we are analysing mutants in FraD interacting proteins via FRAP experiments and ECT imaging.
In conclusion, our study shows that filamentous cyanobacteria possess gated, dynamic septal junctions allowing the regulation of intercellular molecular exchange. This is a unique bacterial survival strategy, by which the filament is protected, when single cells are destroyed, e.g. by predator attack, senescence or shear forces, by closing the cell-cell connections.

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Gut commensal-induced IκBζ expression in dendritic cells influences the Th17 response

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Intestinal commensal bacteria can have a large impact on the state of health and disease of the host. By directly interacting with the host's immune system, they shape the intestinal environment to create more favorable survival conditions. Regulation of Th17 cell development by gut commensals is known to contribute to their dichotomous role in promoting gut homeostasis and host defense, or development of autoimmune diseases. Yet, the underlying mechanisms remain to be fully elucidated. One candidate factor contributing to Th17 differentiation, and the expression of which could be influenced by commensals is the atypical nuclear IκB protein IκBζ. IκBζ acts as a transcriptional regulator of the expression of Th17-related secondary response genes in many cell types including dendritic cells (DCs). Insights into the regulation of IκBζ in DCs could shed light on how these immune sentinel cells at the interface between commensals, innate and adaptive immune system drive an immune-tolerogenic or inflammatory Th17 cell response. In this study, the influence of two gut commensals of low (*Bacteroides vulgatus*) or high (*Escherichia coli*) immunogenicity on IκBζ expression in DCs and its downstream effects was analyzed. We observed that the amount of IκBζ expression and secretion of Th17-inducing cytokines correlated with the immunogenicity of these commensals. However, under immune-balanced conditions, *E. coli* also strongly induced an IκBζ-dependent secretion of anti-inflammatory IL-10, facilitating a counter-regulative Treg response. Yet, in an in vivo mouse model of T cell-induced colitis, prone to inflammatory and autoimmune conditions, the enrichment of the microbiota with *E. coli* promoted an expansion of rather pro-inflammatory T helper cell subsets whereas enrichment with *B. vulgatus* resulted in the induction of protective T helper cell subsets. These findings might contribute to the development of new therapeutic strategies for the treatment of autoimmune diseases using commensals or commensal-derived components.

Reconstitution of the functional Clp protease complex of streptomycetes *in vitro*

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Bacterial Clp proteases are multimeric degradation machines that play a major role in the bacterial protein stress response and survival under adverse conditions. In principle, a proteolytic core composed of 14 ClpP monomers associates with hexameric Clp-ATPases to degrade misfolded and specific regulatory proteins [1, 2]. In contrast to most eubacteria comprising one or two ClpP homologs, streptomycetes rely on a more complex Clp system that is essential for their survival under all conditions. Up to five distinct ClpP homologs are organized in two bicistronic and one monocistronic operon [3]. So far, knowledge on the molecular composition, interaction and operation mode of the Clp system in streptomycetes is rare, and a functional Clp system has not been reconstituted *in vitro.*

In this study, we functionally reconstituted the Clp protease complex of *Streptomyces hawaiiensis* NRRL 15010, the producer of the antibacterial complex A54556 [4], as a prerequisite for investigations of the self-resistance mechanism of the producer strain. The *clpP* genes of *S. hawaiiensis* show high sequence similarities to other streptomycetes [4]. To shed light on the interaction and functional cross-talk between the ClpP proteins in *Streptomyces*, we expressed and purified the Clp proteins of *S. hawaiiensis* for functional studies using different Clp-ATPases as mediators for substrate degradation by different ClpP isoforms. In addition, ADEP1, the main component of complex A54556 known to deregulate ClpP in other bacteria [1, 4], was used to investigate its impact on the *Streptomyces* ClpP proteins in the natural context. Our data reveal the operation mode of the Clp protease in streptomycetes, bacteria with highly complex physiology and diverse secondary metabolism, which is an important step to better understand protein homeostasis and protein stress responses guided by the Clp protease in this genus. Regarding the discovery of a sixth Clp peptidase ($ClpP_{ADEP}$) in close proximity to the ADEP-biosynthetic gene cluster in *S. hawaiiensis* that mediates ADEP-resistance [4], analysis of the house-keeping Clp protease represents an important step towards deciphering a novel mechanism of producer strain survival.

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