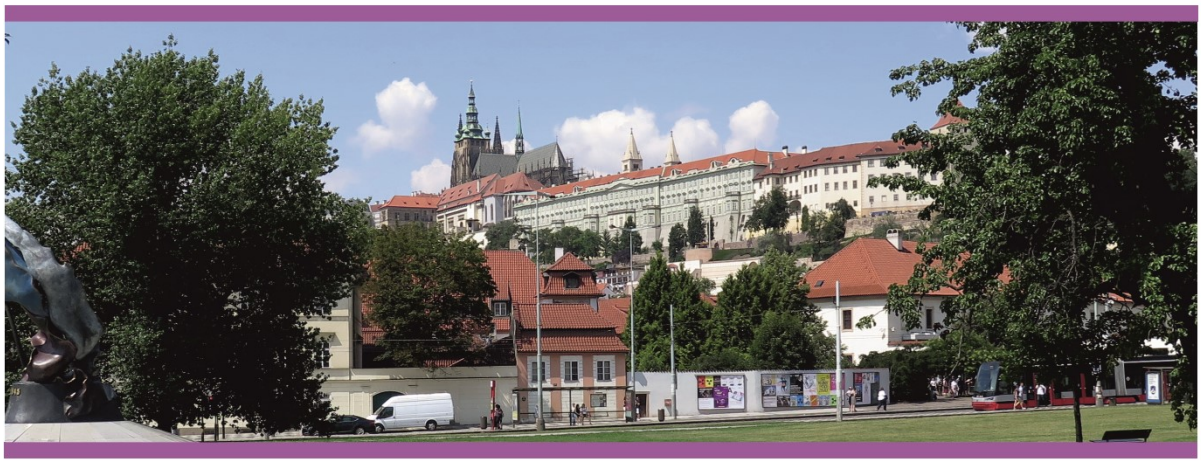
21st International Conference on *Bacilli* and Gram-Positive Bacteria

14 - 17 June 2022

GRAM-POS-2022 @ PRAGUE, CZECH REPUBLIC



Prague, Czech Republic

21st International Conference on *Bacilli* and Gram-Positive Bacteria

14 - 17 June 2022

Prague

Institute of Microbiology

Czech Academy of Sciences

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WELCOME NOTE

Dear Colleagues

It is a great pleasure to welcome you to the 21st International Conference on Bacilli and Gram-Positive Bacteria.

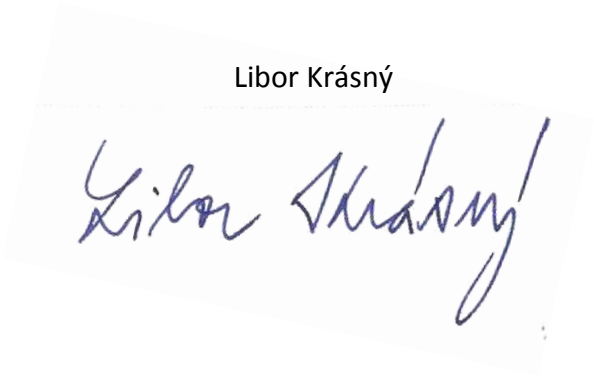
This conference series has a long tradition, spanning about 40 years and taking places both in Europe and America. The last meeting was held in 2019 at the University of Maryland, College Park, Washington, and when Prague was selected as the place of the next meeting, no one expected that it would have to be postponed till 2022.

After those covid years, it is a great satisfaction to finally be able to meet in person. The conference has attracted ~ 200 participants from 25 countries in Europe, Asia, the Americas, and Australia. The topics will cover various aspects of the biology of *Bacilli* and Gram-positive bacteria. Keynote lectures from internationally reknown experts will be complemented by oral presentations selected from submitted abstracts and we will have two poster sessions that promise to provide an excellent platform for interactions between participants. We also expect a fierce competition for the awards in the Best Lecture/Poster by Young Researcher categories.

We are looking forward to stimulating science and, we thank all our sponsors for their support. On behalf of the organizing/scientific committee, I am happy to welcome you here in Prague.

Vítejte!

Libor Krásný



ORGANIZING COMMITTEE

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- **Jan Maarten van Dijl**
  University of Groningen, Groningen, The Netherlands

PROGRAM

Tuesday June 14

12:00 - 14:45 Registration and Check-in
14:45 - 15:00 **Opening Remarks**

15:00 - 16:45 **Session 1: DNA Replication & Transfer/ Cell Division**
Chair: Peter L. Graumann

15:00 - 15:30 How Gram-positive bacteria monitor their cell wall for defects and catalyze their repair
David Rudner

15:30 - 15:45 MurA escape mutations uncouple peptidoglycan biosynthesis from PrkA signaling
Sven Halbedel

15:45 - 16:00 PrkC kinase modulates MreB filament and bacteria growth by monitoring cell wall precursor Lipid II
Yingjie Sun

16:00 - 16:15 Mg2+ modulates *Bacillus subtilis* cell division frequency
Jennifer K. Herman

16:15 - 16:30 Discovery of a novel two-component antitermination system associated with conjugation operons in G+ bacteria
Wilfried J. J. Meijer

16:30 - 16:45 Specific protein-lipid interactions play important role in *Clostridioides difficile* Min-system functioning
Naďa Labajová

17:00 **Welcome drink**

Wednesday June 15

9:00 - 10:45 **Session 2: Gene Expression**
Chair: Yulia Yuzenkova

9:00 - 9:30 Transcription Regulation of Purine Biosynthesis by (p)ppGpp
Jue D. Wang

9:30 - 9:45 Promoter Prediction in Prokaryotes by aid of Machine Learning Technology
Anne de Jong

9:45 - 10:00 SPβ c2 – a heat inducible representative of the SPβ-like phages provides understanding and further exploration of the complex lysogeny management system
***Katharina Kohm**

10:00 - 10:15 Exploring the molecular basis of thermophilicity in a *Bacillus species*
Rajinikanth Mohan

10:15 - 10:30 Gintool; enabling transcriptome analysis using regulon information
Leendert Hamoen

10:30 - 10:45 Repression by DNA looping: Crystal structure and functional analysis of the global transcriptional regulator ScoC
Smadar Shulami

10:45 - 11:30 **Coffee Break**

11:30 – 13:15 **Session 3: RNA, Translation**
Chair: Ciaran Condon

11:30 - 12:00 David vs Goliath: Ribosome-targeting antibiotics and bacterial resistance mechanisms
Daniel N. Wilson

12:00 - 12:15 Dissecting the function of the two paralogous 6S RNAs in an undomesticated *Bacillus subtilis* wild-type strain
Roland K. Hartmann

12:15 - 12:30 Novel RNA molecules interacting with the bacterial transcription machinery
Jarmila Hnilicová

12:30 - 12:45 *B. subtilis* RNase Y and *E. coli* RNase E : Different enzymes – similar strategies
Harald Putzer

12:45 - 13:00 Segregation of transcription and translation in *B. subtilis* spatially organizes ribosome assembly and metabolism
Peter L. Graumann

13:00 - 13:15 Ribosome stalling and collision sensing in bacteria
***Federico Cerullo**

13:15 - 14:30 **Lunch**

13:45 - 14:30 **SubtiWiki Workshop I**

14:30 - 16:00 **Session 4: Cell Morphology**
Chair: Jan Maarten van Dijl

14:30 - 15:00 Homeostatic regulation membrane lipid composition: Is fluidity really the important parameter?
Henrik Strahl

15:00 - 15:15 Identification and characterization of a novel staphylococcal cell morphology determinant
Morten Kjos

15:15 - 15:30 Adaptation of *Bacillus subtilis* to low humidity requires a Sigma B-dependent general stress response
Sjouke Piersma

15:30 - 15:45 Cell Wall Biosynthesis in *Bacillus subtilis*
***Alaa A. Aljohani**

15:45 - 16:00 Takeover of *Bacillus subtilis* cell biology by a phage
Anna Dragoš

16:00 - 16:30 **Coffee Break**

16:30 - 18:00 **Session 5: Cell Metabolism / Biotechnology**
Chair: Ken-ichi Yoshida

16:30 - 17:00 Lanthipeptide biosynthesis: the assembly of the nisin biosynthetic and secretion complex in *B. subtilis* and in *L. lactis*
Oscar P. Kuipers

17:00 - 17:15 Subtilisin-mediated bioplastic degradation: from mechanism to application
***Jordan Cannon**

17:15 - 17:30 Exploring the iron starvation stimulon of *Staphylococcus aureus* using a combination of bioinformatics and experimental approaches
***Larissa Busch**

17:30 - 17:45 System-wide discovery of protein-protein interactions and interfaces in *Bacillus subtilis*
Andrea Graziadei

17:45 - 18:00 Protein arginine phosphorylation and dephosphorylation facilitates protein homeostasis in *Bacillus subtilis*
Kürşad Turgay

18:00 - 19:45 **Poster Session I**
+ Buffet dinner

Thursday June 16

9:00 - 10:45 **Session 6: Cell - Cell Communication**
Chair: Leendert Hamoen

9:00 - 9:30 Intra and interspecies interactions shape the war and peace in the *Bacillus subtilis* world
Ines Mandic-Mulec

9:30 - 9:45 Evidence for SCCmec transfer by natural transformation in *Staphylococcus aureus* biofilms
***Mais Maree**

9:45 - 10:00 Gram positives against Gram negatives: *B. subtilis* PS-216 inhibits *Campylobacter* biofilm formation
Polonca Stefanic

10:00 - 10:15 Cannibalism in *Bacillus subtilis*: Role of programmed cell death in shaping and functionalizing differentiated multicellular populations
Thorsten Mascher

10:15 - 10:30 Controlled interkingdom communication: crosstalk between bacteria *Bacillus subtilis* and the eukaryote *Saccharomyces cerevisiae* by utilizing bacterial quorum sensing peptides
***Tomislav Vološen**

10:30 - 10:45 Single molecule dynamics of the DNA receptor ComEA and DNA uptake in competent *Bacillus subtilis* cells
***Alexandra Kilb**

10:45 - 11:30 **Coffee Break**

11:30 – 13:15 **Session 7: Motility**
Chair: Olga Sotourina

11:30 - 12:00 SwrA increases binding affinity at a subset of DegU-regulated promoters
Daniel B. Kearns

12:00 - 12:15 SwrA: much more than a swarming protein
Cinzia Calvio

12:15 - 12:30 Strategies for improved surfactin production using *Bacillus subtilis*
***Lars Lilge**

12:30 - 12:45 Flagella disruption in *Bacillus subtilis* increases amylase production yield
Thomas B. Kallehauge

12:45 - 13:00 SpoVG – a global regulator of stationary phase processes
Emma L. Denham

13:00 - 13:15 c-di-AMP signaling is required for bile salt resistance and long-term colonization by *Clostridioides difficile*
Johann Peltier

13:15 - 14:30 **Lunch**

13:45 - 14:30 **SubtiWiki Workshop II**

14:30 - 16:15 **Session 8: Biofilm**
Chair: Ulrike Mader

14:30 - 15:00 Modulating cell type distribution in a biofilm
Gürol M. Süel

15:00 - 15:15 Restoring functionality of a compromised *Bacillus subtilis* biofilm activator protein
Felix Dempwolff

15:15 - 15:30 SAOUHSC_00671 contributes to cell splitting and biofilm formation in *Staphylococcus aureus* and its expression is associated with other peptidoglycan hydrolases
Danae Morales Angeles

15:30 - 15:45 Second-generation transfer mediates efficient propagation of ICEBs1 in biofilms
***Jean-Sébastien Bourassa**

15:45 - 16:00 Tackling biofilms: Revealing protective components of *B. subtilis* biofilms in ROS stress
***Erika Muratov**

16:00 - 16:15 Spatial transcriptome profiling unveils multi-scale heterogeneity levels between subpopulations of *Bacillus subtilis* surface-associated communities
***Yasmine Dergham**

16:15 - 18:00 **Poster Session II**
Coffee Break

19:00 **Gala Dinner in the City Center**

Friday June 17

9:00 - 10:45 **Session 9: Bacterial Virulence**
Chair: Monika Ehling Schulz

9:00 - 9:30 *Bacillus anthracis* Branched Chain Amino Acid Transporters: Expression, Function, and Relationship to Virulence
Therese M. Koehler

9:30 - 9:45 Determination of *Staphylococcus aureus* infection-mimicking conditions by CRISPRi-Seq
Maria Vittoria Mazzuoli

9:45 - 10:00 An alternative way of life for *Bacillus thuringiensis* in the late stages of an infection
Leyla Slamti

10:00 - 10:15 Elucidating the mechanisms conferring self-resistance to the emetic toxin cereulide by the opportunistic pathogen *Bacillus cereus*
Sabrina Jenull

10:15 - 10:30 Regulatory RNAs in *Clostridioides difficile*: from genome-wide identification to targeted characterization and applications
Olga Soutourina

10:30 - 10:45 Towards characterizing the putative ABC transporter EslABC in the human pathogen *Listeria monocytogenes*
***Lisa M Schulz**

10:45 - 11:30 **Coffee Break**

11:30 – 13:15 **Session 10: Sporulation**
Chair: Imrich Barak

11:30 - 12:00 The assembly and functional architecture of the spore surface layers
Adriano O. Henriques

12:00 - 12:15 A new role for SR1 from *Bacillus subtilis* – regulation of sporulation by inhibition of kinA
Peter Müller

12:15 - 12:30 Investigation of spore-associated lipoproteins YlaJ, YhcN, YutC, and CoxA suggests existence of a complex that stabilizes the germination apparatus in *Bacillus subtilis* spores
***Matthew J. Flores**

12:30 - 12:45 A novel ribonuclease KapD is integrated into the *B. subtilis* spore surface layers and plays a key role in structuring the outer coat and crust
Ciaran Condon

12:45 - 13:00 Insights into the role of CD25890, a conserved protein that modulates sporulation initiation in *Clostridioides difficile*
***Diogo Martins**

13:00 - 13:15 PrkA regulates *Bacillus subtilis* sporulation through a Lon protease activity
Frederique Pompeo

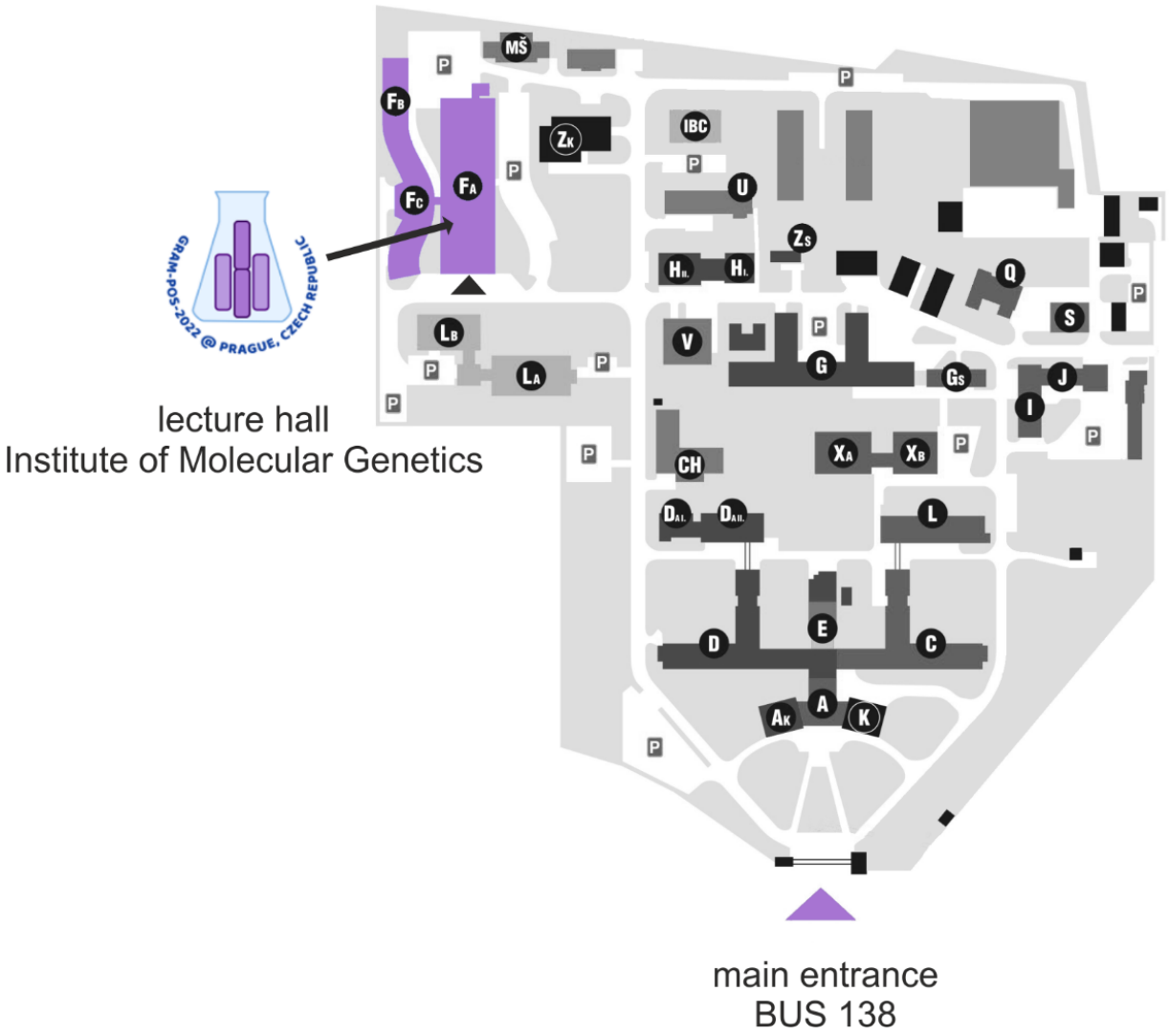
13:15 - 13:30 **Closing Remarks**

13:30 – 14:30 **Lunch**

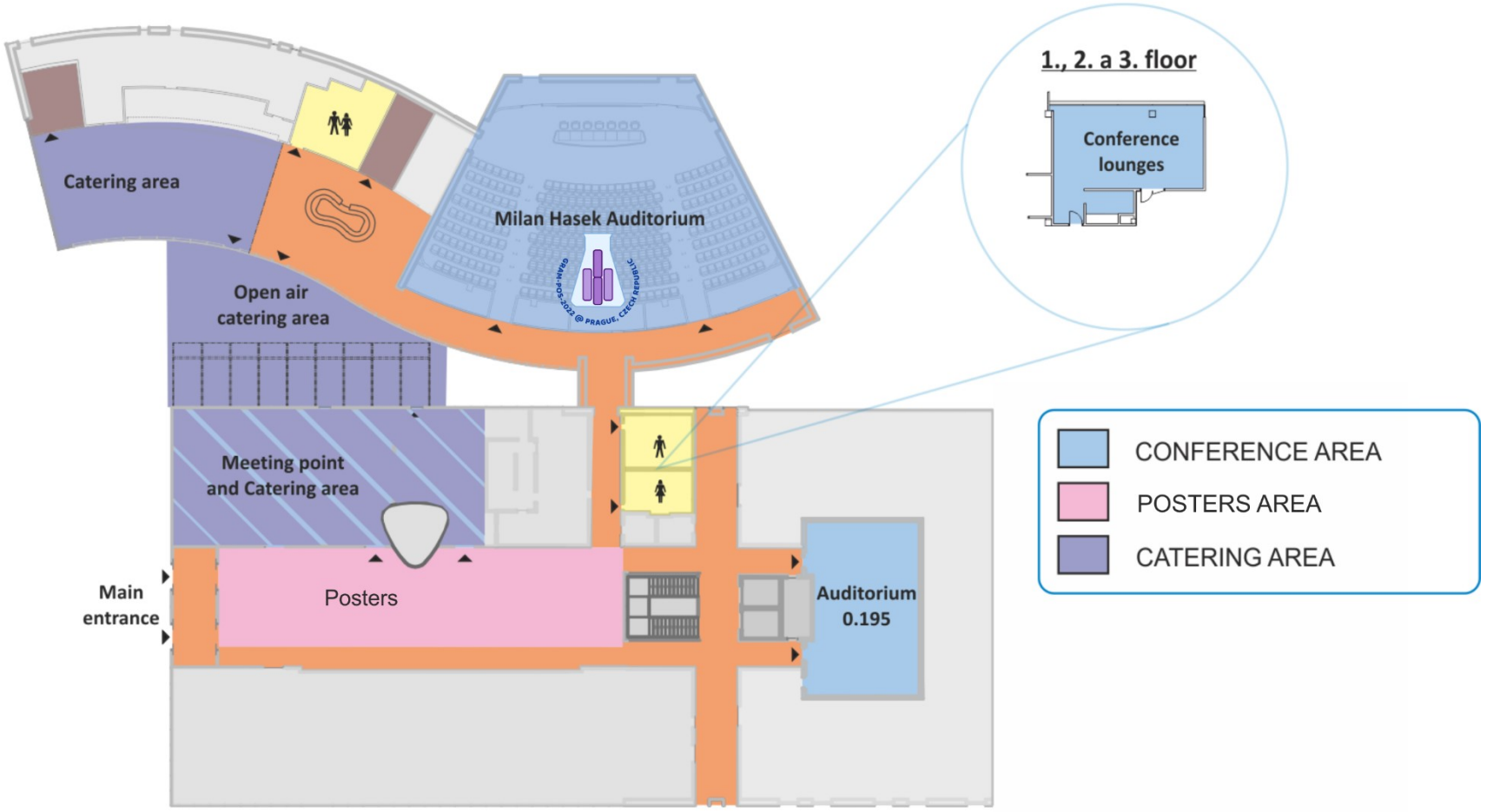
VENUE

Institute of Molecular Genetics

Campus of Czech Academy of Sciences, building F
Vídeňská 1083
Prague 4
142 20



Ground floor



GENERAL INFORMATION

Conference website

https://www.gram-pos-2022.org/

#GramPos2022

Abstracts

The Abstract Book is available also in the pdf format at the Conference web pages. Use your e-mail and PIN from registration to get access.

Lectures

The keynote lectures will be 25 minutes plus 5 minutes for discussion. Other talks will be 12 minutes plus 3 minutes for discussion. We kindly ask all the speakers to download their presentation in the Projection Room at the back of the Lecture Hall (entrance from the outside) and check their functionality before their respective sessions.

Posters

Posters will be displayed in two separate sessions. The first session (**odd numbers**) will be held on Wednesday, June 15 from 18:00 to 19:45. Please note that the first poster session overlaps with the buffet dinner. The posters from the first session have to be removed by the end of the day. The second session (**even numbers**) will be held on Thursday, June 16, from 16:15 to 18:00. It is not allowed to take pictures of the posters without the prior consent of the person presenting the poster.

Awards

There will be awards for two best oral and four best poster presentations (500 and 250 EUR), sponsored by BACIP. The results will be announced at the end of the Conference during Closing Remarks.

*in the Abstract Book denotes that the person is eligible for competition for the Best lecture/poster by a young scientist.

SubtiWiki workshop

There will be two identical SubtiWiki workshops on June 15 and 16, both partially overlapping with lunch. Those interested in the workshop, please sign up at the registration desk. Each session can hold 35-40 participants max.

Catering

Tea, coffee, refreshments and snacks will be available free of charge during all program breaks in the Meeting Point Room.

Special requirements (vegan, lactose intolerance, gluten-free): these meals, snacks, drinks will be prepared for those who requested them in the registration forms. The meals will be labeled as such in the buffets. We kindly ask our omnivorous colleagues to leave these meals for those who have requested them.

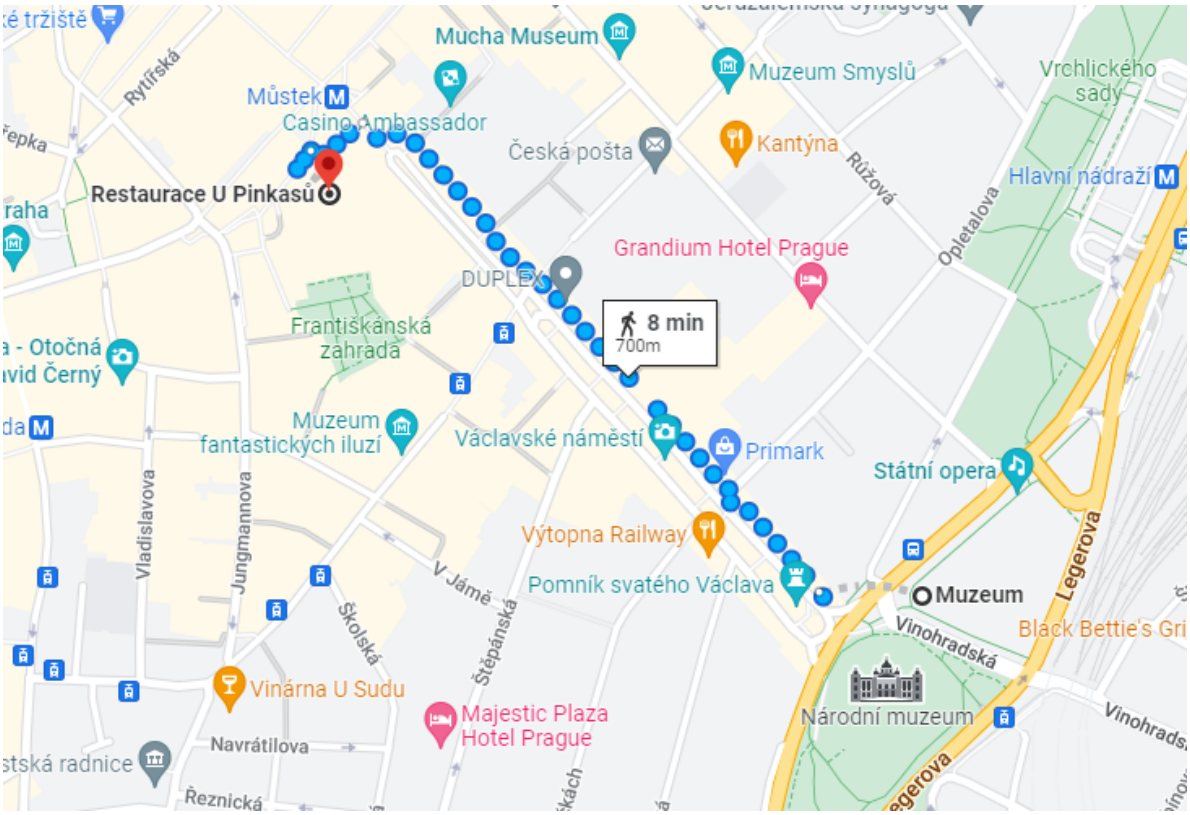
During the first poster session, buffet dinner will be available plus drinks. The second poster session will be held during a coffee break.

Gala dinner

The gala dinner will be held in downtown Prague at the U Pinkasů restaurant (http://www.upinkasu.cz) on June 16, starting at 7 pm, covered by the Conference. You can travel to U Pinkasů on your own (it takes ~ 30-40 minutes by public transport; the map with detailed instructions is below this paragraph. Alternatively, you can join the organizers who will escort you there; ask for details at the registration desk. The departure will be in several groups, starting around 6:10 pm.

For those who will be arriving from the Conference Venue by themselves, we recommend taking bus 138 to the Kačerov and then changing to metro C line (red). Another option is to take bus 193 to Poliklinika Budějovická and then change to the metro C line, get off at Muzeum and then walk ~ 10 minutes to the U Pinkasů restaurant.

The dinner will offer several dishes to choose from; vegetarian, vegan *etc.* options will be available. Each participant will also get two drink coupons at registration. Each coupon can be exchanged for one beer or one soft drink or tea/coffee, depending on your preference. After that, you pay for your drinks.



Cloakroom

You will have the option to use the cloakroom, situated on the outer side of the Lecture Hall. Please note that the organizers assume no liability.

Liability and insurance

The organizers will not assume any responsibility for damage or injuries to persons or property during the conference.

Fist Aid

In case of emergency, please contact the registration desk next to main entrance or any of the organizers or call 112.

Internet

Wireless Internet access (wifi) is available free of charge in the building; use FreeWifiRadio-IMG. You will have to give your consent regarding its use. No password is required.

Language

The conference language is English. No simultaneous translation will be provided.

Smoking policy

No smoking is allowed within any building on the campus. Smoking is permitted outside of the buildings.

Travel within Prague

For more information about the public transport in Prague, please visit https://www.prague.eu/en/practical/getting-around-prague-by-public-transport-17064.

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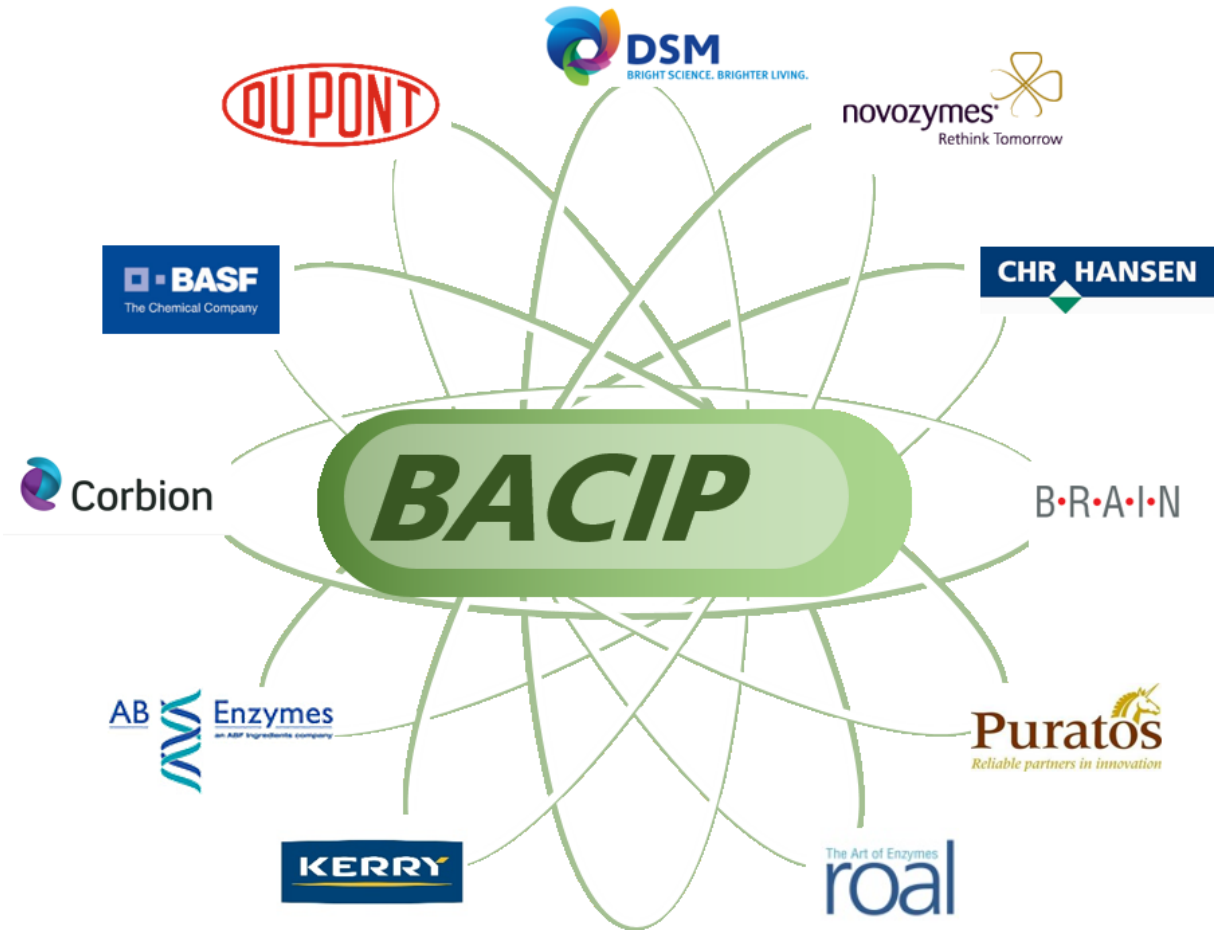

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ABSTRACTS

SESSION 1: DNA Replication and Transfer/Cell Division

K-01

How Gram-positive bacteria monitor their cell wall for defects and catalyze their repair

Yannick Brunet[1], Cameron Habib[1], Anna Brogan[1], Lior Artzi[1], Alex Meeske[1], **David Rudner**[1]

[1] *Harvard Medical School (United States of America)*

Bacteria contain a large set of proteins that monitor their genomes for replication errors and DNA lesions and either directly repair them or recruit repair factors. Do bacteria have analogous proteins that monitor their cell wall for defects or gaps in the meshwork? And if so, how do these proteins catalyze their repair? Here, we describe two complementary pathways that identify and repair gaps in the cell wall meshwork in *B. subtilis* and other gram-positive bacteria. Both pathways use a common mechanism to probe the cell wall. However, in one case, gaps in the meshwork are directly repaired by the protein that does the monitoring. In the other, in response to defects in the peptidoglycan, the monitor transduces this information across the membrane to induce genes that promote repair. The broadly conserved “structural integrity probe” and the mechanism of information transduction will be described in molecular detail.

Keywords: peptidoglycan, cell wall, signal transduction

Notes

T-01

MurA escape mutations uncouple peptidoglycan biosynthesis from PrkA signaling

Sabrina Wamp[1], Patricia Rothe[1], Daniel Stern[2], Gudrun Holland[3], Janina Döhling[1], **Sven Halbedel**[1]

[1] *FG11, Robert Koch Institute, Wernigerode (Germany)*
[2] *ZBS3, Robert Koch Institute, Berlin (Germany)*
[3] *ZBS4, Robert Koch Institute, Berlin (Germany)*

Biosynthesis of peptidoglycan (PG) consumes a high amount of cellular resources and therefore requires careful adjustments to environmental conditions. An important switch in the control of PG biosynthesis of *Listeria monocytogenes* is the serine/threonine protein kinase PrkA. A key substrate of this kinase is the small cytosolic protein ReoM. We have shown previously that ReoM phosphorylation regulates PG formation through control of MurA stability (1). MurA catalyzes the first step in PG biosynthesis and the current model suggests that phosphorylated ReoM prevents MurA degradation by the ClpCP protease. In contrast, conditions leading to ReoM dephosphorylation stimulate MurA degradation. How ReoM controls degradation of MurA and potential other substrates is not understood. Also, the individual contribution of the ~20 other known PrkA targets to PG biosynthesis regulation is unknown.

We have identified murA mutants which escape proteolytic degradation (2). The release of MurA from ClpCP-dependent proteolysis was able to activate PG biosynthesis and further enhances the intrinsic cephalosporin resistance of *L. monocytogenes*. This latter effect required the RodA3/PBP B3 transglycosylase/transpeptidase pair as additional effectors of the PrkA signaling route. One murA escape mutation not only fully rescued an otherwise non-viable prkA mutant during growth in batch culture and inside macrophages but also overcompensated cephalosporin hypersensitivity. Our data indicate that the main purpose of PrkA-mediated signaling in *L. monocytogenes* is control of MurA stability during standard laboratory growth conditions and intracellular growth in macrophages. These findings have important implications for the understanding of PG biosynthesis regulation and β-lactam resistance of *L. monocytogenes* and related Gram-positive bacteria.

(1) Wamp S, Rutter ZJ, Rismondo J, Jennings CE, Möller L, Lewis RJ, Halbedel S. Elife. 2020 May 29;9:e56048.
(2) Wamp S, Rothe P, Stern D, Holland G, Döhling J, Halbedel S. 2022 PLOS Pathogens, accepted.

Keywords: GpsB, ReoY, MurZ, PrpC, PASTA kinase

Notes

T-02

PrkC kinase modulates MreB filament and bacteria growth by monitoring cell wall precursor Lipid II

Yingjie Sun[1], Ethan Garner[1]

[1] *Harvard University, Cambridge (United States of America)*

All cells need to modulate their rate of growth to external nutrient conditions. For bacteria to change their growth rate, they must modulate the rate they add new peptidoglycan into their encapsulating cell wall. In many rod-shaped bacteria, the synthesis of new cell wall is mediated by filaments of MreB, an actin homolog that moves around the rod circumference with the synthetic enzymes. We used Total Internal Reflection Fluorescence Structured Illumination Microscopy (TRIF-SIM) to image the MreB filaments of *Bacillus subtilis* in different growth conditions. Using single-molecule tracking, we quantified the number and the velocity of the directionally moving filaments and enzymes. We found that cells regulate the density of MreB filaments in response to nutrient availability and the number of MreB filaments is controlled via the cell wall precursor Lipid II: increasing or decreasing the activity of the Mur pathway can dial both the number of MreB filaments as well as the rate of growth. We further quantitated the Lipid II levels and showed that MurAA, which catalyzes the first committed step in Lipid II biosynthesis, is the rate limiting step. We found that the levels of Lipid II are sensed by the serine/threonine kinase PrkC, which phosphorylates a variety of targets. Strains that overexpress PrkC or lack the cognate phosphatase PrpC grow faster than wild-type cells in nutrient limited media. One key substrate of PrkC is RodZ, which is a putative MreB filament nucleator. Surprisingly, we found that strains that overexpress PrkC or contain phosphomimetic RodZ mutations have an increased number of MreB filaments, they also grow faster than they would normally in carbon limited media. Together, our experiments demonstrate that bacteria regulate their rate of cell wall synthesis of growth by measuring the level of peptidoglycan precursors Lipid II. Our work reveals that PrkC kinase functions as a cellular rheostat, tuning the activities of cellular processes in response to Lipid II, allowing cells to grow robustly across a broad range of nutrient conditions.

Keywords: Bacteria growth rate, MreB filaments, Mur pathway, Lipid II biosynthesis, Serine/Threonine kinase PrkC, Post-translation

Notes

T-03

Mg2+ modulates *Bacillus subtilis* cell division frequency

Tingfeng Guo[1], **Jennifer K. Herman**[1]

[1] *Texas A&M University, College Station (United States of America)*

We discovered a window of extracellular [Mg2+] that modulates *B. subtilis* cell division frequency in a growth-rate independent manner. In this window, cells grown at higher [Mg2+] divide more frequently and produce shorter cells than those grown at lower [Mg2+]. To explore further, we determined the transcriptional profiles of cells in the two conditions, identified mutants in which cells are constitutively short and no longer Mg2+ responsive, and performed a selection to identify factors that reduce *B. subtilis*' sensitivity to low exogenous [Mg2+]. We find that Mg2+ responsiveness is impacted by multiple pathways, suggesting that magnesium's effect on division frequency is likely homeostatic rather than attributable to a single mechanism. Our results provide insight into not only how cells switch between states of elongation and division, but also how Mg2+ is able to rescue a wide range of growth and envelope integrity defects.

Keywords: *Bacillus*, magnesium, cell division, morphology

Notes

T-04

Discovery of a novel two-component antitermination system associated with conjugation operons in G+ bacteria

Andrés Miguel Arribas[1], David Abia[1], Jorge Val Calvo[1], Ling J Wu[2], Jeff Errington[2], **Wilfried JJ Meijer**[1]

[1] *Centro de Biología Molecular "Severo Ochoa", CSIC, Madrid (Spain)*
[2] *Centre for Bacterial Cell Biology, Newcastle (United Kingdom)*

Conjugation is the process by which a DNA element is transferred from a donor to a recipient cell via a connecting pore. It is the main HGT transfer route responsible for the spread of antibiotic resistance (AR). On the other hand, native conjugative elements may be exploited for the construction of genetic tools to modify bacteria that are reluctant to genetic modification in other ways. For both, a better understanding of conjugation is required. Very little is known about conjugation in G+ bacteria. We study different aspects of conjugation using as a model the conjugative plasmid pLS20 from *Bacillus subtilis*. All pLS20 conjugation genes are located in a very large operon (>30 kb), whose expression is controlled by a single strictly regulated strong promoter. Besides advantages, the organisation of genes in an operon also has disadvantages; e.g. it hampers differential expression of subsets of genes, and strict control of expression is undermined by spurious transcription. We have discovered a processive antitermination (P-AT) system located at the start of the conjugation operon. In combination with multiple terminators present in the conjugation operon, this P-AT system allows for differential expression of subsets of genes, and minimizes the effects of spurious transcription. The pLS20 P-AT system, unlike the known AT systems, is composed of two components: an RNA moiety that exerts AT, and a protein that is required for processivity. We show that similar bipartite P-AT systems are present on many other conjugative elements in G+ bacteria. We also found that P-AT systems of different plasmids have different host-range functionalities, which implies that the potential of the conjugative element to spread AR is directly related to the P-AT system. These studies provide new insights into the regulation of conjugation and its role in spreading AR, and the fundamental process of transcription in general.

Keywords: conjugation, transcription regulation, antitermination, antibiotic resistance, *Bacillus subtilis*, pLS20, G+ bacteria

Notes

T-05

Specific protein-lipid interactions play important role in *Clostridioides difficile* Min-system functioning

Naďa Labajová[1], Natalia Baranova[2,3], Miroslav Jurásek[4], Robert Vácha[4], Martin Loose[3], Imrich Barák[1]

[1] *Institute of Molecular Biology SAS, Bratislava (Slovakia)*
[2] *University of Vienna, Vienna (Austria)*
[3] *Institute of Science and Technology Austria, Klosterneuburg (Austria)*
[4] *CEITEC and Faculty of Science Masaryk University, Brno (Czech Republic)*

Precise division septum positioning is fundamental for effective division and bacterial survival. Min system is an important mechanism involved in regulation of cell division precision and was best characterised in Gram-negative *Escherichia* coli and Gram-positive *Bacillus subtilis*. In *E. coli* it is a very dynamic system that undergoes pole-to-pole oscillation and consists of MinC, MinD and MinE proteins. *B. subtilis* Min system does not oscillate, localizes at the cell poles more stably and consists of MinC, MinD, MinJ and DivIVA proteins. However, Gram-positive human pathogen *Clostridioides difficile* possesses unique Min system composed of MinC, MinD, MinE but also DivIVA. MinE and DivIVA are considered as topological markers of Min system in *E. coli* and *B. subtilis*, respectively. While MinE acts very dynamically, and is essential for Min system oscillation [1], DivIVA attaches the Min system to areas with high negative membrane curvature, cell poles and division site [2]. Here we show that *C. difficile* MinD/MinE, similarly as *E. coli* MinD/MinE homologues [3], are capable of dynamic wave formation in vitro. Importantly, DivIVA can bind to curved but also to planar membranes containing negatively charged phospholipids, especially cardiolipin. Upon binding, DivIVA changes the cardiolipin distribution and alters morphology of these lipid membranes. Our findings indicate that DivIVA has more complex and active role during cell division septal membrane formation [4]. Moreover, as *C. difficile* MinD and DivIVA interact directly [5], DivIVA likely affects MinDE oscillation properties.

1 Raskin & De Boer (1999) J Bacteriol. 181, 6419–6424.
2 Lenarcic et al. (2009) EMBO J 28, 2272–2282.
3 Loose et al. (2008) Science 320, 789–792.
4 Labajová et al. (2021) IJMS 22, 8350.
5 Valenčíková et al. (2018) Anaerobe 50, 22–31.

Keywords: bacterial cell division, Min system, protein self-organization, oscillation

Notes

SESSION 2: Gene expression

K-02

Transcription Regulation of Purine Biosynthesis by (p)ppGpp

Jue D Wang[1]

[1] *University of Wisconsin, Madison (United States of America)*

The nucleotide messenger (p)ppGpp allows bacteria to adapt to fluctuating environments by reprogramming the transcriptome. Despite its well-recognized role in gene regulation, (p)ppGpp is only known to directly affect transcription in Proteobacteria by binding to the RNA polymerase. Here, we reveal a different mechanism of gene regulation by (p)ppGpp in Firmicutes: (p)ppGpp directly binds to the transcription factor PurR to downregulate purine biosynthesis gene expression upon amino acid starvation. We first identified PurR as a receptor of (p)ppGpp in *Bacillus* anthracis. A co-structure with *Bacillus subtilis* PurR reveals that (p)ppGpp binds to a PurR pocket reminiscent of the active site of phosphoribosyltransferase enzymes that has been repurposed to serve a purely regulatory role, where the effectors (p)ppGpp and PRPP compete to allosterically control transcription. PRPP inhibits PurR DNA binding to induce transcription of purine synthesis genes, whereas (p)ppGpp antagonizes PRPP to enhance PurR DNA binding and repress transcription. A (p)ppGpp-refractory purR mutant in *B. subtilis* fails to downregulate purine synthesis genes upon amino acid starvation. Our work establishes the precedent of (p)ppGpp as an effector of a classical transcription repressor and reveals the key function of (p)ppGpp in regulating nucleotide synthesis through gene regulation, from soil bacteria to pathogens.

Notes

T-06

Promoter Prediction in Prokaryotes by aid of Machine Learning Technology

Anne de Jong[1], Auke J. van Heel[1], Hung Chu[1], Oscar P. Kuipers[1]

[1] *University of Groningen (Netherlands)*

Bacteria, archaea, phages and viruses are attracting increased interest with respect to research and applications in health, disease treatment, outbreak management, food production, food spoilage, agriculture, novel antibiotics discovery and novel pharmaceutical compounds. Although a prokaryote or virus is a relatively simple organism/entity compared to eukaryotes, the high diversity and number of different species show a huge amount of unknown and undiscovered features. Every day, new bacteria are discovered and the DNA of genomes can be sequenced even without cultivating the bacterium, phage or virus. Software packages have been developed to annotate the DNA sequence of genomes for genes, encoded proteins and other features. A major unsolved problem of these annotation methods is to find promoter sequences at which RNA expression is initiated. Using RNA-seq data derived from a broad range of bacterial species and machine learning technology we have made a big step forward in correctly predicting promoter regions and the exact starting point of the RNA (Transcription Start Site (TTS)) in genomes of bacteria, which will improve the exact prediction of gene products, solely based on DNA sequences. Also, the annotation of proteins can be influenced by the detection of promoters as especially the N-terminus of proteins is currently often determined incorrectly. Additionally, good promoter prediction can be helpful to identify the presence of smaller RNA molecules that encode peptides or non-coding RNAs, which enable better insight in dynamic processes in microbial cells. For annotating promoters in microbial genomes, our convenient and easy to use tool ‘ProPr’ and associated web server is freely available at http://ppp.molgenrug.nl.

Keywords: promoter, transcription start site, TSS, prokaryote, annotation, machine learning, CNN

Notes

*T-07

SPβ c2 – a heat inducible representative of the SPβ-like phages provides understanding and further exploration of the complex lysogeny management system

Katharina Kohm[1,2], Syamantak Basu[2], Fabian M Commichau[1], Robert Hertel[2]

[1] *FG Molekulare Mikrobiologie (190h), Universität Hohenheim (Germany)*
[2] *FG Synthetische Mikrobiologie, BTU Cottbus-Senftenberg (Germany)*

The temperate *Bacillus* phage SPβ is known for half a century. Despite over 50 years of research on SPβ, only individual aspects of its biology are understood. SPβ-like phages exhibit a novel lysogeny management system regulated at various levels. The lysis-lysogeny decision and phage-host recombination are well-investigated. The arbitrium-system is responsible for the lysis-lysogeny decision process, and recent reports indicate a connection with the lysogeny maintenance and resolvement system. However, SPβ prophage establishment, maintenance and switch to lytic replication is not understood and requires further investigation (Kohm and Hertel, 2021). To gain more insides into the lysogeny management system of SPβ, we study the genotype of the c2 mutant. This mutant cannot maintain its lysogenic status upon heat induction, implying alteration of a core regulatory element.

We discovered that the genetic origin of the SPβ c2 phenotype is located on the prophage and is associated with the point mutation G136E in the yopR gene. Furthermore, we identified that the conserved genes yopR, yokI and yopQ are transcribed from the dormant prophage and are involved in the SPβ lysogeny management (Kohm et al., 2022). By isolation and sequencing of heat-insensitive suppressors of the c2 mutant, we identified YosL as an additional component of the induction cascade. Deletion mutants of yosL neither react to Mitomycin C nor heat treatment and prevent excision of the prophage from the host genome. The artificial expression of YosL during logarithmic growth of an SPβ c2 lysogen did not induce the lytic pathway. However, by combining artificial YosL expression with heat based YopRG136E activation we re-established the heat inducibility. In summary, we can conclude that YosL - while not being an inducer - is essential for lysis. Its function requires other phage components present during infection or formed after prophage activation.

Kohm, K., Hertel, R. (2021). Arch Virol 166: 2119-2130
Kohm et al. (2022). Environ Microbiol (publishing pending)

Keywords: *Bacillus*, phage, lysogeny, arbitrium, yopR, yosL

Notes

T-08

Exploring the molecular basis of thermophilicity in a *Bacillus* species.

Rajinikanth Mohan[1], Zachary Buchman[1], Ava Bianchi[1], Colin Brown[1], Collin Olson[1]

[1] *Mercyhurst University, Erie, Pennsylvania 16546 (United States of America)*

Many species of *Bacillus* are known to be thermophiles but the understanding of the molecular basis of thermophilicity is limited. We isolated a thermophilic strain of *Bacillus velezensis* that can tolerate temperatures up to 54°C. Microscopy analysis revealed a tendency of rods to form chains and filaments at the highest survival temperatures. To explore the molecular basis of the heat adaptation, we performed RNA sequencing and found enrichment of 1764 genes (1.5-fold cutoff) that were differentially expressed during heat stress. Gene ontology analysis revealed a surprising enrichment of several clusters involved in metabolic processes. Characterization of the mutants of some of these genes is ongoing with an aim to identify genetic deficits that point to a function of these genes in heat tolerance. These results could shed light on how these bacteria are adapted for survival at higher temperatures that are lethal to many others.

Keywords: *Bacillus*, heat, thermophile, gene expression, metabolic proces

Notes

T-09

Gintool; enabling transcriptome analysis using regulon information

Leendert Hamoen[1], Frans Van der Kloet[1], Biwen Wang[1]

[1] *University of Amsterdam, Amsterdam (Netherlands)*

When analyzing transcriptome data certain threshold values are chosen to decide whether the regulation of a gene is relevant or not. These choices are arbitrary and may result in the loss of valuable information. To overcome this limitation, we developed a software tool called Gintool that quantitatively and graphically analyses key regulatory units, i.e. regulons, instead of individual genes. This provides direct insights into the activity of known regulatory pathways, and does not require a preselection of relevant genes based on threshold levels. We developed Gintool as an add-in for Excel and added additional features including the possibility to evaluate transcriptome data based on functional categories and operon structure. The use of Gintool is illustrated by the identification and comparison of cellular stresses in *Bacillus subtilis*.

Keywords: transcriptome analysis, regulons, *Bacillus subtilis*

Notes

T-10

Repression by DNA looping: Crystal structure and functional analysis of the global transcriptional regulator ScoC

Smadar Shulami[1], Noam Hadad[2], Sergei Pomyalov[2], Noa Lavid[1], Abraham L Sonenshein[3], Yuval Shoham[1], Gil Shoham[2]

[1] *Technion-Israel Institute of Technology, Haifa (Israel)*
[2] *The Hebrew University of Jerusalem, Jerusalem (Israel)*
[3] *Tufts University School of Medicine, Boston (United States of America)*

The global transcriptional regulator ScoC belongs to a group of transition state regulators controlling genes at the transition from exponential growth to stationary phase. In *Bacillus subtilis*, ScoC controls directly or indirectly the transcription of more than 500 genes. Using gel retardation assays, isothermal titration calorimetry, fluorescence resonance energy transfer (FRET) analyses and transcriptional lacZ-fusions in *B. subtilis* background, we demonstrate that ScoC from *Geobacillus stearothermophilus* T-6 binds to two operator sites in the oppA promoter region, and both binding sites are necessary for high repressive effect.

The 3D crystal structures of ScoC and ScoC complexed with DNA were determined at 2.8 Å resolution, revealing a tetrameric X-shape assembly composed of two dimers. Each dimeric unit comprises a winged helix-turn-helix DNA-binding motif. FRET analyses and gel retardation assays indicate that a single tetrameric ScoC binds two operator sites and can induce DNA looping. The initial rate of fluoresce intensity (F) in the presence of increasing concentrations of the ScoC protein can be modeled into a typical Michaelis-Menten kinetics, exhibiting uncompetitive substrate inhibition with kinetic constants of Fmax, Km, and Ki of 0.55 1/sec, 9.5 µM, and 9.8 µM, respectively. These results support an inhibition mechanism in which at high concentrations of ScoC, two ScoC tetramers (rather than a single tetramer) bind to the two operators, thus preventing DNA loop formation. Specific amino acid replacements in ScoC, which interact with the DNA bases in the wing recognition site (R100A, D98A) and which create hydrogen bonds in the major groove via the recognition helix (75SerA, 79AsnA), resulted in reduced ability of ScoC to bind DNA.

In conclusion, functional analysis together with crystal structures of ScoC and ScoC complexed with DNA showed that ScoC can bind to two operator sites in the oppA promoter region so as to allow full repression of transcription, most likely by inducing DNA looping.

Keywords: ScoC, global regulator, crystal structure, *Geobacillus stearothermophilus*

Notes

SESSION 3: RNA, Translation

K-03

David vs Goliath: Ribosome-targeting antibiotics and bacterial resistance mechanisms.

Daniel N. Wilson[1]

[1] *University of Hamburg, Hamburg (Germany)*

Protein synthesis is a major target within the bacterial cell for antibiotics (1,2). Investigations into ribosome-targeting antibiotics have provided much needed functional and structural insight into their mechanism of action (1,2). However, the increasing prevalence of multi-drug-resistant bacteria has limited the utility of our current arsenal of clinically relevant antibiotics, highlighting the need for the development of new classes (3,4). In this presentation, I will discuss our recent structural insights into the mechanism of action of novel ribosome-targeting compounds as well as novel molecular mechanisms of bacterial resistance, with an outlook as to how this information could be used for the production of improved drugs that inhibit bacterial protein synthesis (3,4).

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Keywords: Antibiotics, Ribosome, RNA, Resistance, Translation, Cryo-electron microscopy

Notes

T-11

Dissecting the function of the two paralogous 6S RNAs in an undomesticated *Bacillus subtilis* wild-type strain

Roland K. Hartmann[1], M. Amri C. Schlüter[1], Jana C. Wiegard[1], Ahmad Altoun[1]

[1] *Philipps-Universität Marburg, Marburg (Germany)*

Bacterial 6S RNAs regulate transcription by binding to the active site of RNA polymerase holoenzymes. Unlike most other bacteria, *B. subtilis* encodes two 6S RNA paralogs, 6S-1 and 6S-2 RNA, that differ in their expression profiles and several aspects of their structure and function; furthermore, phenotypes of 6S-1/2 RNA single and double knockout strains are diverse. We aim at deciphering their biological roles using the following approaches: (1) transcriptomics (RNA-seq, qRT-PCR), proteomics (MS) and ChIP-seq (distribution of RNAP molecules on the *B. subtilis* genome) of the wild-type strain NCIB 3610 and derivative strains with 6S-1/2 RNA single and double knockouts; (2) mechanistic and kinetic studies as well as in vitro iCLIP (mapping of 6S-1/2 RNA interaction sites on σA-RNAP) to understand the functional cycle of 6S RNAs; (3) 6S-1/2 RNA interaction with RNA poymerase (RNAP) holoenzymes carrying σA or alternative sigma factors to determine the holoenzyme specificity of the two 6S RNAs. Our recent findings and state of knowledge will be discussed.

Keywords: non-coding 6S RNAs in *B. subtilis*, functional analysis of 6S-1 and 6S-2 RNAs, regulation of transcription, sigma factors

Notes

T-12

Novel RNA molecules interacting with the bacterial transcription machinery

Viola Vaňková Hausnerová[1], Mahmoud Shoman[1], Dilip Kumar[1], Jitka Jirát Matějčková[1], Libor Krásný[1], **Jarmila Hnilicová**[1]

[1] *Institute of Microbiology of the CAS, v. v. i. (Czech Republic)*

Regulatory 6S RNA molecules that interact with the RNA polymerase are widespread among bacteria. We discovered a new type of regulatory RNA in mycobacteria, named it Ms1 and showed that Ms1 regulates the amount of RNA polymerase in nonpathogenic *Mycobacterium smegmatis*. In addition, we found Ms1 homologs among other actinobacteria using bioinformatic search. This phylum includes severe human pathogens (for example *Mycobacterium tuberculosis, Mycobacterium leprae, Corynebacterium diphtheria*), industrially important producers of aminoacids (*Corynebacterium glutamicum*) and antibiotics (*Streptomycetes*) or probiotic bacteria (*Bifidobacterium*).

We performed RIP-seq (RNA immunoprecipiation coupled with next-generation sequencing) and identified a complete set of regulatory RNAs interacting with the transcription machiery in several bacterial species - *Mycobacterium smegmatis, Streptomycetes celicolor* and well established model organism *Bacillus subtilis*. Our data show that in addition to 6S and Ms1 RNA, other RNAs associate with the different forms of bacterial RNA polymerase. These novel RNAs expand the portfolio of possible mechanisms of bacterial transcription regulation. We propose that 6S RNA and Ms1 were the first RNAs to be identified due to their high abundance; however, other; less abundant regulatory RNAs are waiting to be discovered.

Keywords: sRNA, 6S RNA, Ms1, RNA polymerase, sigma factor, transcription

Notes

T-13

B. subtilis RNase Y and *E. coli* RNase E : Different enzymes – similar strategies

Soumaya Laalami[1], Marina Cavaiuolo[1], Anna Dudnic[1], **Harald Putzer**[1]

[1] *CNRS UMR8261 IBPC, Université Paris Cité, Paris (France)*

Metabolic instability of mRNA is fundamental to the control of gene expression in all organisms. In bacteria, mRNA degradation generally follows an “all-or-none” pattern. This implies that if control is to be efficient, it must occur at the initiating (and presumably rate-limiting) step of the degradation process. RNase Y and RNase E are disparate endoribonucleases that govern global mRNA turnover/processing in the two evolutionary distant bacteria *Bacillus subtilis* and *Escherichia coli*, respectively. To evaluate the potential equivalence in biological function between the two enzymes in vivo we analyzed whether and to what extent RNase E is able to replace RNase Y in *B. subtilis*. We observed a surprising reversal of transcript profiles both of individual genes and on a genome-wide scale. The important parameters to efficient complementation will be discussed.

We have also studied how the expression of *B. subtilis* RNase Y is controlled and present evidence for an autoregulatory mechanism.

Our data confirm the notion that RNase Y and RNase E have evolved through convergent evolution towards a low specificity endonuclease activity universally important in bacteria.

Keywords: mRNA degradation, rnase Y, rnase E

Notes

T-14

Segregation of transcription and translation in *B. subtilis* spatially organizes ribosome assembly and metabolism

Joshua Stoll[1], Daniel O Rotter, **Peter L Graumann**

[1] *Biochemie, Fachbereich Chemie, Philipps-Universität Marburg (Germany)*

In many, if not most, bacteria transcription takes place on the centrally located nucleoid(s), while actively transcribing ribosomes are located at subcellular spaces surrounding the nucleoids, i.e. mostly at polar sites, and directly underneath the cell membrane. Nucleoids do not impose diffusion barriers for free ribosomal subunits, but even allow for fast diffusion of proteins. Polar accumulation of ribosomes is likely due to exclusion of mRNA from the nucleoids, and indeed depends on active transcription. We show that GTPases involved in the maturation of ribosomal subunits in *Bacillus subtilis* also accumulate at translation zones, indicating that ribosome assembly takes place at sites of translation, rather than in a freely diffusive manner. Moreover, we found that large enzymes also show slowed-down diffusion within ribosome-dense areas, and fast diffusion within the nucleoids, again dependent on active transcription. These findings suggest that bacteria having chromosomes compacted into nucleoids employ the segregation of RNA and of DNA to generate areas of high protein density at polar regions, which in turn lead to the accumulation of enzymes, likely affecting metabolic efficiency of cells. *B. subtilis* cells keep up polar translation zones during slow growth, indicating that segregation of transcription and translation is not only an adaptation to high doubling times, but a general subcellular process. These findings suggest that bacteria utilize general entropic principles to generate heterogeneity of diffusion in the cytosol, and thereby generate self-organization independent of cytoskeletal elements.

Keywords: Subcellular organization, heterogeneous diffusion, self-organization, transcription, translation, ribosome assembly

Notes

*T-15

Ribosome stalling and collision sensing in bacteria

Federico Cerullo[1], Sebastian Filbeck[1], Pratik Rajendra Patil[1], Hao-Chih Hung[1], Haifei Xu[2], Florian W. Hofer[1], Jaro Schmitt[1], Guenter Kramer[1], Bernd Bukau[1], Kay Hofmann[3], Stefan Pfeffer[1], Claudio A.P. Joazeiro[1,2]

[1] *ZMBH, Heidelberg University, Heidelberg (Germany)*
[2] *Department of Molecular Medicine, Scripps Florida, Jupiter (United States of America)*
[3] *Institute for Genetics, University of Cologne, Cologne (Germany)*

Stress conditions, faulty mRNA translation and other mechanisms can lead to ribosomal stalling, which is a source of truncated, potentially toxic polypeptides. We had originally discovered that in eukaryotes, such aberrant translation products are targeted for degradation by a pathway now known as Ribosome-associated Quality Control (RQC). In RQC, large ribosomal subunits obstructed with nascent chains are sensed by NEMF/Rqc2, which then recruits the E3 ligase Ltn1/Listerin for nascent chain ubiquitylation. We subsequently found that an ancestral RQC pathway is also active in bacteria, mediated by the NEMF/Rqc2 homolog, RqcH. Strikingly, with Listerin and a ubiquitin-like system absent in bacteria, RqcH promotes degradation of stalled nascent chains through alanine tailing, a non-canonical proteolytic tagging mechanism.

How ribosome stalling is sensed and elicits RQC is poorly understood in any organism. We have now uncovered the poorly characterized *Bacillus subtilis* MutS2, a member of the conserved MutS-family of ATPases, as an unexpected ribosome-binding protein with an essential function in translational quality control. Cryo-EM analysis of affinity-purified native complexes reveals that MutS2 functions in sensing collisions between stalled and translating ribosomes and suggests how ribosome collisions can serve as platforms to deploy downstream processes: MutS2 has an RNA endonuclease Small MutS-Related (SMR) domain, as well as an ATPase/clamp domain that is properly positioned to promote ribosomal subunit dissociation, which is a requirement both for ribosome recycling and for initiating RQC. Accordingly, MutS2 promotes nascent chain modification with C-terminal alanine tails in an ATPase domain-dependent manner. The relevance of these observations is underscored by evidence of strong co-occurrence and genomic synteny of MutS2 and RqcH genes across bacterial phyla. Overall, the findings reveal a deeply-conserved role of ribosome collisions in mounting a complex response to the interruption of translation within open reading frames.

Keywords: RQC, Ribosome-associated Quality Control, MutS2, RqcH, Cryo-EM

Notes

SESSION 4: Cell Morphology

K-04

Homeostatic regulation membrane lipid composition: Is fluidity really the important parameter?

Marvin Gohrbandt[1], Andrew Watson[3], André Lipski[2], James W Grimshaw[3], Jessica A Buttress[3], Zunera Baig[1], Brigitte Herkenhoff[1], Stefan Walter[1], Rainer Kurre[1], Gabriele Deckers-Hebestreit[1], **Henrik Strahl**[3]

[1] *Osnabrück University (Germany)*
[2] *University of Bonn (Germany)*
[3] *Newcastle University (United Kingdom)*

Homeostatic regulation of membrane properties is a universally conserved biological process. Indeed, all living organisms adapt their membrane lipid composition in response to changes in their environment or diet. Due to difficulties of modifying lipid composition in living cells, key concepts of membrane biology such as homeoviscous adaptation maintaining stable levels of membrane fluidity, and gel-fluid phase separation resulting in domain formation, heavily rely upon observational in vivo studies, and in vitro experiments with model membranes or lipid extracts. Using the bacterial model organisms *Escherichia coli* and *Bacillus subtilis*, can now demonstrate in vivo that strongly reduced membrane fluidity interferes with essential and complex cellular processes including cytokinesis, envelope expansion, chromosome replication/segregation and maintenance of membrane potential. Furthermore, low membrane fluidity is indeed capable of triggering large-scale lipid phase separation and protein segregation in intact, protein-crowded membranes of living cells; a process that coincides with the minimal level of fluidity capable of supporting growth. Importantly, the in vivo lipid phase separation is not associated with a breakdown of the membrane diffusion barrier function, thus explaining why the phase separation process is biologically reversible. Crucially, however, such effects are only induced by drastic changes in fluidity that go well beyond those observed upon physiologically relevant environmental stresses. Hence, these findings directly question the prevailing dogma that careful regulation of membrane fluidity is critical for cell function. We postulate that, rather than maintaining stable membrane fluidity levels, cells instead carefully regulate their lipid composition in order to maintain stable membrane thickness; a property that is critical for the function of integral membrane proteins.

Keywords: bacterial membranes, membrane fluidity, lipid adaptation, phase separation, membrane thickness

Notes

T-16

Identification and characterization of a novel staphylococcal cell morphology determinant

Ine S. Myrbråten[1], Danae Morales Angeles[1], Maria D. Barbuti[1], Gro A. Stamsås[1], Helena Chan[2,3], Tiril M. Knutsen[1], Volha Shapaval[1], Daniel Straume[1], **Morten Kjos**[1]

[1] *Norwegian University of Life Sciences, Ås (Norway)*
[2] *Okinawa Institute of Science and Technology, Okinawa (Japan)*
[3] *University of Technology Sydney (Australia)*

Cell division and cell wall synthesis in staphylococci need to be precisely coordinated and controlled to allow the cell to multiply while maintaining their nearly spherical shape. The mechanisms ensuring correct placement of the division plane and synthesis of new cell wall have been studied intensively, however, hitherto unknown factors and proteins are likely to play key roles in this complex interplay. From a combined gene knockdown and subcellular localization screen of essential proteins with unknown function, we identified a protein with major influence on cell morphology in *Staphylococcus aureus*. The protein, named SmdA (for staphylococcal morphology determinant A), is a membrane-protein with septum-enriched localization. By CRISPR interference knockdown and overexpression combined with different microscopy techniques, we demonstrate that proper levels of SmdA is necessary for cell division, including septum formation and cell splitting. We also identified conserved residues in SmdA that are critical for its functionality. Pulldown- and bacterial two-hybrid interaction experiments showed that SmdA interacts with several known cell division- and cell wall synthesis proteins, including penicillin binding proteins (PBPs) and EzrA. Notably, SmdA also affects susceptibility to cell wall targeting antibiotics, particularly in methicillin-resistant *S. aureus* (MRSA). Together, our results show that *S. aureus* is dependent on balanced amounts of membrane-attached SmdA in order to carry out proper cell division.

Keywords: Staphylococcus, cell morphology

Notes

T-17

Adaptation of *Bacillus subtilis* to low humidity requires a Sigma B-dependent general stress response

Xiao-Mei Yang[1,2], Marcus C de Goffau[1,6], Emma L Denham[1,3], **Sjouke Piersma**[1], Haike Antelmann[4,5], Michael Hecker[4], Hermie JM Harmsen[1], Jan Maarten van Dijl[1]

[1] *University of Groningen, University Medical Center Groningen (Netherlands)*
[2] *Shanghai Medical College, Fudan University, Shanghai (China)*
[3] *University of Bath, Bath (United Kingdom)*
[4] *Institute for Microbiology, University of Greifswald (Germany)*
[5] *Institute of Biology, Freie Universität Berlin (Germany)*
[6] *University of Cambridge, Cambridge (United Kingdom)*

Bacillus subtilis displays massive morphological changes in response to reduced water availability as it becomes filamentous, shorter, curved and eventually almost coccoid at decreasing relative humidity (RH) values. In the studies we present it is shown that these changes are completely reversible in the presence of water as rod-like cells grew out of coccoid cells in a germination-like process. Alterations in the cell wall were subsequently confirmed using transmission electron microscopy. To obtain a better understanding of underlying adaptive mechanisms, a proteomics analysis was performed. Intriguingly, cells grown at 100% RH contained many fragments of large proteins, which were absent from water-limited cells. Furthermore, the proteomic signatures of water-limited cells indicated a σB-dependent general stress response. This response was shown to be essential for the survival of *B. subtilis* cells under conditions of limited water availability, since deletion mutants lacking the sigB gene or the σB-regulated gene for the catalase KatE were unable to adapt to low RH values. Taken together, our findings suggest that *B. subtilis* needs to protect itself against oxidative stress under water-limited growth conditions.

Keywords: *Bacillus*, subtilis, relative humidity, proteomics, SigB, KatE

Notes

*T-18

Cell Wall Biosynthesis in *Bacillus subtilis*

Alaa A Aljohani[1], Richard Daniel [1]

[1] *Newcastle Univesity, Newcastle upon Tyne (United Kingdom)*

A bacterial cell's integrity is maintained by an extracellular matrix that encases the cytoplasmic membrane, generally referred to as the cell envelope. This structure protects the cell from rupturing through its internal osmotic pressure. The structural component of the cell envelope, the peptidoglycan, is a matrix composed of glycan polymers that are cross-linked together by peptide side chains. In Gram-positive bacteria, the cell envelope is dynamic with the "old" outer material degrading and new peptidoglycan synthesized near the cell membrane. Since the wall degrading enzymes are secreted through the cell membrane there must be topologically coordinated regulation of the peptidoglycan hydrolases to prevent degradation of the newly synthesized material or the random breakdown of the peptidoglycan resulting in cell lysis. Using *Bacillus subtilis* as the model system, we can show genetically that post-synthesis "maturation" of the peptidoglycan is specifically required for one important autolytic enzyme. Our data also shows that specific functions can be assigned to specific sets of autolytic enzymes. In summary, our results, combined with other work in the group, provide a way to explain how turnover of the PG may be coordinated to prevent futile synthesis and degradation in a way that permits controlled cell envelope enlargement.

Keywords: Cell wall-Synthases- Hydrolyase

Notes

T-19

Takeover of *Bacillus subtilis* cell biology by a phage

Valentina Floccari[1], Helge Feddersen[2], Anna Munk[3], Paul J. Kempen[3], Tomaž Accetto[1], Ákos T. Kovács[3], Robert Hertel[4], Marc Bramkamp[2], **Anna Dragoš**[1]

[1] *University of Ljubljana, Ljubljana (Slovenia)*
[2] *Kiel University, Germany (Germany)*
[3] *Technical University of Denmark, Kongens Lyngby (Denmark)*
[4] *Brandenburg University of Technology Cottbus-Senftenberg (Germany)*

Phages are killers and genetic parasites, but there are phages that exhibit an alternative lifestyles of so phage regulatory switches (phage RS). For instance, large SPβ and related phages of *Bacillus subtilis*, regulate gene expression by reversible integration and excision from functional loci. Here we report an extreme effect of one of the SPβ-like phages on morphology of *B. subtilis* cell, where during lysogeny, a phage turns rod-shaped bacterium into a sphere. The phenotype of the lysogen manifests in stationary phase and especially during sporulation, resulting in spherical and enlarged spores. Interestingly, the superinfection with closely-related phage RS rescues the cell from the shape-shifting effects of the first phage. We further inspect several aspects of cell biology, starting from the cell wall via thin sectioning and transmission electron microscopy, followed by dynamics of cell division, cell elongation and sporulation via time-lapse fluorescence microscopy methods. To better understand the rescue effect of the superinfection, we also compare genomes of single and double lysogens using de novo genome sequencing. Finally, we report the impact of this peculiar phage-host interaction on host and phage fitness. Our study demonstrates a new phenomenon, where temperate phage remodels cell biology of the host during long-term relationship. We believe it is a tip of the iceberg among *B. subtilis* traits that can be controlled by phage RS.

Keywords: prophage, SPbeta-like, regulatory switch, cell morphology, phage-host interaction

Notes

SESSION 5: Cell Metabolism/Biotechnology

K-05

Lanthipeptide biosynthesis: the assembly of the nisin biosynthetic and secretion complex in *B. subtilis* and in *L. lactis*

Jingqi Chen[1], Auke J. van Heel[1], **Oscar P. Kuipers**[1]

[1] *Molecular Genetics, University of Groningen (Netherlands)*

Nisin, a class I lanthipeptide, is synthesized as a precursor peptide by a putative membrane-associated lanthionine synthetase complex consisting of the dehydratase NisB, the cyclase NisC and the ABC transporter NisT. Here, we characterize the subcellular localization and the dynamic assembly process of the nisin biosynthetic machinery by fluorescence microscopy in coccoid and rod-shaped bacteria respectively. In *Lactococcus lactis*, the nisin precursor, NisB and NisC were found to be mainly co-localized at the cell poles, with a preference for the old poles. In contrast, the transporter NisT is distributed uniformly and circumferentially in the membrane. When nisin secretion was blocked by mutagenesis of NisT, the nisin biosynthetic machinery was visualized directly also at a polar position. The interactions between NisB and other components indicated that NisB directly or indirectly plays a role as polar "recruiter" in the initial assembly process. We propose a model wherein the nisin precursor is first completely modified by NisBC, preventing premature secretion of partially modified peptides, and subsequently secreted by recruited NisT, preferentially at the old pole regions in coccoid cells. In *Bacillus subtilis*, we observed that NisT as well as NisB and NisC were highly co-localized in a punctate pattern along the cell periphery. A strategy of differential timing of expression demonstrated the in vivo dynamic assembly of NisBTC, revealing the recruitment by NisT of NisBC to the membrane. By use of mutated proteins, the nucleotide binding domain (NBD) of NisT was found to function as a membrane anchor for NisB and/or NisC. In rod-shaped cells, we propose a model for a three-phase production of modified nisin precursor, emphasizing the crucial role of NisBC, next to NisT, in the process of nisin precursor translocation.

Keywords: nisin, biosynthesis, subcellular localisation, complex formation, GFP, fluorescence microscopy, NisBTC

Notes

*T-20

Subtilisin-mediated bioplastic degradation: from mechanism to application

Jordan Cannon[1], Todd Reynolds[1]

[1] *University of Tennessee Knoxville (United States of America)*

Subtilases are a ubiquitous superfamily of proteases that span all domains of life. In *Bacillus* spp., subtilisins serve as major extracellular proteases involved in a wide variety of cellular processes (nutrient acquisition, quorum sensing, competence, sporulation, etc.). Unsurprisingly, subtilisins can be promiscuous with regard to substrate specificity. In our lab, we have identified a subtilisin, AprBp, from a soil isolate of *Bacillus pumilus* that has the ability to degrade high molecular weight polylactic acid (PLA). PLA is a biodegradable plastic that is one of the most promising candidates to replace non-renewable, fossil fuel-based plastics. In contrast to B. pumilus, a lab strain of *Bacillus subtilis* carries a homolog of AprBp, called AprE, that shows strong protease activity, but has limited ability to degrade PLA. Elucidating the mechanisms by which some subtilisins have activity toward PLA will provide novel insights into substrate preference in subtilisins and have biotechnological significance with regard to enzymatic recycling practices for PLA waste products.

To better understand how AprBp degrades PLA, we have compared the active site residues of *B. pumilus* AprBp and *B. subtilis* AprE, leading to the identification of several residues hypothesized to favor PLA degradation. Using site directed mutagenesis and an overexpression/secretion system in a protease-deficient strain of *B. subtilis*, we have generated and characterized AprE variants that are more similar to AprBp, and vice versa. This approach has led to the identification of several key residues that are favored for PLA degradation. Using in silico methods, we have expanded this work by identifying other subtilisins that contain these specific residues to predict other potential degraders. We are currently validating our predictions by screening PLA degrading activity from these enzymes. This information will validate the residues allowing for PLA substrate specificity in subtilisins and help identify subtilisins that are suited for new biotechnological applications in PLA recycling.

Keywords: Subtilisin, plastic biodegradation, metabolism, *Bacillus* pumilus, biotechnology

Notes

*T-21

Exploring the iron starvation stimulon of *Staphylococcus aureus* using a combination of bioinformatics and experimental approaches

Larissa Busch[1], Alexander Ganske[1], Christian Hentschker[1], Karen Methling[2], Manuela Gesell Salazar[1], Stephan Michalik[1], Michael Lalk[2], Kristin Surmann[1], Uwe Völker[1], Ulrike Mäder[1]

[1] *University Medicine Greifswald, Dept. of Functional Genomics (Germany)*
[2] *University of Greifswald, Inst. of Biochemistry (Germany)*

Pathogenic bacteria have to cope with host-mediated iron limitation, and therefore the iron stimulon is of special interest for understanding *S. aureus* physiology. Fur is the main regulator of iron homeostasis, classically acting as repressor of iron-uptake systems under iron-rich conditions. A recent global analysis of *S. aureus* gene expression revealed new aspects of iron regulation by predicting an updated Fur regulon and identifying the iron-responsive sRNA S596 [1]. Since S596 was shown to be a Fur-regulated sRNA, it was renamed FsrA. Additionally, modes of action differing from the classical Fur regulation mechanism are known for various organisms and iron-regulated genes clearly outweigh direct Fur targets. We therefore aimed to experimentally validate predicted Fur regulon members and to derive models of accessory iron-responsive regulatory mechanisms in *S. aureus*.

Initially proteome profiles were recorded for *S. aureus* HG001 and isogenic mutants deficient in Fur, FsrA, or both cultivated in iron-rich and iron-limited medium. Candidates of Fur regulon members and of the FsrA targetome were determined by statistical analyses. Of the Fur regulon candidates, 49 proteins represent previously predicted members. Combination of these data sets with an analysis of conserved Fur boxes in 21 staphylococcal genomes suggested a positive role of Fur in regulation of sdhCAB and citB. This finding was supported by experimental data including Northern blot and extracellular metabolome analyses. Thereby, by acting as a positive regulator of the TCA cycle, Fur is directly linked to the central metabolism. Moreover, based on the data set in combination with robust in silico target prediction, nine very likely FsrA targets were identified, revealing that FsrA mediates an iron-sparing response as described for e.g. *B. subtilis*.

In summary, the iron starvation stimulon of *S. aureus* involves more complex regulatory layers than the canonical Fur regulon.

[1] Mäder et al. PLoS Genet. 2016;12:e1005962

Keywords: *Staphylococcus aureus*, iron starvation, iron stimulon, metal ion homeostasis, Fur, ferric uptake regulator, FsrA, sRNA

Notes

T-22

System-wide discovery of protein-protein interactions and interfaces in *Bacillus subtilis*

Andrea Graziadei[1], Francis O'Reilly[1], Christian Fohrbig[1], Rica Bremenkamp[2], Joerg Stuelke[2], Juri Rappsilber[1]

[1] *TU Berlin, Berlin (Germany)*
[2] *University of Goettingen, Goettingen (Germany)*

System-wide discovery of protein-protein interactions (PPIs) has been driven by developments in affinity purification mass spectrometry, bacterial two-hybrid and similar approaches. In recent years, crosslinking mass spectrometry has been developed into a tool for system-wide determination of PPI topologies. Moreover, the dawn of AI-assisted accurate protein structure prediction with Aphafold-multimer now allows identification of the interaction interfaces and their likely structure. In order to derive protein-protein interactions and topologies, we performed a deep crosslinking mass spectrometry analysis of *B. subtilis*, which revealed 6751 residue pairs in 311 PPIs involving known and novel protein complexes. This analysis was supplemented by a co-fractionation-MS analysis that generated 637 additional candidate PPIs. We then sought to leverage Alphafold multimer to generate interface predictions for experimentally-derived and previously reported PPIs in Subtiwiki, yielding a comprehensive picture of PPIs in *B. subtilis*, including the characterisation of several novel interactions, both between characterised and uncharacterised proteins. We validate the structure of these interactions against crosslinking-MS residue-residue pairs. We further characterise the function of high-scoring candidates by mutational and biochemical analysis, which highlights the role of some of the high-scoring candidates PPIs in iron metabolism and cellular redox balance.

Keywords: *Bacillus subtilis*, systems biology, structural biology, crosslinking mass spectrometry, alphafold, structure prediction

Notes

T-23

Protein arginine phosphorylation and dephosphorylation facilitates protein homeostasis in *Bacillus subtilis*

Regina Alver[1,2], Ingo Hantke[2], Fabián A Cornejo[1], Katrin Gunka[2], Sebastian Rämisch[1], Noël Molière[2], **Kürşad Turgay**[1,2]

[1] *Max Planck Unit for the Science of Pathogens, Berlin (Germany)*
[2] *Leibniz University Hannover, Hannover (Germany)*

In the Gram-positive model organism *Bacillus subtilis*, the AAA+ unfoldase ClpC associated with the ClpP protease plays an important role in cellular protein homeostasis and stress response. Here, we could demonstrate that the protein arginine kinase and adaptor protein McsB, its activator McsA, and the phosphatase YwlE form with ClpC a unique chaperone system necessary for protein aggregate removal in vivo and in vitro. We observed that aggregated substrate proteins were phosphorylated and targeted by activated McsB for extraction and unfolding by ClpC. Only sub-stoichiometric amounts of catalytically active YwlE significantly enhanced ClpC/McsB-mediated disaggregation activity and facilitated the refolding of unfolded, arginine-phosphorylated substrate proteins in vitro. These refolded substrate proteins were protected from degradation, depending on the strength of ClpC-ClpP interaction. In this unique chaperone system, protein unfolding, coordinated with protein arginine phosphorylation and de-phosphorylation, facilitates efficient protein refolding and homeostasis.

Notes

SESSION 6: Cell-Cell Communication

K-06

Intra and interspecies interactions shape the war and peace in the *Bacillus subtilis* world

Ines Mandic-Mulec[1,2], Katarina Belcijan[1], Polonca Štefanić[1], Barbara Kraigher[1], Eli Podnar[1], Eva Kovačec[1], Tjaša Danevčič[1], Iztok Dogša[1], Katarina Šimunović[1]

[1] *University of Ljubljana, Biotechnical faculty, Ljubljana (Slovenia)*
[2] *University of Ljubljana-COMPETE, Ljubljana (Slovenia)*

Bacteria live in diverse communities where they engage in rich social lives involving cooperative and antagonistic cell-cell interactions. We study interactions between genotypes within *B. subtilis* species and between different species (e.g *B. subtilis* and *Salmonella Typhimurium*). We have recently shown that phylogenetic kinship between *B. subtilis* isolates directs their swarm compatibility; hence *Bacillus subtilis* excludes non-kin from common groups. In contrast, highly related cells merge their swarms. This differential behaviour is an example of kin discrimination, for which Hamilton predicted to limit the expansion of cheaters and thus stabilize cooperative behaviours. We tested this prediction by mixing mutants (cheaters) that do not produce public goods (e.g. surfactants) and thus cannot swarm with their kin or non-kin wild type swarmers. We confirmed that mutants were helped only by their wild type kin, who accepted them within their swarms. In contrast, they did not mix with non-kin surfactant producers, which efficiently excluded them without help. Kin discrimination also affects horizontal gene transfer at the meeting area of two swarms. Interestingly, non-kin encounters induce a competition sensing response in the attacked swarm cells that generate an unknown signal, which then activates the competence genes and the DNA uptake in the attacker, potentially leading to its ecological advantage. While we are just beginning to understand mechanisms and consequences of *B. subtilis* kin discrimination, interspecies antagonism has been addressed extensively, often to identify potential antibiotics for pathogen control. In contrast, competition sensing mechanisms between distant species, such as *Bacillus* and *Salmonella*, were less studied and hence are not well understood. We find that nutrients and extracellular matrix components of *B. subtilis* can shape competition sensing between the two species in coculture and thus affect peace and war in the *Bacillus subtilis* world.

Keywords: *Bacillus subtilis*, kin discrimination, swarming, cheaters, horizontal gene transfer, competition sensing, *Salmonella*

Notes

*T-24

Evidence for SCCmec transfer by natural transformation in *Staphylococcus aureus* biofilms

Mais Maree[1], Le Thuy Thi Nguyen[2], Ryosuke L Ohniwa[1], Masato Higashide[3], Tarek Msadek[4], Kazuya Morikawa[1]

[1] *University of Tsukuba, Tsukuba (Japan)*
[2] *Biotechnology Centre of Ho Chi Minh City (Vietnam)*
[3] *University of Tsukuba, Tsukuba (Japan)*
[4] *Kotobiken Medical Laboratories, Inc., Kamiyokoba, Tsukuba (Japan)*
[5] *Institut Pasteur, Université de Paris, CNRS UMR6047, Paris (France)*
[6] *University of Tsukuba, Tsukuba (Japan)*

Staphylococcus aureus is an opportunistic pathogen that can rapidly acquire resistance to antibiotics. The emergence and dissemination of drug resistant strains, especially methicillin-resistant *S. aureus* (MRSA), is a global health concern. Methicillin resistance is conferred by the mecA gene, which is carried by the mobile staphylococcal cassette chromosome (SCC) element that transfers among staphylococci through an unknown horizontal gene transfer mechanism. Here, we present evidence for the development of natural transformation in *Staphylococcus aureus* and show its relevance in SCCmec transmission. We found that biofilm growth conditions increase the transformation efficiency of the unmodified *S. aureus* cells. Under these biofilm conditions, the transfer of SCCmec elements (types I to IV) from MRSA or methicillin-resistant coagulase negative staphylococci donors to methicillin-sensitive *S. aureus* recipients by natural transformation could be demonstrated. The transfer was dependent on the site-specific insertion/excision system mediated by the cassette chromosome recombinases, and the stability of SCCmec in the transformants varied depending on SCCmec types and the recipients. Taken together, our results suggest that natural transformation may be a key process in MRSA emergence.

Keywords: *Staphylococcus aureus*, MRSA, natural competence, biofilm, SCCmec, horizontal gene transfer

Notes

T-25

Gram positives against Gram negatives: *B. subtilis* PS-216 inhibits Campylobacter biofilm formation

Polonca Stefanic[1], Andi Erega[1], Katarina Šimunovič[1], Tjaša Danevčič[1], Anja Klančnik[2], Sonja Smole Možina[2], Ines Mandic-Mulec[1]

[1] *Department of Microbiology, BF, UNI Ljubljana (Slovenia)*
[2] *Department of Food Science and Technology, BF, UNI Ljubljana (Slovenia)*

Multi-species biofilms are the predominant bacterial life forms and can protect microorganisms from environmental stresses. Biofilms play an essential role in the survival of enteric pathogens that contaminate food. Therefore, studying the underlying mechanisms of multispecies biofilm formation and survival is critical for food safety and human health.

Here, we present an experimental model system consisting of *Bacillus subtilis*, a Gram-positive, beneficial spore-forming probiotic for humans and animals, and the Gram-negative foodborne pathogen *Campylobacter jejuni*, and test the ability of the natural isolate *B. subtilis* PS-216 to negatively affect *C. jejuni* biofilm formation and its adhesion to abiotic surfaces. In addition, the disruption of an already established *C. jejuni* biofilm by PS-216 was investigated using confocal laser scanning microscopy (CLSM).

We demonstrated that the presence of *B. subtilis* PS-216 reduced the growth of the *C. jejuni* biofilm by 3-4 logarithms. *B. subtilis* PS-216 impairs *C. jejuni* adhesion to abiotic surfaces, prevents *C. jejuni* biofilm formation, and disperses already formed *C. jejuni* biofilms. Furthermore, using transwell experiments, we show, that this inhibitory effect of *B. subtilis* PS-216 on *C. jejuni* biofilm is contact-independent. By testing the inhibitory effect of *B. subtilis* knockout mutants in genes encoding nonribosomal peptides and polyketides, we discovered that bacillaene and bacilysin contribute significantly to the inhibitory effect of *B. subtilis* PS-216 and using CLSM, we also show they reduce the thickness of the pathogen's biofilm and the distribution of *C. jejuni* and *B. subtilis* cells in the submerged biofilm.

B. subtilis has been known as an effective biocontrol agent for more than 50 years and is a recognized probiotic for animals and humans. Here we show strong potential for the use of *B. subtilis* PS-216 against *C. jejuni* biofilm formation and adhesion to abiotic surfaces, which could enable new applications of *B. subtilis* in animal production and support food safety in food processing.

Keywords: Antibiotics, secondary metabolites, *Campylobacter jejuni*, *Bacillus subtilis*, biofilm formation, bacillaene, bacilysin

Notes

T-26

Cannibalism in *Bacillus subtilis*: Role of programmed cell death in shaping and functionalizing differentiated multicellular populations

Thorsten Mascher[1]

[1] *Technische Universität Dresden, Institute of Microbiology (Germany)*

On solid surfaces, undomesticated strains of *B. subtilis* form intricately structured colonies, in which phenotypically different cell types are spatiotemporally coordinated to form robust microbial tissues and fruiting bodies. These structures provide a multicellular form for controlling population expansion, balancing growth with survival, and ultimately culminating in the formation of dormant endospores. In the context of such multicellular differentiation, antimicrobial compounds can play a role in intraspecies competition, resulting in the death of a sub-population of genetically identical siblings for the benefit of the population. Cannibalism is a unique bacterial form of programmed cell death that links multicellular differentiation to endospore formation in *Bacillus subtilis*.

Cannibalism was originally associated with the production of two toxins, the sporulation killing factor SKF and the sporulation delay protein SDP, which both target the cell envelope of *B. subtilis* [1]. More recently, we have described a comparable role for a third antimicrobial compound, the epipeptide EPE, which is produced by the gene products of the epeXEPAB operon [2, 3]. EPE targets the cell envelope of *B. subtilis* and results in severe perturbations of the cytoplasmic membrane, including dissipating the membrane potential via membrane permeabilization, accompanied by a rapid reduction of membrane fluidity and lipid domain formation. Alterations of the balance between autoimmunity and production of both EPE and SDP result in dramatic changes in differentiation and hence colony morphology. Taken together, cannibalism seems to represent a central checkpoint in tissue formation, which (i) provides resources to either delay or ultimately fuel sporulation, (ii) structures and functionalizes the colonies, and might ultimately serve to (iii) balance the ratio of different cell types in structured multicellular populations.

[1] Höfler et al. (2016), Microbiology 162:164-176.
[2] Popp et al. (2020) Front Microbiol. 11:151.
[3] Popp et al. (2021) Microb Physiol. 31:306-318.

Keywords: differentiation, programmed cell death, envelope stress response, antimicrobial peptide

Notes

*T-27

Controlled interkingdom communication: crosstalk between bacteria *Bacillus subtilis* and the eukaryote *Saccharomyces cerevisiae* by utilizing bacterial quorum sensing peptides

Tomislav Vološen[1], Antonia Stammberger[2], Juliane Korp[2], Linda Schuster[3], Uta Gey[2], Kai Ostermann[2], Diana Wolf[1]

[1] *TU Dresden, Group of General Microbiology (Germany)*
[2] *TU Dresden, Group of Biological Sensor-Actor systems (Germany)*
[3] *TU Dresden, Institute of Water Chemistry (Germany)*

Quorum sensing is substantial for cell differentiation within a bacterial population and is mediated by small self-produced secreted peptides. In the Gram positive bacterium *Bacillus subtilis* W168, some of these quorum sensing peptides are CSF (competence and sporulation factor) and PhrF. CSF indirectly regulates the activity of the transcriptional regulator ComA and thereby the expression of ComA-dependent genes responsible for e.g. secondary metabolites, natural competence development or sporulation.

In this study, we used CSF and PhrF to establish a controlled interkingdom communication between yeast and *B. subtilis*. For this purpose, we have engineered *B. subtilis* to serve as a reporter strain, which responds to the CSF and PhrF heterologously produced by the yeast Saccharomyces cerevisiae.

The reporter strain was constructed by fusing the ComA-dependent srfAA promoter with the luxABCDE cassette as a bioluminescence reporter of promoter activity. The generated fragment was then integrated into the genome of *B. subtilis*. Properties of different reporter strains were tested through a multi-mode microplate reader assay by measuring OD600 and bioluminescence.

First results of promoter activity measurements in combination with CSF and/or PhrF-producing yeast culture supernatant or synthetic peptides showed specific respond of the reporter strain to both sources of CSF and PhrF. Then, systematic mutagenesis of the genes that regulate the activity of ComA in the reporter strain resulted in an increased sensitivity of the promoter and thereby higher activity to the heterologously produced or synthetic CSF/PhrF.

Altogether, a quorum sensing-driven interkingdom crosstalk between bacteria and yeast was successfully generated. Currently, we are doing promoter engineering to increase activity and sensitivity to quorum sensing peptides. In future, we hope to fine-tune this communication in bacteria-yeast co-cultures and the use of it for downstream processes, like controlled protein expression and establishing biological sensor-actor systems.

Keywords: *B. subtilis*, *S. cerevisiae*, quorum sensing, CSF, PhrF, srfA, interkingdom communication

Notes

*T-28

Single molecule dynamics of the DNA receptor ComEA and DNA uptake in competent *Bacillus subtilis* cells

Alexandra Kilb[1,2], Marie Burghard-Schrod[1,2], Peter L. Graumann[1,2]

[1] *Fachbereich Chemie Philipps-Universität Marburg (Germany)*
[2] *Zentrum für Synthetische Mikrobiologie (Germany)*

Many bacteria can take up double stranded DNA from the environment, in a process called natural competence. This results in the uptake of novel genetic information leading to horizontal gene transfer. The model organism *Bacillus subtilis* uses a cell envelope-spanning machinery to take up exogenous DNA at the onset of stationary phase. This multiprotein complex imports DNA into the cytoplasm where it can integrate into the genome through homologous recombination. According to a prevailing model, DNA is transported across the cell wall by a pseudopilus that is formed by polymerization of the major pilin ComGC together with minor pilins. After transport, DNA is likely bound by the membrane receptor ComEA, a membrane protein with a DNA binding domain located in the periplasm, before it is eventually transported through the membrane by the ComEC channel. We wished to determine the spatio-temporal dynamics of the path of DNA into competent *B. subtilis* cells. Using in vivo labelling via cysteine substitution in the major pilin and a maleimide dye, we show that indeed, *B. subtilis* has a pseudopilus. We observed that cells had one or more labelled filaments exposed to the surface, of a size of roughly 200 to 300 nm, suggesting that cells can take up DNA at many places on their surface. In order to follow the motion and dynamics of fluorescently labelled DNA and the DNA receptor mVenus-ComEA, we used single molecule tracking. By tracking fluorescently labelled DNA we found that taken-up DNA diffuses through the entire periplasm, with a mobility similar that of a large protein complex, and also comparable to that of mVenus-ComEA. Our data suggest that most of taken-up DNA molecules are directly bound to ComEA. DNA/ComEA complexes can diffuse through the entire periplasm, indicating that this subcellular space can act as reservoir for taken-up DNA, before its entry into the cytosol, occurring through the polar ComEC protein, which finds its place by a diffusion/capture mechanism. Thus, DNA uptake in *B. subtilis* is spatially, but not temporally coupled.

Keywords: Competence; DNA uptake; *Bacillus subtilis*; single-molecule tracking

Notes

SESSION 7: Motility

K-07

SwrA increases binding affinity at a subset of DegU-regulated promoters

Ayushi Mishra[1], Xindan Wang[1], **Daniel Kearns**[1]

[1] *Indiana University, Bloomington (United States of America)*

Swarming motility is a flagellar-mediated form of surface migration and requires activation of the fla/che flagellar biosynthesis operon by the protein SwrA. SwrA has been reported to interact with the response regulator DegU which is also required for swarming. Here we perform ChIP-Seq analysis on both the SwrA and DegU proteins and find that SwrA interaction with DNA is indirect and DegU-dependent. Using EMSA and footprinting at multiple targets, we show that SwrA increases affinity for DegU binding to DNA and is required for DegU binding at certain promoters. How SwrA governs specificity and the DegU binding consensus, or lack thereof, will be discussed.

Keywords: flagella, swarming, motility, swrA, degU

Notes

T-29

SwrA: much more than a swarming protein

Cinzia Calvio[1], Lisa Gollinucci[1], Simone Russo[1]

[1] *Pavia University, Pavia (Italy)*

SwrA encodes a small protein essential for swarming motility and for poly-γ-glutamate biosynthesis which is not functional in domestic strains. Previous work indicated that SwrA interacts with DegU~P, the response regulator of two-component system DegS/U of *Bacillus subtilis*, to be able to exert its role on both motility and γ-PGA biosynthesis (1,2). Recently we have shown that SwrA, jointly with the phosphorylated form of DegU, also modulates subtilisin and other degradative enzymes expression, as well as DNA uptake via transformation (3).

Now, the role of SwrA in competence has been deepened by analyzing its contribution on the expression of comS and comK, the key genes for the development of the competent state.

Taken globally, our results allow to reconsider the overarching impact of the DegS/U system on the physiology of *B. subtilis*.

1. The Role of SwrA, DegU and PD3 in fla/che Expression in *B. subtilis*. 2013. Mordini S, Osera C, Marini S, Scavone F, Bellazzi R, Galizzi A, Calvio C. 2013. PLoS ONE: 8(12):e85065. DOI:10.1371/journal.pone.0085065.
2. SwrAA activates poly- -glutamate synthesis in addition to swarming in *Bacillus subtilis*. 2009. Osera C, Amati G, Calvio C, Galizzi A. Microbiology 05/2009; 155(Pt 7):2282-7. DOI:10.1099/mic.0.026435-0.
3. SwrA as global modulator of the two-component system DegSU in *Bacillus subtilis*. 2021. Ermoli F, Bontà V, Vitali G, Calvio C. Research in Microbiology 172: 103877, DOI: 10.1016/j.resmic.2021.103877.

Keywords: swrA, competence, DegS/U, gene expression regulation, motility

Notes

*T-30

Strategies for improved surfactin production using *Bacillus subtilis*

Lars Lilge[1], Peter Klausmann[1], Mareen Hoffmann[1], Chantal Treinen[1], Maliheh Vahidinasab[1], Jens Pfannstiel[1], Marius Henkel[1], Rudolf Hausmann[1]

[1] *University of Hohenheim, Stuttgart (Germany)*

Bacillus subtilis, known as microbial cell factory, offers great potential for the production of bioactive metabolites. Regarding this, the lipopeptide surfactin has often been described as a powerful microbial surfactant. However, bottlenecks, such as insufficient productivity of *Bacillus* wild-type strains and need for adapted bioprocesses due to features like strong foaming require further research. Details on process and genetic engineering approaches to establish efficient and robust surfactin production are addressed here. A common drawback in surfactin bioproduction is excessive foaming during aerated bioreactor cultivation. For this reason, various strategies have been developed to solve this issue. For example, oxygen-reduced cultivation processes with anaerobically inducible promoter systems have been established to decouple aerobic biomass formation from anaerobically stimulated surfactin production. Conversely, foam can be beneficial for surfactin enrichment using a foam fractionation column that allows semi-continuous product removal. Secondly, molecular mechanisms need to be understood in order to develop *Bacillus* production strains. In case of surfactin biosynthesis, the srfA operon encoding surfactin-producing non-ribosomal peptide synthetase is controlled by several regulators, including ComX-mediated quorum sensing. High cell density fermentation using the sporulation-deficient *B. subtilis* strain 3NA and the *B. subtilis* DSM10T wild-type strain allowed the determination of the ComX time course during bioreactor cultivation. Moreover, effects of several global regulators involved in cell differentiation, such as Spo0A, AbrB and DegU, on srfA operon expression, surfactin bioproduction and yields (YP/X and YP/S) were determined. Overall, the strategy of high cell density fed-batch cultivation allowed surfactin production up to 26.5 g/L, which is currently the highest surfactin titre known in the scientific literature. These results enable the combination of beneficial process and molecular adaptations for economic surfactin production in future.

Keywords: *Bacillus subtilis*, secondary metabolites, biosurfactants, surfactin, strain engineering, bioprocess engineering

Notes

T-31

Flagella disruption in *Bacillus subtilis* increases amylase production yield

Annaleigh O Fehler[1], **Thomas B Kallehauge**[2], Adrian S Geissler[3], Enrique González-Tortuero[3], Stefan E Seemann[3], Jan Gorodkin[3], Jeppe Vinther[1]

[1] *Dept. of Biology, Uni. of Copenhagen (Denmark)*
[2] *Novozymes A/S, Bagsværd (Denmark)*
[3] *Dept. of Veterinary and Animal Sciences, Uni. of Copenhagen (Denmark)*

Background: *Bacillus subtilis* is a Gram-positive bacterium used as a cell factory for protein production. Over the last decades, the continued optimization of production strains has increased yields of enzymes, such as amylases, and made commercial applications feasible. However, current yields are still significantly lower than the theoretically possible yield based on the available carbon sources. In its natural environment, *B. subtilis* can respond to unfavorable growth conditions by differentiating into motile cells that use flagella to swim towards available nutrients.

Results: In this study, we analyze existing transcriptome data from a *B. subtilis* α-amylase production strain at different time points during a five-day fermentation. We observe that genes of the fla/che operon, essential for flagella assembly and motility, are differentially expressed over time. To investigate whether expression of the flagella operon affects yield, we performed CRISPR-dCas9 based knockdown of the fla/che operon with sgRNA target against the genes flgE, fliR, and flhG, respectively. The knockdown resulted in inhibition of mobility and a striking 2-3 fold increase in α-amylase production yield. Moreover, replacing flgE (required for flagella hook assembly) with an erythromycin resistance gene followed by a transcription terminator increased α-amylase yield by about 30 %. Transcript levels of the α-amylase were unaltered in the CRISPR-dCas9 knockdowns as well as the flgE deletion strain, but all manipulations disrupted the ability of cells to swim on agar.

Conclusions: We demonstrate that the disruption of flagella in a *B. subtilis* α-amylase production strain, either by CRISPR-dCas9-based knockdown of the operon or by replacing flgE with an erythromycin resistance gene followed by a transcription terminator, increases the production of α-amylase in small-scale fermentation.

Keywords: *Bacillus subtilis*, Industrial production, CRISPR-dCas9, Flagella, motility, Biotechnology.

Notes

T-32

SpoVG – a global regulator of stationary phase processes

Swati Mahapatra[1,2], Jack Crook[2], Niki Cristopoulou[3], Pedro Arede Rei[3], Tetyana Sukhodub[4], Juan H Fernaud[1], Tauqeer Alam[1], Chrystala Constantinidou[1], Nicola Stanley-Wall[4], Sander Granneman[2], **Emma L Denham**[2]

[1] *University of Warwick, Coventry (United Kingdom)*
[2] *University of Bath, Bath (United Kingdom)*
[3] *University of Edinburgh, Edinburgh (United Kingdom)*
[4] *University of Dundee, Dundee (United Kingdom)*

SpoVG was first identified in *Bacillus subtilis* as a stage V sporulation protein and was shown to mildly affect the process of spore development. However, its role in this process has never been fully described. SpoVG is highly conserved across many bacteria including some Gram-negative species. In *Listeria monocytogenes* SpoVG was described as an RNA-binding protein and was shown to have pleiotropic effects. To explore the role of SpoVG in the biology of *B. subtilis* we have used a combination of omics analyses. Using the RNA-protein interaction technique CRAC we have shown that SpoVG is a global RNA-binding protein with affinity for specific RNA-binding motifs. Many of the RNAs highly bound by SpoVG encode antimicrobial compounds, secondary metabolites, non-coding RNAs and metabolic enzymes. Proteomics comparing wild-type and a spoVG deletion strain showed many of the equivalent biological processes were also significantly altered in these data sets, suggesting SpoVG plays a role in *B. subtilis* biology beyond sporulation. Using phenotypic assays we show that SpoVG is involved in the development of several stationary phase behaviours. The spoVG deletion strain is perturbed in biofilm formation, migration, production of antimicrobial compounds and protein secretion. This suggests that although SpoVG plays a role in sporulation, it is not just a sporulation protein and is likely to be a novel post-transcriptional global regulator. To explore it's regulatory role in the cell we have used a combination of transcriptional and translational reporter assays, competition assays and point mutations.

Keywords: RNA, regulation, development, stationary phase

Notes

T-33

c-di-AMP signaling is required for bile salt resistance and long-term colonization by *Clostridioides difficile*

Marine Oberkampf[1], Audrey Hamiot[1], Pamela Altamirano-Silva[2], Paula Belles-Sancho[1], Yannick DN Tremblay[1], Nicholas DiBenedetto[3], Roland Seifert[4], Olga Soutourina[5], Lynn Bry[3,6], Bruno Dupuy[1], **Johann Peltier**[1,5]

[1] *LPBA, Institut Pasteur, Université de Paris, Paris (France)*
[2] *Universidad de Costa Rica, San José (Costa Rica)*
[3] *Harvard Medical School, Boston (United States of America)*
[4] *Hannover Medical School, Hannover (Germany)*
[5] *Université Paris-Saclay, CEA, CNRS, I2BC, Gif-sur-Yvette (France)*
[6] *Brigham and Women's Hospital, Boston (United States of America)*

To colonize the host and cause disease, the human enteropathogen *Clostridioides difficile* must sense, respond, and adapt to the harsh environment of the gastrointestinal tract. Nucleotide second messengers are signaling molecules that mediate bacterial responses to changing environmental conditions. In this study, we showed that *C. difficile* produced cyclic diadenosine monophosphate (c-di-AMP) that was essential for growth because it controlled the uptake of potassium. We found that c-di-AMP was also involved in biofilm formation, cell wall homeostasis, osmotolerance, and resistance to detergents and bile salts. The c-di-AMP–binding transcriptional repressor BusR repressed the expression of the compatible solute transporter BusAA-AB. Compared to the parental strain, a busR mutant was highly resistant to hyperosmotic and bile salt stresses, whereas a busAA mutant was more susceptible. A short exposure of *C. difficile* cells to bile salts decreased intracellular c-di-AMP concentrations, suggesting that changes in membrane properties due to variations in cellular turgor or membrane damage is a signal for the adjustment of the intracellular c-di-AMP concentration. In a mouse gastrointestinal colonization model, a *C. difficile* strain that is unable to degrade c-di-AMP failed to persist in the gut as long as did the wild-type strain. Thus, c-di-AMP in *C. difficile* has pleiotropic effects, including the control of osmolyte uptake to confer osmotolerance and bile salt resistance, and is important for host colonization.

Keywords: *Clostridioides difficile*, second messenger, regulation, colonization

Notes

SESSION 8: Biofilm

K-08

Modulating cell type distribution in a biofilm

Gurol Suel[1]

[1] *UCSD, San Diego (United States of America)*

I will be sharing our recent efforts to uncover the mechanisms and principles that drive the spatial and temporal organization of cell types during biofilm development. I will also present how we can use these insights to exert control over biofilm organization.

Notes

T-34

Restoring functionality of a compromised *Bacillus subtilis* biofilm activator protein

Felix Dempwolff[1], Patricia Bedrunka[1], Pietro Giammarinaro [1], Gert Bange[1], Erhard Bremer[1]

[1] *Center for Synthetic Microbiology (SYNMIKRO) Marburg (Germany)*

Undomesticated strains of the Gram-positive soil bacterium *Bacillus subtilis* can differentiate into complex structures, referred to as biofilms. In these biofilms, genetically identical but physiologically different cell types are encased in a self-produced matrix consisting of extracellular polysaccharides and the fibrillar TasA protein. Biofilm formation relies on a highly integrated network of signaling cascades and involves a multitude of transcriptional regulators. Mutational analysis has previously identified a new component of this network, RemA, a crucial activator for biofilm formation. Great strides have recently been made in our understanding of RemA function through its crystallographic analysis. This approach revealed a unique structure of a bacterial transcriptional activator, as RemA displays an octameric organization into a ring-like complex. Eight LytTR-related DNA binding motif are exposed on the surface of the ring. Although no structure of a RemA:DNA complex is available, the overall topology of the octamer suggest that the RemA activator protein wraps DNA around it. The Arg-18/Trp variant of RemA is defective in biofilm formation, yet it can still bind to DNA. This single amino acid substitution causes the conversion of the authentic RemA octamer into a heptamer. To further our understanding of the structure/function relationship of RemA, we exploited the inability of this RemA variant to promote biofilm formation by selecting *B. subtilis* strains that had regained the ability to form biofilms on solid surfaces and at liquid/air interfaces. Intragenic suppressors were found in this screen that contained, in addition to the original Arg-18/Trp mutation, single amino acid substitutions at different positions of the RemA protein. Biochemical and structural analysis of some of them revealed the potential to form either heptameric or octameric assemblies. We will discuss these findings in light of the structural plasticity of the RemA protein and its biological consequences for gene regulation and the cellular differentiation of *B. subtilis*.

Keywords: biofilms, gene regulation, DNA binding

Notes

T-35

SAOUHSC_00671 contributes to cell splitting and biofilm formation in *Staphylococcus aureus* and its expression is associated with other peptidoglycan hydrolases

Danae Morales Angeles[1], Rebekka Moe, Marita Torrissen Mårli, Morten Kjos

[1] *Norwegian University of Life Science (Norway)*

Peptidoglycan hydrolases (PHs) play a critical role during bacterial cell wall synthesis and biofilm formation . PHs digest the cell wall to allow the insertion on peptidoglycan subunit resulting in cell expansion. In addition, PHs are responsible for splitting during cell division. The overproduction of PHs results in cell lysis, while a downregulation result in defects in cell expansion and in splitting. Therefore, it is crucial that the expression and the activity of PHs are tightly regulated.

Staphylococcus aureus has 11 peptidoglycan hydrolases under regulation of WalKR two-component system. WalKR controls the expression of PH in response to changes in cell wall structure. However, which other factors that affects the WalKR pathway and regulate PHs are not completely understood. Here, we focus on try to understand the role of SAOUHSC_00671, a PH with two LysM domain and a CHAP domain.

We initially identified SAOUHSC_00671 as factor important for biofilm formation in *S. aureus*. Our single cell analyses show that deletion of SAOUHSC_00671 produces bigger cells and an increase of cells with a complete septum, suggesting that cell splitting in Δ00671 is affected. To understand the regulatory interplay between staphylococcal PHs, a CRISPRi system was used to systematically knock down each of the hydrolases regulated by WalKR while following SAOUHSC_00671 transcription with a luciferase reporter. These results show that SAOUHSC_00671 transcription is reduced by the depletion of atl or sle1, but exacerbated in a ssaA knockdown. Interestingly, ssaA transcription is increased on Δ00671 both in cultures and biofilms, indicating that the expression of SAOUHSC_00671 and ssaA is tightly connected.

In addition, we show that SAOUHSC_00671 contributes to cell division in *S. aureus* as the reduced growth and cell splitting observed upon knockdown of atl and/or sle1, is further exacerbated in Δ00671. Together, these results demonstrate overlapping roles of PHs and highlight a regulatory interplay between PHs in response to changes in the peptidoglycan in *S. aureus*.

Keywords: cell splitting, cell wall, peptidoglycan hydrolase, biofilm, WalKR

Notes

*T-36

Second-generation transfer mediates efficient propagation of ICEBs1 in biofilms

Jean-Sébastien Bourassa[1], Gabriel Jeannotte[1], Sandrine Lebel-Beaucage[1], Pascale B. Beauregard[1]

[1] *Université de Sherbrooke (Canada)*

Antimicrobial resistance propagation by horizontal gene transfer (HGT) is a major contributor to the increasing prevalence of resistant bacteria. While the molecular mechanisms involved in HGT are extensively examined, only a few studies have looked at the impact of more environmentally relevant conditions, such as biofilms, on this process. We took advantage of the well-characterized integrative and conjugative element ICEBs1 and of its host *Bacillus subtilis* to determine if these multicellular communities affect ICEBs1 conjugative properties. Previously, we showed that biofilm formation increased ICEBs1 propagation at least a hundred-fold. Using a fluorescently marked ICEBs1, we observed that propagation of ICEBs1 in biofilm seemed to be concentrated in clusters of cells showing a very high transfer. We then methodically evaluated the localization of the conjugative clusters using thin sections displaying the entire depth of the biofilm. Surprisingly, we observed that conjugative clusters appeared to be mostly located at the air/biofilm interface, insinuating that the metabolic state of bacteria in this region favored propagation of ICEBs1. Further analysis of the conjugative clusters revealed that most of the transconjugant bacteria are not next to a donor cell, suggesting that they acquired ICEBs1 via transfer by another transconjugant. To validate this epidemic mode of transfer, we performed a conjugation assay in which the second-generation transfer is blocked. We observed that second-generation transfer of ICEBs1 in biofilm accounts for 99% of total transfers, stressing its importance for the remarkable transfer efficiency in biofilm, which differs from other elements where second-generation transfer is insignificant. The ICEBs1 regulation mechanism which allow a delay between acquisition of the element and repression of its transfer operon might be at the origin of this important transfer capacity from transconjugants.

Keywords: Biofilm, *Bacillus subtilis*, ICEBs1, horizontal gene transfer

Notes

*T-37

Tackling biofilms: Revealing protective components of *B. subtilis* biofilms in ROS stress

Erika Muratov[1], Julian Keilholz[1], Felix M. Fuchs[2], Andrew R. Gibson[2,3], Ralf Moeller[1]

[1] *German Aerospace Center (DLR), Cologne (Germany)*
[2] *Institute for Electrical Engineering and Plasma Technology (Germany)*
[3] *Research Group for Biomedical Plasma Technology (Germany)*

Biofilms are highly resistant microbial communities that withstand extreme conditions and disinfectants. These surface-associated consortia are embedded in extracellular polymeric substances (EPS), a self-built matrix made up of different biopolymers. Due to these defense mechanisms, biofilm inactivation remains a challenging task. Hence, there is an urgent need for reliable sterilization approaches. Plasma could be a promising next generation sterilization method of biofilms. Its antimicrobial properties are achieved by a mixture of reactive oxygen/nitrogen species (RONS), excited atoms/molecules, charged particles and UV-photons. This project aims to improve the decontamination processes by cold plasmas. Therefore, it is crucial to uncover underlying protection mechanisms and understand interactions between individual plasma elements and biofilm structures.

Since biofilms are highly heterogeneous communities developing over time, various maturity levels were treated with hydrogen peroxide (H2O2). Here, H2O2 represents a source for reactive oxygen species (ROS) and one of the major biocidal parts formed during gas plasma discharge. Since *Bacilli* are used as quality control strains for conventional sterilization methods, *B. subtilis* was chosen as bioindicator. Lethal effects were assessed by calculating colony forming units (CFU) of spores as well as vegetative cells. To confirm if exopolysaccharides and sporulation contribute to ROS resistance, strains lacking corresponding genes (epsA-O and sigG) were tested and compared to *B. subtilis* NCIB3610

Planktonic cells of the sporulation-deficient strain were not able to survive ROS exposure but since consortia are formed, no reduction in CFU can be detected. Biofilms without EPS survived ROS stress worse than the biofilms free of spores, regardless of the maturity degree. These results indicate that intact EPS is more protective than sporulation. To verify whether both, sporulation and EPS are essential to survive H2O2 exposure, a strain lacking ability to sporulate and EPS production will be generated and tested.

Keywords: Biofilms, sterilization, plasma inactivation, ROS stress, exopolysaccharides, sporulation

Notes

*T-38

Spatial transcriptome profiling unveils multi-scale heterogeneity levels between subpopulations of *Bacillus subtilis* surface-associated communities

Yasmine Dergham[1,2], Dominique Le Coq[1,3], Pierre Nicolas[4], Julien Deschamps[1], Eugénie Huillet[1], Pilar Sanchez-Vizuete[1], Kassem Hamze[2], Romain Briandet[1]

[1] *Université Paris-Saclay, INRAE, Micalis, Jouy en Josas (France)*
[2] *Lebanese University, Beirut (Lebanon)*
[3] *Université Paris-Saclay, CNRS, INRAE, Micalis, Jouy-en-Josas (France)*
[4] *Université Paris-Saclay, INRAE, MAIAGE, Jouy-en-Josas (France)*

Many bacterial species adapt to the environmental fluctuations by forming multicellular heterogeneous communities surrounded by a complex matrix of exopolymeric substances, which are collectively referred to as biofilms. Over the last decades, *Bacillus subtilis* has been extensively used as a model for molecular studies on biofilm formation. These studies encompassed the development of complex macro-colonies on either solid or semi-solid agar, the formation of pellicles at the air-liquid interface, and lately the formation of submerged architectural biofilms at the solid-liquid interface. Beside similarities, these multicellular communities also display considerable heterogeneity at the structural, chemical and biological levels. We used RNA-seq to analyze nine different spatio-physiological conditions, including the three biofilm models (colony, pellicle, and submerged). The transcriptome data gave a global landscape characterization of gene expression profiles for each of the differently localized selected populations. Transcriptional reporter fusions of genes involved in different physiological functions associated with confocal laser scanning microscopy, allowed to visualize in situ heterogeneous gene expression at a cell level. Furthermore, a temporal scale (4D-CLSM) observation of the submerged model showed spatio-temporal expression patterns of genes required for various cellular differentiations during biofilm development.

Keywords: *Bacillus subtilis*, biofilm, swarming, transcriptome, CLSM, fluorescent transcriptional fusions, heterogeneity

Notes

SESSION 9: Bacterial Virulence

K-09

Bacillus anthracis Branched Chain Amino Acid Transporters: Expression, Function, and Relationship to Virulence

Soumita Dutta[1], Ileana D Corsi[1,2], Naomi Bier[1,2], **Theresa M Koehler**[1,2]

[1] *McGovern Medical School, Univ of Texas Health Science Center (United States of America)*
[2] *MD Anderson UTHealth Graduate School of Biomedical Sciences (United States of America)*

Studies of the *Bacillus anthracis* virulence arsenal center on the protective capsule and the anthrax toxin proteins. Less discerned are metabolic attributes of the bacterium that permit proliferation to high numbers in diverse niches of mammalian hosts. In recent work, we have established that branched-chain amino acid (BCAA) biosynthesis by *B. anthracis* is insufficient for robust growth; access to BCAAs is necessary for proliferation during culture and for colonization in a murine model for anthrax. Despite the presence of BCAA biosynthesis genes, *B. anthracis* growth is severely restricted in defined media lacking exogenous BCAAs, indicating that BCAA transport is required for optimal growth in vitro. *B. anthracis* harbors an unusually large repertoire of potential BCAA transporters, including six BrnQ-type transporters. Results of BCAA transport assays using recombinant brnQ mutants indicate a high degree of functional redundancy among the transporters during culture of the bacterium. For example, BrnQ3, BrnQ4, and BrnQ5 were each associated with isoleucine and valine transport. Intriguingly, deletion of BrnQ3, but not BrnQ4 or BrnQ5, resulted in attenuated virulence, suggesting differences in BrnQ function and/or expression during infection. Gene expression studies showed that AtxA, the major activator of capsule and toxin gene transcription, regulates BCAA transport genes indirectly via the small regulatory RNA XrrA. AtxA positively controls expression of XrrA, and XrrA negatively regulates BCAA genes. To address relationships between BCAAs and AtxA that could be associated with the attenuated virulence of the brnQ3-null mutant, we measured atxA expression and AtxA activity during culture of *B. anthracis* with different levels of BCAAs. BCAAs, and specifically valine, increased AtxA activity in a dose-dependent manner, supporting a model in which BCAAs serve as a signal for AtxA-controlled virulence gene expression. Overall these investigations further our understanding of *B. anthracis* nutritional requirements and virulence signaling during infection.

Keywords: *anthracis*, anthrax, branched chain amino acid, amino acid transport, sRNA, BrnQ, AtxA, XrrA

Notes

T-39

Determination of *Staphylococcus aureus* infection-mimicking conditions by CRISPRi-Seq

Maria Vittoria Mazzuoli[1], Jessica Burnier[1], Clement Gallay[1], Xue Liu[2], Alejandro Gómez-Mejia[3], Morten Kjos[4], Annelies Zinkernagel[3], Jan-Willem Veening[1]

[1] *University of Lausanne, Lausanne (Switzerland)*
[2] *International Cancer Center, Shenzhen (China)*
[3] *University Hospital Zurich, Zurich (Switzerland)*
[4] *Norwegian University of Life Sciences, Oslo (Norway)*

Staphylococcus aureus is an important human pathogen and a major cause of a wide range of infections. *S. aureus* infections range from asymptomatic colonisation, skin and soft tissue infections to life-threatening pneumonia and bacteremia. Deep-seated infections are becoming increasingly difficult to eradicate in part due to the spread of strains with resistance to various classes of antibiotics, including methicillin-resistant strains (MRSA). Even when the drug seems appropriate based on in vitro susceptibility, antibiotic therapy often fails to cure these infections. Depending on environmental conditions (e.g. synthetic media or infection settings), different genes are important for survival and fitness. Here, we designed a doxycycline-inducible CRISPR interference system (CRISPRi) for *S. aureus* to conditionally downregulate genes on a genome-wide level and determine the bacterial fitness landscape both in vitro and in vivo. In our system, inducible dCas9 is integrated into the chromosome, allowing for tighter expression than in previous studies. We established pooled CRISPRi libraries targeting representative strains of clinical *S. aureus*, including MRSA and deep tissue isolates and provide an updated in vitro gene essentiality map for several strains. Ongoing CRISPRi screens in both in vitro and in vivo models will further define the list of essential genes for each strain at different levels of complexity. The resulting essentiality maps will provide crucial insights in relevant differences between models and thus into the mechanisms contributing to treatment failure of 'susceptible' strains in the host. Importantly, we aim to identify conserved targets that exhibit essentiality in vivo as potential candidates for the development of novel antimicrobial strategies.

Keywords: *Staphylococcus aureus*, clinical isolates, CRISPRi-Seq, bacterial fitness , antibiotic resistance

Notes

T-40

An alternative way of life for *Bacillus thuringiensis* in the late stages of an infection

Hasna Toukabri[1], Didier Lereclus[1], **Leyla Slamti**[1]

[1] *Micalis Institute, INRAE, Jouy-en-Josas (France)*

The formation of endospores has always been considered as the unique mode of survival and transmission of sporulating Firmicutes due to the exceptional resistance and persistence of this bacterial cell form. *Bacillus thuringiensis* (Bt) is a Gram-positive spore-forming bacterium used as a bio-control agent against insect pests. It belongs to the *Bacillus cereus* group that includes the foodborne pathogen *B. cereus* sensu stricto and the agent of anthrax, *Bacillus* anthracis. It was shown that Bt was able to complete a full infectious cycle in its host by sequentially activating virulence, necrotrophism (Nec) and sporulation (Spo) genes. These studies reported that the bacterial population was heterogeneous in the host cadaver and that spores constituted only 30% of the total cell load. In this study, we investigated the behavior of a bacterial population in the late stages of an infection as well as the characteristics and the importance of the Spo- bacterial form in the Bt/Galleria mellonella infection model. Using fluorescent reporters coupled to flow cytometry as well as molecular markers, we demonstrated that the Spo- cells compose the majority of the population two weeks post-infection (pi) and that these bacteria present vitality signs. However, a protein synthesis and a growth recovery assay indicated that they are in a metabolically slowed-down state. Interestingly, they were found to be extremely resistant to the cadaver environment which proved deadly for in-vitro grown vegetative cells and, more strikingly, for spores. We were able to perform a transcriptomic analysis of this subpopulation at 7 days pi that revealed a signature profile of this state. Analysis of the expression profile of individual genes at the cell level suggested that iron homeostasis is important at all stages of the infection, whereas the oxidative stress response seems of particular importance as the survival time increases. Altogether, these data indicate that non-sporulated bacteria engage in a profound adaptation process that leads to their persistence in the host cadaver.

Keywords: phenotypic heterogeneity, survival, non-sporulated bacteria, *Bacillus*, infection

Notes

T-41

Elucidating the mechanisms conferring self-resistance to the emetic toxin cereulide by the opportunistic pathogen *Bacillus cereus*

Sabrina Jenull[1], Agnieszka Gacek-Matthews [1], Stelli Stancheva[1], Alexei Sorokine[2], Didier Lereclus[2], Monika Ehling-Schulz[1]

[1] *University of Veterinary Medicine, Vienna (Austria)*
[2] *University Paris-Saclay, Jouy-en-Josas (France)*

Most bacterial species produce an arsenal of toxins. Thereby, the producer cell entails different and often multiple strategies to confer self-resistance to its toxin(s) such as transporters mediating toxin efflux. The foodborne pathogen *Bacillus cereus* is an endospore-forming bacterium displaying a wide array of phenotypic and pathogenicity traits, depending on the strain background. It can cause different types of food-associated diseases, manifested in diarrheal or emetic symptoms. Emesis is triggered by the heat- and acid-stable cyclic peptide toxin cereulide. The genetic elements facilitating its biosynthesis are encoded by the ces gene cluster (cesPTABCD) located on the megaplasmid pCER270. Cereulide interferes with cellular and mitochondrial membrane potentials and is highly cytotoxic. Besides, cereulide shows antimicrobial activity against certain fungi and bacteria, while emetic *B. cereus* displays resistance. However, the mechanisms conferring self-resistance remain elusive.

Here, we explore how emetic *B. cereus* resists against cereulide. We confirm that emetic *B. cereus* isolates from different origins are less susceptible to cereulide exposure than non-emetic isolates. Analysis of *B. cereus* mutants revealed that inactivation of the ces gene cluster or the ABC transporter cesCD does not affect cereulide resistance. However, complete loss of pCER270 abrogates cereulide resistance, which is partially restored by ectopic expression of cesCD. This indicates that cereulide self-resistance is a multi-factorial process with pCER270 encoding several putative resistance elements. In line with this, the transfer of pCER270 to otherwise susceptible species substantially decreases cereulide susceptibility of the recipient. These data highlight the dominant role of pCER270 in conferring cereulide resistance, which acts independently from genomic backgrounds. Given the concerns of clinical antibiotic resistance, dissecting the molecular mechanisms mediating intrinsic toxin resistance will aid in understanding the development of new resistance traits in pathogens.

Keywords: *Bacillus cereus*, cereulide, self-resistance

Notes

T-42

Regulatory RNAs in *Clostridioides difficile*: from genome-wide identification to targeted characterization and applications

Emma Piattelli[1], Victor Kreis[1], Polina Muzyukina[1], Adeline Humbert[1], Johann Peltier[1], **Olga Soutourina**[1]

[1] *I2BC, CNRS, University Paris-Saclay, Gif-sur-Yvette (France)*

During infection, bacteria reprogram their gene expression in response to environmental constraints. Non-coding RNAs (ncRNAs) play key roles in the regulation of adaptive responses. We are interested in the roles of ncRNAs in the pathophysiology of major human enteropathogen *Clostridioides difficile*. Deep sequencing (Transcriptional Start Site (TSS) mapping and RNA-seq) led to the identification of a great number (>200) and a large diversity of ncRNAs of different classes in *C. difficile*. These ncRNAs should be included into global regulatory network controlling gene expression in *C. difficile* in concert with specific sigma factors and protein regulators. A part of these RNAs could require the RNA chaperone protein Hfq for their action. We performed RNA immunoprecipitation high-throughput sequencing (RIP-Seq) analysis to identify Hfq-associated RNAs in *C. difficile*. Our work revealed a large set of Hfq-interacting ncRNAs and mRNAs, with a number of cis-antisense RNAs including antitoxins from type I toxin-antitoxin (TA) modules, riboswitches and CRISPR RNAs as new categories of Hfq ligands.

To analyse simultaneously host and pathogen transcriptomes, we recently performed dual RNA-seq in a mouse model of *C. difficile* infection. We identified a number of *C. difficile* ncRNAs among differentially expressed *C. difficile* genes that could mediate the adaptation of *C. difficile* inside the host and the crosstalk with the host immune response.

Altogether, the genome-scale data on ncRNA expression in in vitro conditions and in vivo during infection and on their interactions with Hfq combined with targeted ncRNA characterization provide essential basis for further studies on post-transcriptional regulatory network in *C. difficile*. This increasing knowledge has a great potential for future development of new antibacterial strategies based on antisense RNA targeting, TA and CRISPR applications to limit the pathogen development. Promising advances in *C. difficile* genome editing could be already achieved by using endogenous CRISPR-Cas and TA systems.

Keywords: regulatory RNAs, infection, human pathogen

Notes

*T-43

Towards characterizing the putative ABC transporter EslABC in the human pathogen *Listeria monocytogenes*

Lisa M Schulz[1], Patricia Rothe[2], Sven Halbedel[2], Angelika Gründling [3], Jeanine Rismondo[1,3]

[1] *Georg-August-Universität Göttingen (Germany)*
[2] *Robert Koch-Institut, Werningerode (Germany)*
[3] *Department of Infectious Disease, Imperial College London (United Kingdom)*

Transporters mediate many crucial processes in the cell. One conserved group of transporters are ATP-binding cassette (ABC) transporters, which facilitate the translocation of their cognate substrate across the membrane via ATP hydrolysis. Interestingly, recent studies have revealed alternative mechanisms not directly linked to import or export, resulting in a more dynamic use of the term. One candidate for this is the putative ABC transporter EslABC, which was recently identified in the human pathogen *Listeria monocytogenes*. Preliminary experiments demonstrate the involvement of the transporter in creating intrinsic lysozyme resistance and ensuring correct cell elongation. As part of the innate immune system, lysozyme serves as a natural antibiotic, thus, identifying proteins involved in its resistance will aid efforts to counteract pathogenic bacteria. Our findings also reveal that the transporter plays a crucial role in peptidoglycan biosynthesis and cell wall integrity. Even though, the mode of action of EslABC, as well as its exact connection to peptidoglycan biosynthesis and remodeling remains elusive, EslABC sheds light on cognate transporter functions and their versatility in cellular processes.

Keywords: *Listeria monocytogenes*, peptidoglycan biosynthesis , lysozyme, ABC transproter

Notes

SESSION 10: Sporulation

K-10

The assembly and functional architecture of the spore surface layers

Adriano O. Henriques[1]

[1] *Instituto de Tecnologia Química e Biológica, Oeiras (Portugal)*

Endosporulation, which emerged at the basis of the Firmicutes phylum, some 2.8 billion years ago is a developmental pathway by which certain bacteria convert vegetative cells into highly resistant dormant endospores (spores for simplicity) within a mother cell. The resilience of the spores allows their persistence in the environment for long periods of time and contributes to their broad geographic distribution in Earth's ecosystems. Most of the species that have been studied in some way belong to the Clostridia (anaerobic) and *Bacilli* (aerobic) classes of the Firmicutes. However, endospore formers are found in other classes within the Firmicutes and encompass aerobic or anaerobic organisms with a wide range of morphologies, lifestyles, and metabolic traits, including rods, cocci, branching species, plant or animal pathogens or symbionts, syntrophs, sulfate reducers, phototrophs and some organisms are diderms. Despite the diversity of spore-forming bacteria, the basic architecture of the spore is conserved across species, reflecting the general conservancy of a core machinery that propels progress through the morphological stages in the cell differentiation process. Spores have a core compartment, delimited by a lipid bilayer, that contains one copy of the genome. The core is surrounded by a series of concentric structures that function in the maintenance of dormancy and protection. Two spore structures are the cortex peptidoglycan, which is essential for the acquisition and maintenance of heat resistance, and a protein coat which surrounds the cortex and protects it from the action of peptidoglycan-breaking enzymes. The coat and the additional structures that may enclose it also mediate environmental transactions of the spore, including adhesion to host cells or abiotic surfaces and the evaluation of the ability of the environment to support growth. As such, the composition and structure of the spore surface layers is extraordinarily diverse and the mechanisms underpinning their assembly and functional architecture remain an absorbing area of study.

Keywords: spores, spore surface, *Bacillus subtilis*, *Clostridium difficile*

Notes

T-44

A new role for SR1 from *Bacillus subtilis* – regulation of sporulation by inhibition of kinA

Inam Ul Haq[1], Sabine Brantl[1], **Peter Müller**[1]

[1] *Friedrich Schiller University, Jena (Germany)*

SR1 is a dual-function sRNA from *Bacillus subtilis*. It inhibits translation initiation of ahrC mRNA encoding the transcription activator of the arginine catabolic operons. Base-pairing is promoted by the RNA chaperone CsrA, which induces a slight structural change in the ahrC mRNA to facilitate SR1 binding. Additionally, SR1 encodes the small protein SR1P that interacts with glyceraldehyde-3P dehydrogenase A to promote binding to RNase J1 and enhancing J1 activity. Here, we describe a new target of SR1, kinA mRNA encoding the major histidine kinase of the sporulation phosphorelay. SR1 and kinA mRNA share 7 complementary regions. Base-pairing between SR1 and kinA mRNA decreases kinA translation without affecting kinA mRNA stability and represses transcription of the KinA/Spo0A downstream targets spoIIE, spoIIGA and cotA. The initial interaction between SR1 and kinA mRNA occurs 10 nt downstream of the kinA start codon and is decisive for inhibition. The sr1 encoded peptide SR1P is dispensable for kinA regulation. Deletion of sr1 accelerates sporulation resulting in low quality spores with reduced stress resistance and altered coat protein composition which can be compensated by sr1 overexpression. This indicates, that the sRNA SR1 is involved in sporulation regulation by affecting the timing of spore generation. In addition, we will present our recent data on a new potential target of SR1.

Keywords: *Bacillus subtilis*, sporulation, SR1, sRNA, kinA, regulation

Notes

*T-45

Investigation of spore-associated lipoproteins YlaJ, YhcN, YutC, and CoxA suggests existence of a complex that stabilizes the germination apparatus in *Bacillus subtilis* spores

Matthew J Flores[1], Kate Duricy[1], David L Popham[1]

[1] *Virginia Tech, Blacksburg Va (United States of America)*

In order to return to a vegetative state, dormant bacterial spores must undergo the process of germination, which can be divided into two stages. In most species, stage I is triggered by sensing of nutrient germinants and is characterized by the release of calcium-dipicolinic acid (DPA) and various cations, while stage II is characterized by spore cortex degradation and full rehydration of the spore core. All of these steps are mediated by membrane-associated proteins, including germinant receptors, the major DPA channel, and a key cortex lytic enzyme, SleB, and all of these proteins have exposure on the outer surface of the membrane, a hydrated environment where they are potentially subject to damage during dormancy. A family of spore lipoproteins, including YlaJ, which is expressed from the sleB operon in some species, are present in all *Bacillus* and *Clostridium* species that express SleB. *B. subtilis* possesses four proteins in this family, and prior studies have demonstrated that two of these are required for the most efficient spore germination. Genetic studies of strains lacking all combinations of these four genes now reveal that all four play roles in ensuring efficient germination, and that they affect multiple steps in this process, including germinant sensing, DPA release, and SleB stability. Various protein-protein interaction studies indicate that the lipoproteins are able to self-associate, interact with their paralogues, and interact with SleB, and give possible insight to preferential interactions between the lipoproteins themselves. These results are consistent with predicted multimerization domains within the lipoproteins. All of these data suggest a model in which the lipoproteins form a macromolecular structure on the outer surface of the inner spore membrane, where they act to stabilize and/or promote the function of multiple components of the germination machinery. Continuing studies are examining the assembly of such a structure and potential effects on membrane structure and germination protein function.

Keywords: spore germination, heat resistance, *Bacillus*, *Clostridium*, sporulation

Notes

T-46

A novel ribonuclease KapD is integrated into the *B. subtilis* spore surface layers and plays a key role in structuring the outer coat and crust

Anastasia Tolcan[1], Laetitia Gilet[1], Magali Ventroux[2], Olivier Pellegrini[1], Mónica Serrano[3], Sylvain Durand[1], Sabine Figaro[1], Adriano O. Henriques[3], Rut Carballido-Lopez[2], **Ciaran Condon**[1]

[1] *UMR8261 (CNRS, Université de Paris-Cité), Paris (France)*
[2] *Micalis, INRAE, U. Paris-Saclay, Jouy-en-Josas (France)*
[3] *ITQB, Universidade Nova de Lisboa, Oeiras (Portugal)*

When facing severe nutritional stress, the Gram-positive model bacterium *Bacillus subtilis* forms highly resistant endospores. Although the pathway to sporulation is one of the best-studied developmental programs in bacteria, the function of many genes expressed during this process remains a mystery. We have identified a new 3'-exoribonuclease, KapD, that is expressed specifically in the mother cell, first under Sigma E control and then Sigma K later in sporulation. Remarkably, KapD dynamically assembles over the spore surface with kinetics very similar to that of outer spore coat proteins. A yeast two-hybrid screen and in vitro pull-down assays show that KapD interacts with the major spore crust protein CotY and we demonstrate that this accounts for its localisation in vivo. Deletion of the kapD gene or inactivation of its catalytic site strongly decreases the adhesiveness of the coat and crust layers, suggesting that in addition to being an integral component of the crust layer, KapD ribonuclease activity is import for normal spore morphogenesis. We analyse several potential mRNA substrates of KapD and present possible models for its role in the sporulation process.

Keywords: Ribonuclease, sporulation, spore coat, *Bacillus*

Notes

*T-47

Insights into the role of CD25890, a conserved protein that modulates sporulation initiation in *Clostridioides difficile*

Diogo Martins[1], Bruno Salgueiro[1], Daniel Sobral[2], Célia V. Romão[1], Adriano O. Henriques[1], Mónica Serrano[1]

[1] *Instituto de Tecnologia Química e Biológica António Xavier (Portugal)*
[2] *Faculdade de Ciências e Tecnologia NOVA University (Portugal)*

The YicC-like family of proteins is widespread in eubacteria and includes *Escherichia coli* YicC, *Bacillus subtilis* YloC and *Clotridioides difficile* CD25890. This family includes poorly characterized proteins that play a role in survival during the stationary phase of growth. The *E. coli* and *B. subtilis* proteins were recently described as a novel type of endoribonucleases with a role in silencing small RNAs. We show that CD25890 is involved in the control of sporulation initiation in *C. difficile* under certain nutritional conditions. Spo0A is the main regulatory protein controlling entry into sporulation and deletion of CD25890 increases expression of spo0A per cell and enhances sporulation. The effect of CD25890 on spo0A is likely indirect. Comparative transcriptomic analysis identifies three small RNAs that are upregulated in the CD25890 deletion mutant compared to wild type, suggesting that they are CD25890 substrates. We show that 35.4 kDa purified CD25890 exists in solution predominantly in an oligomeric form with a molecular weight of approximate 245 kDa, presumably an hexamer. These results were confirmed by CryoEM and supported by bacterial two hybrid assays that detected strong self-interaction of CD25890 through a C- terminal domain. Results indicate that in vivo CD25890 also accumulates as an hexamer. Collectively, these findings contribute to our understanding of the role of CD25890 in the control of sporulation initiation and the functions of this new endoribonuclease family.

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Keywords: *Clostridioides difficile*, YicC-like family, sporulation, gene regulation

Notes

T-48

PrkA regulates *Bacillus subtilis* sporulation through a Lon protease activity

Ao Zhang[1], Regine Lebrun[2], Leon Espinosa[1], Anne Galinier[1], **Frederique Pompeo**[1]

[1] *LCB, IMM, CNRS, AMU - Marseille (France)*
[2] *Plateforme Protéomique, IMM, MaP, CNRS, AMU, - Marseille (France)*

In *Bacillus subtilis*, sporulation is a sequential and highly regulated process. Phosphorylation events by Histidine or Serine/Threonine protein kinases are key points in this regulation. PrkA has been proposed to be an essential Serine protein kinase for the initiation of sporulation but its kinase activity has not been clearly demonstrated so far. Indeed, neither its autophosphorylation nor identification of a *B. subtilis* phosphorylated substrate was unambiguously established. Bioinformatic homology searches revealed sequence similarities with the AAA+ ATP-dependent Lon protease family. We were indeed able to demonstrate that PrkA could hydrolyse α-casein, an exogenous substrate of Lon proteases, in an ATP-dependent manner. We also showed that this ATP-dependent protease activity was essential for PrkA function in sporulation since mutation in the Walker A motif (characteristic of nucleotide binding) led to a sporulation defect. Furthermore, we found that PrkA protease activity was tightly regulated by phosphorylation events involving one of the Ser/Thr protein kinases of *B. subtilis*, PrkC. We finally demonstrated that PrkA regulation of the sporulation transcriptional factor σK via the transition phase regulator ScoC was certainly indirect.

Keywords: *Bacillus subtilis*, sporulation, phosphorylation, protease

Notes

POSTERS

P-01

*P-02

Regulatory network controlled by sigma factors of RNA polymerase in *Rhodococcus erythropolis*

Václav Štěpánek[1], Hana Dostálová[1], Tobias Busche[2], **Jan Blumenstein**[1,3], Michal Grulich[1], Lukáš Plašil[1,4], Lenka Rucká[1], Jan Nešvera[1], Miroslav Pátek[1], Jörn Kalinowski[2]

[1] *Institute of Microbiology of the CAS, v.v.i., Prague (Czech Republic)*
[2] *Center for Biotechnology, Bielefeld University, Bielefeld (Germany)*
[3] *Faculty of Science, Charles University, Prague (Czech Republic)*
[4] *University of Chemistry and Technology, Prague (Czech Republic)*

Rhodococcus erythropolis is a gram-positive non-sporulating bacterium with a wide potential in biodegradation of various pollutants. The general aim of our work was to uncover promoters conrolled by various stress sigma factors and propose their consensus sequences. Since many stress sigma genes are autoregulated, i.e. expressed from promoters recognized by the sigma factors in question, we tried to localize promoters of the respective *R. erythropolis* CCM2595 sigma genes. Using 5´-specific RNA-sequencing technique, we determined the transcriptional start sites of the sigA, sigB, sigD, sigE, sigG, sigH, sigJ and sigK genes. We identified the promoters of these genes and defined their -35 and -10 elements by comparison with the related bacteria *Mycobacterium tuberculosis* and *Corynebacterium glutamicum*. Promoter activity was measured by in vitro and in vivo methods. These assays confirmed the association of proper promoters and sigma factors. The analysis of the promoter sequences showed that most studied sigma genes are - in addition the stress sigma factors - also under the control of the housekeeping sigma factor SigA. Based on the classification of sig gene promoters, a basic model of the transcriptional regulatory network controlled by sigma factors in *R. erythropolis* was designed.

Keywords: *Rhodococcus erythropolis*, sigma factor, transcriptional regulatory network, RNA-seq

*P-03

Y-Complex Proteins Show RNA-Dependent Binding Events at the Cell Membrane and Distinct Single-Molecule Dynamics

Rebecca Hinrichs[1,3], Nadiia Pozhydaieva[1,2], Katharina Höfer[1,2], Peter L. Graumann[1,3]

[1] *SYNMIKRO, Zentrum für Synthetische Mikrobiologie (Germany)*
[2] *Max-Planck-Institut für Terrestrische Mikrobiologie (Germany)*
[3] *Fachbereich Chemie, Philipps Universität Marburg (Germany)*

Bacteria are dependent on rapid alterations in gene expression. A prerequisite for rapid adaptations is efficient RNA turnover, with endonuclease RNase Y playing a crucial role in mRNA stability, as well as in maturation. In *Bacillus subtilis*, RNase Y in turn interacts with the so-called "Y-complex" consisting of three proteins, which play important functions in sporulation, natural transformation and biofilm formation. It is thought that the Y-complex acts as an accessory factor in RNase Y regulation but might also have independent functions. Using single-molecule tracking, we show that all three Y-complex proteins exhibit three distinct mobilities, including free diffusion through the cytosol and confined motion, predominantly at membrane-proximal sites but also within the cell center. A transcriptional arrest leads to a strong change in localization and dynamics of YmcA, YlbF and YaaT, supporting their involvement in global RNA degradation. However, Y-complex proteins show distinguishable protein dynamics, and the deletion of yaaT or ylbF shows a minor effect on the dynamics of YmcA. Cell fractionation reveals that YaaT displays a mixture of membrane association and presence in the cytosol, while YlbF and YmcA do not show direct membrane attachment. Taken together, our experiments reveal membrane-associated and membrane-independent activities of Y-complex proteins and of RNase Y. Also, we found a dynamic interplay between them, with indirect membrane association of YmcA and YlbF via YaaT. Based on the amphipathic behaviour of the Y-complex, we propose it is involved in mRNA maturation and riboswitch control on the nucleoids, and may be involved in transfer of soluble mRNA to the membrane-associated RNA degradosome.

Keywords: RNA degradation; riboswitch; *Bacillus subtilis*; Y-complex; RNase Y; single-molecule tracking;

P-04

Small RNA S313 regulates the elemental iron transport system EfeUOB in *Bacillus subtilis*

Bimal Prajapati[1], Rocio Aguilar Suarez[1], Ruben AT Mars[1], **Jolanda Neef**[1], Marina López-Álvarez[1], Pierre Nicolas[2], Emma L Denham[1,3], Marc Schaffer[4], Leif Steil[4], Uwe Völker[4], Ulrike Mäder[4], Jan Maarten van Dijl[1]

[1] *University Medical Center Groningen (Netherlands)*
[2] *Université Paris-Saclay, INRAE (France)*
[3] *University of Bath (United Kingdom)*
[4] *University Medicine Greifswald (Germany)*

Small regulatory RNA (sRNA) molecules play increasingly recognized roles in bacterial gene regulation, especially in adaptive responses to changing environmental conditions. The regulatory networks in which sRNAs participate are complex and largely unexplored in most bacterial species. Thus, the specific biological functions of many sRNA molecules have remained enigmatic. In the present study, we addressed the role of the sRNA S313 of *Bacillus subtilis*, which is one of more than 150 different sRNAs known to be produced by this resident of the soil and plant rhizosphere. Our investigation was motivated by a genome-wide sRNA target prediction, which suggested a role of S313 in modulating the expression of the elemental iron transport system EfeUOB. As shown by deletion of the S313 gene, this sRNA stabilizes the efeU transcript. Accordingly, the absence of S313 leads to a specific growth phenotype that is also observed for efeU mutant strains when introduced into medium lacking NaCl. This phenotype is characterized by stalled growth, lysis and subsequent recovery of the S313 or efeU mutant bacteria. Importantly, in the absence of NaCl, the S313-deficiency has additional far-reaching consequences, as evidenced by major rearrangements in bacterial metabolism and decreased resistance to antibiotics. Altogether, our present observations show that S313 is crucial for growth in conditions of low salinity and plays a key role in cellular homeostasis and cefuroxime resistance in *B. subtilis*.

Keywords: *Bacillus subtilis*, sRNA, S313, iron transport

P-05

Insights into the role of lipoteichoic acids in *Bacillus subtilis*- a new function for MprF

Aurelie Guyet[1], Amirah Alofi[1], Richard A Daniel[1]

[1] *Newcastle University, Newcastle (United Kingdom)*

In *Bacillus subtilis*, the cell is protected from the environment by a cell envelope, which comprises of layers of peptidoglycan that maintain the cell shape and anionic teichoic acids polymers whose biological function remains unclear. In *B. subtilis*, loss of all Class A Penicillin-Binding Proteins (aPBPs) which function in peptidoglycan synthesis is conditionally lethal, with cell viability maintained by the SEDS proteins and also extracellular ions. Here, we show that lethality of strains lacking aPBPs on glucose rich medium is associated with an alteration of the lipoteichoic acids (LTA) and the accumulation of the major autolysin LytE in the cell wall. We provide evidence that the length and abundance of LTA acts to regulate the cellular level of LytE. Importantly, we identify a novel function for the aminoacyl-phosphatidylglycerol synthase MprF which is to modulate LTA biosynthesis in *B. subtilis* and in the pathogen *Staphylococcus aureus*. This finding has implications for our understanding of antimicrobial peptide resistance (particularly daptomycin) in clinically relevant bacteria, such as methicillin resistant *S. aureus* and our understanding of MprF-associated virulence in pathogens.

Keywords: *Bacillus*, Cell Evnvelope, Peptidoglycan, Teichoic acid, Autolysin

P-06

Polymerization of a bacterial actin-like MreB reveals actin-like and actin-unlike properties

Wei Mao[1], Lars Renner[2], Charlene Cornilleau[1], Ines Li de la Sierra[3], Sarah Benlamara[1], Sylvie Nessler[3], Aurélie Bertin[4,5], **Arnaud Chastanet**[1], Rut Carballido-Lopez[1]

[1] *MICALIS Institute, INRAE, AgroParisTech, Université UPsay (France)*
[2] *Leibniz Institute for Polymer Research (Germany)*
[3] *I2BC, University Paris-Saclay, CEA, CNRS (France)*
[4] *Institut Curie, PSL Research University, CNRS (France)*
[5] *Sorbonne Université (France)*

The bacterial homologue of actin, MreB, is an essential protein at the core of the proteinaceous complex building and maintaining the rigid protective scaffold enclosing most bacterial cells, the cell wall. The structural parenthood between MreB proteins and eukaryotic actin is long established but their physiological functions and dynamic properties in vivo seem always more different with growing knowledge. While actin is directly shaping eukaryotic cells by constituting an internal cytoskeletal scaffold, MreB forms short discontinuous and mobile assemblies that regulate the activity of the enzymatic complex assembling the external cell wall. A tremendous amount of data was collected along the years to describe in fine details the structure of actin polymers, their properties and how ATP degradation in actin filaments prompt their depolymerization. At the contrary, little is known on MreB polymeric structures in vitro, how they assemble or the purpose of the binding and degradation of nucleotides.

To get insight into MreB properties, we purified MreB from a Gram-positive thermophilic bacterium, *Geobacillus stearothermophilus*. We solved the first tri-dimensional structure of a Gram-positive MreB, revealing the hydrophobic subdomain required for its interaction with the membrane. Using soluble MreB, we observed straight pairs of protofilaments of various sizes, forming sheets through lateral association, similarly to structures obtained with MreBs from Gram-negative bacteria. Finally, our results show that, at the contrary to eukaryotic actin, the hydrolysis of ATP is a prerequisite to MreB interaction with lipids, and that this binding to lipids is then potentiating the polymerization of the protein.

Keywords: actin mreB cytoskeleton polymerization cell-wall

P-07

Image-guided in situ detection of Gram-positive bacterial biofilms in a human prosthetic knee infection model and on extracted osteosynthesis devices

Marina López-Álvarez[1], Jorrit W. Schoenmakers, Marjolein Heuker, Paul C. Jutte, Frank F. Ijpma, Marleen van Oosten, **Jan Maarten van Dijl**

[1] *University Medical Center Groningen (Netherlands)*

Due to increased human life expectancy, the need to replace arthritic or dysfunctional joints by prosthetics, or to support broken bones with osteosynthesis devices, is higher than ever before. Such implanted devices are unfortunately inherently susceptible to bacterial infection accompanied by biofilm formation. Early and accurate diagnosis of such biofilms is vital to increase the therapeutic success. However, established diagnostic modalities cannot directly detect bacterial biofilms on implanted devices. Therefore, the present study was aimed at investigating whether optical imaging can accurately detect bacterial biofilms on prosthetic joints and osteosynthesis devices. To this end, we developed a conjugate of the antibiotic vancomycin and the near-infrared fluorophore IRDye800CW, in short vanco-800CW. We show in a human post-mortem prosthetic knee infection model that a staphylococcal biofilm is accurately detected in real-time and distinguished from sterile sections in high-resolution using vanco-800CW and image-guided arthroscopy. Furthermore, compared to diagnostic culturing, the bacteria-targeted fluorescence imaging with vanco-800CW of extracted osteosynthesis devices from patients in need of revision surgery allows for a prompt diagnosis of clinical Gram-positive bacterial biofilms, reducing the time-to-result from days to less than 30 min. Lastly, we demonstrate that biofilms associated with the clinically most relevant Gram-positive bacterial species can be detected using vanco-800CW. We conclude that imaging with vanco-800CW can provide early, accurate and real-time visual diagnostic information of biofilms in the clinical setting, even in case of low-grade infections.

Keywords: Biofilm, Gram-positive, prosthetic joints, osteosynthesis devices

*P-08

Regulation of the plastic degrading protease AprE in *Bacillus pumilus* B12

Elise K Phillips[1], Jordan A Cannon[1], Todd B Reynolds[1]

[1] *University of Tennessee, Knoxville (United States of America)*

To mitigate the environmental impacts associated with petroleum-based plastics, biobased biodegradable plastics are being adopted. However, despite their many benefits, biodegradation of bioplastics is inefficient in a number of conditions, and is still poorly understood. This results in inconsistent degradation outcomes and challenges for widespread implementation. We recently showed *Bacillus pumilus* B12 can degrade the bioplastic polylactic acid (PLA), and have seen that the protease AprE is involved in this process. Despite being closely related to *B. subtilis*, our *B. pumilus* B12 has not been as easily genetically manipulated. We have developed a suite of novel conjugal vectors to explore the transcriptional regulation of aprE in response to conditions that affect PLA degradation. We have generated an aprE transcriptional reporter construct where 700bp of DNA upstream of aprE (PaprE) drives expression of the green fluorescent protein gene (GFP). Systemic deletions of this putative aprE promoter revealed regions that alter expression of PaprE-GFP. Several predicted transcription factor binding sites are in these regions, including homologs of transcription factors Spo0A, SinR, ScoC, and AbrB. We previously reported that adenine causes a reduction in PLA degradation, and have observed that exogenous adenine causes a commensurate reduction in PaprE-GFP expression and inosine has a stronger effect. All PaprE-GFP deletion mutants are impacted by inosine addition, suggesting that the point at which adenine and inosine regulate aprE expression is high up in the regulatory hierarchy. One potential point could be Spo0A, which directly or indirectly regulates many *B. subtilis* AprE regulatory proteins including SinR, ScoC and AbrB homologs. We hypothesize that Spo0A, via downstream transcription factors, directs the cell towards PLA- degrading protein secretion through regulation of AprE and that the addition of exogenous nucleotides affects this balance. Disentangling this regulatory mechanism will help inform strategies to improve efficiencies of bioplastic degradation.

Keywords: plastic degradation, regulation, stationary phase

*P-09

Microbial lipopeptides produced by *Bacillus* strains: a future solution against plant pathogenic fungi

Maliheh Vahidinasab[1], Lars Lilge[1], Stephen Akintayo[1], Chantal Treinen[1], Marius Henkel[1], Rudolf Hausmann[1]

[1] *University of Hohenheim (Germany)*

The maintenance of food security is a global concern, especially in the context of population growth. At the same time, the demand for an effective, environmentally friendly alternative to chemical fungicides is increasing. *Bacillus* strains are among the beneficial microorganisms known as effective cell factories and can produce many secondary antimicrobial metabolites, including lipopeptides. Iturin and fengycin are two important antimicrobial lipopeptide families produced by *Bacillus* strains that display additional biological activities besides their amphiphilic properties. However, the industrial application of these lipopeptides has not yet occurred, mainly because of the low yield in the production by wild-type strains and the unknown biosynthetic regulation pathways, especially in the case of fengycin. Therefore, a main goal of this research project is to find high antimicrobial lipopeptide producer strains. In order to elucidate the growth and lipopeptide production process, wild-type *Bacillus velezensis* strains were used as model strains for production of antimicrobial lipopeptides in bioreactors. To increase the overall iturin and fengycin production, several different bioprocess development strategies such as modeling a feeding profile during cultivation in fed-batch bioreactors and supplementation of putative critical precursors were utilized. In addition, the non-sporulating strain *B. subtilis* 3NA has been engineered to gain further insights about the mono-production of plipastatin (a member of fengycin family). We have substantiated that a full plipastatin production requires surfactin synthetase or some of its components and degQ expression followed by ensuring activation of DegU-P response regulator stimulates the native plipastatin production. Furthermore, the provision of ornithine is an indispensable constituent for plipastatin mono-production by *B. subtilis*. In future studies, novel strategies such as targeting the ornithine metabolic flux might be a promising strategy to increase plipastatin/fengycin mono-production.

Keywords: *Bacillus subtilis*, *Bacillus velezensis*, secondary metabolite, iturin, fengycin, biosurfactant, strain engineering

*P-10

Development of *Bacillus licheniformis* as alternative host for functional metagenomics

Luis Roberto Serrano-Garcia[1], Tu-An Vu-Dinh[1], Lukas Maisch[1], Sonja Vanderhaeghen[1], Wolfgang Liebl[1]

[1] *Technical University of Munich, Freising (Germany)*

Due to their high microbial diversity, natural habitats harbor pools of bacterial enzymes degrading a wide variety of materials, which represent a rich reservoir of enzymes for industrial applications. Novel bacterial enzymes can be identified more efficiently through function-based metagenomic studies (FBMS), avoiding the challenging step of bacteria isolation. In FBMS, the functional expression of enzymes of interest by a host bacterium is essential. However, E. coli, the most frequently used host, is not able to express all genes present in the sample. To extend the number of enzyme-encoding genes retrieved and provide an alternative Gram-positive host for FBMS that is additionally able to efficiently secrete enzymes, we established *B. licheniformis* as a new host for FBMS.

We designed the mobilizable, replicative pREFOBA vector to detect enzymatic activities using *B. licheniformis* as host in FBMS. pREFOBA includes the commercial fosmid PCC1FOS as a backbone, allowing the construction of metagenomic libraries in E. coli. In order to enable library transfer from *E. coli* to *B. licheniformis*, a fragment from the *Bacillus* vector pBACOV was inserted into pREFOBA. The performance of the pREFOBA/*B. licheniformis* system was tested constructing a genomic library from the thermophilic soil strain *Geobacillus stearothermophilus* DSM22. Upon transfer of the recombinant fosmids from *E. coli* to *B. licheniformis* through triparental conjugation, lipolytic and amylolytic activities were screened in both hosts using agar plates. A total of 384 *E. coli* clones were screened, identifying two clones with lipolytic activity. For *B. licheniformis* as a host, 239 transconjugants were obtained. After screening, the same lipolytic activities observed in *E. coli* were also detected in the corresponding *B. licheniformis* transconjugants. However, one additional transconjugant with amylolytic activity was identified, suggesting differences in gene expression between *E. coli* and *B. licheniformis* using the same library, supporting the relevance of using different host bacterium in FBMS.

Keywords: function based metagenomic studies, host bacterium, *Bacillus* licheniformis

P-11

Toward improved terpenoids biosynthesis in *B. subtilis*: strategies to enhance the methylerythritol phosphate (MEP) pathway

Siqi He[1], Wim J Quax[1]

[1] *University of Groningen, Groningen (Netherlands)*

Terpenoids, also known as isoprenoids, are a large group of natural products that are extensively used in food, cosmetic, pharmaceutical, and agricultural industries due to their versatile bioactivities. With the rapidly increasing demands for terpenoids, microbial cells have garnered vast attention as hosts for the production of valuable natural products. The Generally Regarded As Safe (GRAS) *Bacillus subtilis* 168 with its broad metabolic potential has demonstrated high possibility as a bacterial platform for terpenoids' production. In the effort toward engineering *B. subtilis* as a cell factory for the production of terpenoids, we exploited several engineering strategies to improve terpenoids' production in *Bacillus subtilis* on engineering its innate methylerythritol phosphate (MEP) pathway.

A synthetic operon was developed to allow the coordinated expression of multiple MEP pathway genes in *B. subtilis*. Together with its GRAS status, this could make *B. subtilis* a preferable cell factory for the production of different valuable terpenoids.

The CRISPR-Cas9 system was established in *B. subtilis* to facilitate precise and efficient genome editing. This system was employed to engineer three more modules to improve terpenoid production, including the terpene synthase module, the branch pathway module, and the central metabolic pathway module.

The use of spatial clustering of enzymes to enhance metabolite transfer between enzymes in synthetic metabolic pathways has shown to be an effective approach. Enzyme-fusion strategy has been employed in *B. subtilis* to ensure FPP is channeled from FPPs active site to GGPPs active site while also preventing competition for GGPP accumulation, this makes *B. subtilis* a suitable host for diterpenoid production.

Keywords: *Bacillus subtilis*; terpenoid; synthetic operon; CRISPR-Cas; enzyme fusion

*P-12

EFFECT OF PROTEASE-DEFICIENT HTRA ON SECRETION STRESS AND RECOMBINANT PROTEIN YIELD IN *BACILLUS SUBTILIS*

Ayşegül Öktem[1], David Núñez Nepomuceno[2], Jonathan Walgraeve[3,1], Borja Ferrero Bordera[4], Sandra Maaß[4], Dörte Becher[4], Ulrike Mäder[2], Uwe Völker[2], Jan Maarten van Dijl[1]

[1] *University of Groningen, Groningen (Netherlands)*
[2] *University Medicine Greifswald, Greifswald (Germany)*
[3] *AB Enzymes, Darmstadt (Germany)*
[4] *Universität Greifswald, Greifswald (Germany)*

Bacillus subtilis is a Gram-positive bacterium, which is known for its high secretory capacity. Although secretion of homologous proteins is extremely efficient, secretion of heterologous proteins imposes what is known as 'secretion stress' on the cells. This is not desirable in an industrial setting, because it can adversely affect product yields. The main approach to reduce potentially detrimental secretion stress responses so far has been to remove components of the CssRS two-component regulatory system. However, there are several examples indicating that a complete loss of the CssRS-regulated quality control proteases HtrA and/or HtrB does not correlate with improved protein secretion. In this study, an alternative approach to reduce secretion stress by modulating the protease activity of HtrA rather than completely removing this protease was investigated. Secretion stress, as indicated by the activity of the htrA promoter, was significantly higher in the strain with wild-type HtrA than the strain with proteolytically inactive HtrA when the heterologous alpha-amylase AmyQ was produced. The strain producing mutant HtrA displayed higher secreted amylase yields, which correlated with superior alpha-amylase enzymatic activity. Proteome analyses of the respective strains revealed critical differences in stress responses and metabolism. Our findings provide insights into how proteolytically inactive HtrA can contribute to lowered secretion stress and improve heterologous protein production.

Keywords: *Bacillus subtilis*, HtrA, secretion stress, protease, recombinant protein production

*P-13

The role of YlaN in iron homeostasis in *Bacillus subtilis*

Rica Bremenkamp[1], Lorenz Demann[1], Francis O'Reilly[2], Juri Rappsilber[2], Jörg Stülke[1]

[1] *Department of General Microbiology, University Göttingen (Germany)*
[2] *Bioanalytics Unit, Institute of Biotechnology, TU Berlin (Germany)*

Iron is required for growth in nearly all living organism and across all domains of life. When life first developed on this planet, the conditions were anoxic which led to iron to be present in its ferrous (Fe2+) form. Nowadays, earth is predominantly aerobic leading to the subsequent oxidation of iron into its ferric form (Fe3+). This results in iron starvation and oxidative stress since the ferric form of iron is highly insoluble and therefore less available for the cells. Therefore, bacteria have evolved multiple systems for the control of iron homeostasis. The main regulators for iron homeostasis are known as ferric uptake regulator (Fur) proteins. In a global protein-protein interaction study by in cell cross-linking and mass spectrometry we observed binding of the unknown essential protein YlaN to Fur. It has been shown that the deletion of ylaN is possible if Fe3+ is supplemented to the medium (1). The aim of this work is to further investigate this interaction and the role of YlaN in the cell. We were able to demonstrate that the deletion of the essential ylaN gene is possible even without a need for high iron concentrations if the fur gene is also absent. This observation suggests that the Fur protein becomes toxic for the cell in the absence of YlaN and suggests an important function of YlaN in the regulation of Fur. Further growth evaluations as well as suppressor screens and gel shift assays will shed more light on the nature of this interaction.

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Keywords: iron, Fur, protein-protein interaction

P-14

A novel method for transforming *Geobacillus kaustophilus* with a chromosomal segment of *Bacillus subtilis* transferred via pLS20-dependent conjugation

Ryotaro Amatsu[1], Shu Ishikawa[1], Valeria Verrone[2], Anil Wipat[2], Wilfried JJ Meijer[3], **Ken-ichi Yoshida**[1]

[1] *Kobe University (Japan)*
[2] *Newcastle University (United Kingdom)*
[3] *Universidad Autónoma Madrid (Spain)*

Geobacillus kaustophilus is a thermophilic Gram-positive bacterium. Methods for its transformation are still under development. Earlier studies have demonstrated that pLS20catΔoriT mobilized the resident mobile plasmids from *Bacillus subtilis* to *G. kaustophilus* [1] and transferred a long chromosomal segment from one cell to another between *B. subtilis* [2]. In this study, we applied mobilization of the *B. subtilis* chromosome mediated by pLS20catΔoriT to transform G. kaustophilus. We constructed a gene cassette to be integrated into *G. kaustophilus* and designed it within the *B. subtilis* chromosome. The pLS20catΔoriT-mediated conjugation successfully transferred the gene cassette from the *B. subtilis* chromosome into the *G. kaustophilus* allowing for the desired genetic transformation [3]. When we added 0.5 M sorbitol, 0.02 M MgCl2, and 0.02 M malate into the medium used for conjugation to adjust the osmotic pressure, the CFU of transconjugants increased approximately 130-fold, establishing the transformation of *G. kaustophilus* by this method to an almost practical level. This transformation approach described here will provide a new tool to facilitate the flexible genetic manipulation of G. kaustophilus, which could also be applied to other *Bacillus* species that are difficult to transform.

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Keywords: *Geobacillus* kaustophilus, conjugation, transformation

*P-15

Nutrient availability and biofilm polysaccharide shape the bacillaene-dependent antagonism of *Bacillus subtilis* against *Salmonella Typhimurium*

Eli Podnar[1], Andi Erega[1], Tjaša Danevčič[1], Eva Kovačec[1], Bram Lories[2], Hans Steenackers[2], Ines Mandic-Mulec[1]

[1] *University of Ljubljana, Biotechnical Faculty, Ljubljana (Slovenia)*
[2] *KU Leuven, Leuven (Belgium)*

Salmonella enterica is one of the most common foodborne pathogens affecting both humans and animals. Due to the spread of antibiotic resistance, new strategies are needed to limit foodborne pathogens. The vast majority of the literature dealing with *B. subtilis-Salmonella* interactions is conducted in broilers, and although these studies are essential for the development of probiotics, they do not address the mechanisms that mediate pathogen-probiotic interactions. In this study, we investigated the ability of a potential probiotic strain *Bacillus subtilis* PS-216 to inhibit the growth and biofilm formation of *S. Typhimurium* SL1344. The results show that *B. subtilis* PS-216 inhibits the growth of *S. Typhimurium*, its adhesion to the polystyrene surface and reduces *S. Typhimurium* biofilm thickness. *B. subtilis* mediated antagonism was lost under nutrient-restricted conditions and in a mutant with an inactivated pks operon essential for bacillaene synthesis. Moreover, the presence of *S. Typhimurium* in static co-culture activated the PpksC promoter of PS-216, which controls bacillaene production, indicating that *B. subtilis* senses and responds to a Gram-negative competitor. As *S. Typhimurium* inhabits nutrient-rich intestine and nutrient-poor aquatic environments, it is important to address pathogen-probiotic competition in relation to nutrient concentrations. We also provide evidence for the loss of bacillaene-mediated antagonism in nutrient starvation, which is mediated in part by the extracellular matrix polysaccharide. In conclusion, we show that nutrient availability, competition sensing, and extracellular biofilm polysaccharide contribute to social outcomes of bacillaene dependent competition between *B. subtilis* and *S. Typhimurium*.

Keywords: *Salmonella*, *Bacillus subtilis*, pathogen, probiotic, bacterial interactions, competition sensing, biofilm, nutrients

*P-16

Distribution of fitness effects of cross-species transformation reveals potential for fast adaptive evolution

Isabel Rathmann[1], Mona Förster[1], Melih Yüksel[1], Lucas Horst[1], Tobias Bollenbach[1], Berenike Maier[1]

[1] *Institute for Biological Physics, University of Cologne (Germany)*

Bacterial transformation, a common mechanism of horizontal gene transfer, can speed up adaptive evolution. Yet, how its costs and benefits depend on the growth environment is poorly understood. Here, we characterize the distribution of fitness effects (DFE) of transformation and test whether it predicts conditions in which transformation is beneficial. We generate hybrid libraries between the recipient *Bacillus subtilis* and different donor species and determine the DFE by measuring the selection coefficient of each hybrid strain. In complex medium, the donor *Bacillus* vallismortis confers larger fitness effects than the more closely related donor *Bacillus spizizenii*. For both donors, the DFEs show strong effect beneficial transfers, indicating potential for fast adaptive evolution. In different growth conditions, transfers of B. vallismortis DNA show pleiotropic effects including a fitness trade-off. In particular, in minimal medium, the DFE is shifted to negative selection coefficients and we find no evidence for strong effect beneficial transfers. We test the prediction that gene transfer speeds up adaptation in complex but not minimal medium by comparing evolution of transformed and untransformed populations in the respective media. After 400 generations, transformed populations are fitter than untransformed populations in complex medium and the effect is reversed in minimal medium. We conclude that transformation has strong potential for speeding up adaptation by letting bacteria tap into a gene pool shared between related species.

Keywords: evolution, adaptation, transformation, *Bacillus subtilis*, distribution of fitness effects, horizontal gene transfer

*P-17

Teichoic acid modifications are required for intercellular competition in *Bacillus*

Biwen Wang[1], Zihao Teng[1], Frans van der Kloet[1], Leendert Hamoen[1]

[1] *University of Amsterdam (Netherlands)*

We used genome wide transposon insertion sequencing (Tn-seq) to measure the fitness of genes in the Gram-positive model system *Bacillus subtilis* grown in rich medium. We applied an alternative fitness calculation method that makes use of the predicted transposon insertion frequency. The advantage of this method is that it provides fitness values for single conditions. Unexpectedly, we found that transposon insertions in lipoteichoic acid biosynthesis and teichoic acid D-alanylation genes strongly reduce cell fitness. Since isogenic cultures of these mutants do not show a reduction in growth rate, we assumed that the effect is caused by a reduced resistance against autologous antimicrobial compounds. To test this, we grew different dilutions of the transposon library on agar plates to compare growth of single colonies and confluent growth. Indeed, optimal growth under the latter condition also required the presence of lipoteichoic acids and teichoic acid D-alanylation, confirming the idea that *B. subtilis* requires these cell wall components for resistance against its own antimicrobials. It also shows that transposon libraries prepared as a mixed culture can influence the fitness of genes.

Keywords: Teichoic acid, *Bacillus*, transposon sequencing (Tn-seq), antibiotic, intercellular competition

*P-18

Consequences of pCER270 megaplasmid transfer in various hosts within the *Bacillus cereus* group

Alicia Nevers[1], Markus Kranzler[2], Stéphane Perchat[1], Michel Gohar[1], Alexei Sorokin[1], Vincent Sanchis-Borja[1], Serina Karuthedom[2], Agnieszka Gacek-Matthews[2], Monika Ehling-Schulz[2], Didier Lereclus[1]

[1] *INRAE, Jouy-en-Josas (France)*
[2] *University of Veterinary Medicine, Vienna (Austria)*

The *Bacillus cereus* group includes genetically close bacteria with a wide variety of ecological niches, life cycles and hosts. Despite the high genome conservation, extra-chromosomal genetic material diverges between these species. Actually, the plasmid genes are responsible for their discriminative properties, especially toxin genes. For instance, the production of the dodecadepsipeptide cereulide, which is responsible for emetic syndrome in foodborne intoxication, requires the non-ribosomal peptide synthetase Ces encoded by the ces locus on the pCER270 megaplasmid. The 270 kb pCER270 shows 222 coding-sequences, with only 39% having an assigned function.

To determine the role of pCER270 in strain adaptation, virulence and ecological niche specificity, we tagged pCER270 in the emetic reference strain F4810/72 with an antibiotic-resistance cassette using CRISPR/Cas9 technology, and transferred it to other *B. cereus* group members by conjugation. Since pCER270 is not conjugative by itself, we used a pXO16-derivative to mobilize its transfer. Transconjugants and their parental strains were subjected to Fourier Transformed Infrared (FTIR-) spectroscopy for metabolic fingerprinting. The chemometric analysis of the respective FTIR spectra indicated specific metabolic traits linked to the presence of the mega-plasmid.

Furthermore, we performed RNA sequencing experiments in the different strains to investigate gene regulations related to the presence of pCER270. Transcriptomic analysis suggested the existence of a strain specificity in chromosome-pCER270 transcriptional crosstalk. Moreover, we could observe that, despite a constant global expression level of pCER270 in all hosts, some specific loci were differentially regulated depending on the genomic context.

Keywords: *Bacillus* cereus, emetic, pCER270, metabolomics, transcriptomics

*P-19

An organogold compound as potential antimicrobial agent against drug resistant bacteria: initial mechanistic insights

Parichita Chakraborty[1], Dorenda Oosterhuis[2], Riccardo Bonsignore[3], Angela Casini[3], Peter Olinga[2], Dirk-Jan Scheffers[1]

[1] *GBB, University of Groningen, Groningen (Netherlands)*
[2] *GRIP, University of Groningen, Groningen (Netherlands)*
[3] *Technical University Munich, Munich (Germany)*

The rise of antimicrobial resistance has necessitated novel strategies to efficiently combat pathogenic bacteria. Metal-based compounds have been proven as a possible alternative to classical organic drugs. Here, we have assessed the antibacterial activity of seven gold complexes of different families. One compound, a cyclometalated Au(III) C^N complex, showed activity against Gram-positive bacteria, including multi-drug resistant clinical strains. The mechanism of action of this compound was studied in *Bacillus subtilis*, which included analyzing the effect of the compound on different cell membrane properties and on the energy status of the cell. Overall, the studies point towards a complex mode of antibacterial action, which does not include induction of oxidative stress or cell membrane damage. Total RNA sequencing of compound-treated cells was performed to determine a possible target. A number of genes related to metal transport and homeostasis were upregulated upon short treatment of the cells with gold compound. Toxicity tests conducted on precision-cut mouse tissue slices ex vivo revealed that the organogold compound is poorly toxic to mouse liver and kidney tissues, and may thus, be treated as an antibacterial drug candidate.

Keywords: Antibiotics, gold compounds, organometallic drugs, mode of action.

P-20

Profiling the cell wall proteome of Mexican *Staphylococcus aureus* isolates associated with bovine mastitis

Yaremit Mora Hernández[1], Jasper Stinenbosch[1], Elias Vera Murguia[1], Sandra Maaß[2], Stefano Grasso[1], Dörte Becher[2], Jan Maarten van Dijl [1], **Girbe Buist**[1]

[1] *Department of Medical Microbiology, University of Groningen (Netherlands)*
[2] *Institute of Microbiology, University of Greifswald (Germany)*

Mastitis is a common problem in dairy farms that leads to large economic losses. This infection of the bovine mammary gland is often caused by the Gram-positive bacterial pathogen *Staphylococcus aureus*. Mastitis is usually treated with antibiotics, but a vaccine to prevent this infection would be preferable, because *S. aureus* rapidly acquires antibiotic resistances. However, no anti-staphylococcal vaccine is currently available. This relates to huge genomic plasticity of the different *S. aureus* lineages, and an even higher variability in the presentation of potential antigens. Importantly, the best vaccine targets are exposed on the bacterial cell surface, where they are directly accessible to complement and immunoglobulins. Therefore, the present study was aimed at profiling the cell wall proteome of six different *S. aureus* isolates associated with bovine mastitis. To this end, we performed in silico target predictions, and identified cell surface-exposed protein domains by surface shaving with trypsin and subsequent mass spectrometry (MS). In parallel, non-covalently cell wall-bound proteins were extracted with potassium thiocyanide and identified by MS. To mimic conditions in the bovine mammary gland, the bacteria were grown in whey permeate. Altogether, 258 different cell wall-associated proteins were identified, including 42 bovine proteins that form a 'corona' on the bacterial surface. Notably, merely 47 proteins were shared by all six investigated *S. aureus* isolates of which 39 were exposed on the cell surface. Altogether, our observations argue in favor of proteomic profiling to identify the best possible candidate proteins for development of future vaccines that protect against bovine mastitis.

Keywords: Mastitis, *Staphylococcus aureus*, antigen, cell wall proteome

*P-21

RCd21 is a non-coding RNA with a cis and trans regulatory mechanism in the human pathogen *Clostridioides difficile*

Emma Piattelli[1], Adeline Humbert[1], Johann Peltier[1,2], Olga Soutourina[1]

[1] *Université Paris-Saclay, CEA, CNRS, I2BC (France)*
[2] *LPBA, Institut Pasteur, Université de Paris (France)*

Clostridioides difficile is the major cause of nosocomial infections associated with antibiotic treatment and spores are the main vector of transmission. Virulence of this human pathogen is finely regulated and many aspects of pathogenesis control have yet to be discovered. Recent transcriptomic analyses have identified a great number of potential regulatory RNAs in *C. difficile* including trans encoded RNA requiring the RNA chaperone protein Hfq. These ncRNAs might play important roles in the control of gene expression during the infection cycle and we are now characterizing a ncRNA named RCd21. RCd21 is a highly structured ncRNA with a housekeeping sigma factor dependent promoter and a Rho independent terminator. RCd21 structure prediction revealed the presence of C-rich loops important for targets recognition. A short RCd21 transcript is detected during different stages of growth in clinically relevant strains. It is enriched when RNA is extracted and sequenced after the coimmunoprecipitation with Hfq, suggesting that RCd21 binds Hfq. This data classified RCd21 as a potential trans encoded ncRNA. Interestingly, the deletion of RCd21 strongly decreases the expression of the downstream gene encoding a metal transporter, proposing a potential cis-regulation by RCd21. Metals are important cofactors during infection and their homeostasis is tightly regulated because metal excess is toxic. The long transcript covering RCd21 and the metal transporter gene is detectable suggesting their co-transcription. We then confirmed the regulation of the metal transporter gene by the promoter upstream of RCd21 during the exponential phase of growth. We also identified an additional active promoter associated with an alternative sporulation sigma factor, located directly upstream of the metal transporter gene. This study reveals an original mechanism of regulation mediated by RCd21 that can function as a trans encoded and cis acting ncRNA and a metal transporter that could be important during a critical step of *C. difficile* infection such as sporulation.

Keywords: *Clostridioides difficile*, non-coding RNAs, metal transporter

*P-22

Evolution of sporulation loss in *Bacillus subtilis*

Corinna Schwarz[1], Javier Lopez-Garrido[1]

[1] *Max-Planck-Institute for Evolutionary Biology, Plön (Germany)*

Endospore formation (sporulation) is a developmental process that culminates in the formation of resilient spores. Sporulation occurs in a broad range of bacterial species from an ancient and exceptionally diverse bacterial phylum, the Firmicutes. Due to the conservation of the regulatory and structural architecture of the sporulation pathway among distantly related species, it is widely accepted that sporulation arose when the Firmicutes emerged more than 2 billion years ago. In spite of the seemingly obvious advantage conferred by the ability to form spores, sporulation has been repeatedly lost in several Firmicute branches throughout evolution. The selective conditions and the genetic changes that underpin sporulation loss in an evolutionary context remain poorly understood. We are using experimental evolution to track sporulation loss in populations of *Bacillus subtilis*. We propagated seven populations of *B. subtilis* PY79 through daily transfer to medium in which the majority of the cells sporulated within twenty-four hours. We did not select for spores or vegetative cells, so that both cell types were allowed to propagate in subsequent transfers. We marked our starting strain with lacZ under control of a promoter that is activated upon sporulation initiation, so that we could follow the emergence of clones with different sporulation initiation frequencies by simply viewing colonies on agar plates containing X-Gal. We have seen a rapid adaptive radiation in which clones with different sporulation frequencies spread through the population. Ultimately asporogenous clones appeared, and all seven populations lost the ability to sporulate in less than ten weeks. Ongoing whole population DNA sequencing is providing us with insights into the genetic changes that lead to sporulation loss in bacterial populations.

Keywords: sporulation, *Bacillus subtilis*, sporulation loss, experimental evolution

*P-23

In-silico modeling of Parasporins obtained from PS2Aa1 and determination of its cytotoxic potential in colorectal cancer cell lines

Miguel O Suárez-Barrera[1,3], Lydia Visser[1], Paola Rondón-Villareal[2], Efraín H Pinzón-Reyes[2], Jahir Orozco[3], Nohora J Rueda-Forero[2]

[1] *University Medical Center Groningen, University of Groningen (Netherlands)*
[2] *Universidad de Santander UDES (Colombia)*
[3] *Universidad de Antioquia UdeA (Colombia)*

Recently, Parasporins PS have been discovered, which have been of great interest due to their selectivity with cancer cells without affecting healthy cells. Within this new group, PS2Aa1 stands out, characterized by strong cytotoxic effects against colon cancer cell lines. However, to date, its mechanism of action is not well defined. In this study, site-directed mutagenesis was used to obtain PS2Aa1 mutants, most with variation in domain I at positions 256 and 257, involved in interaction and binding with the membrane receptors including APN. Cytotoxicity tests were performed to determine cell viability in SW480, SW620, and CaCo-2. Four mutants were shown to act better on colorectal cancer cell lines (3-3, 3-35, and 3-45); while two of them (0015 and 002) presented low or null levels of cytotoxicity compared to the controls. Next, Annexin V/PI assays were performed, and 3-35 showed increased apoptosis. The activated form of caspase 3 and PARP was upregulated when mutant 3-35 was used; in addition, this mutant revealed an increased induction of γH2AX. In parallel, an APN receptor blockade assay was performed showing that the cytotoxic activity of PS2Aa1, as well as its mutants, was markedly reduced. Molecular Dynamics assays were performed to analyze interaction stability and identify residues that are highly involved in interactions for the PS2Aa1-APN complex at times of 100 ns. Molecular docking and molecular dynamics simulations showed an interaction between domain I of PS2Aa1 and APN is likely and stable. Moreover, the mutation in 3-35 was performed in the region of residues involved in molecular interactions. During Molecular Dynamics simulations, residue 256 was present in all replicates, and its mutation reduces the interaction and its cytotoxicity, as obtained in mutants 0015 and 002. On the contrary, a mutation at position 257, which was involved in two replicas of molecular dynamics simulations, leads to an improvement in the interaction and therefore enhanced cytotoxicity, as shown in mutant 3-35 the most promising parasporin so far in this study.

Keywords: Site-directed Mutagenesis, Parasporin PS2Aa1, Colo-rectal cancer.

*P-24

Role of Asp23 family proteins in fatty acid acquisition in *Bacillus subtilis*

Dennis Wicke[1], Sabine Lentes[1], Jörg Stülke[1]

[1] *University of Göttingen, Göttingen (Germany)*

Fatty acids are essential precursors for the synthesis of phospholipids that serve as the main components in biological membranes of virtually all organisms. The acquisition of fatty acids in *B. subtilis* is based on either essential de novo biosynthesis or the uptake of exogenous fatty acids. The highly specific acetyl-CoA-carboxylase (ACCase) complex catalyzes the conversion of acetyl-coA to malonyl-CoA to initiate the biosynthesis of fatty acids. Exogenous fatty acids are phosphorylated by a two-component fatty acid kinase (Fak) prior to incorporation as acyl-phosphates and further processing. The regulation of both committed steps in fatty acid acquisition, however, remains unknown.

We have identified the unknown and highly expressed proteins YqhY and YloU of the conserved Asp23 protein family as promising candidates to regulate fatty acid acquisition. The genes yqhY and yloU encoding the paralogous Asp23 family proteins share conserved operons with accBC and fakA, respectively. Based on the genomic co-localization, protein-protein interaction experiments revealed physical interactions between YqhY and AccBC, and YloU and FakA, respectively. This work aims at characterizing the putative regulatory effects of the Asp23 family proteins on fatty acid acquisition in *B. subtilis*.

Keywords: fatty acid acquisition, unknown proteins, asp23 family proteins

P-25

The relationship between the adaptor protein GpsB and the protein kinase StkP in *Streptococcus pneumoniae*.

Linda Doubravová[1], Václava Stauberová[1], Aleš Ulrych[1], Karolína Buriánková[1], Orietta Massidda[2], Pavel Branny[1]

[1] *Institute of Microbiology, Czech Academy of Sciences, Prague (Czech Republic)*
[2] *CIBIO, University of Trento, Trento (Italy)*

Streptococcus pneumoniae is an important opportunistic G+ pathogen that colonizes the nasopharynx of healthy children and adults. However, it can also cause a variety of diseases ranging from middle ear infections and sinusitis to pneumonia, bacteraemia and meningitis. Cell growth and division in pneumococci are mediated by two distinct molecular complexes: the divisome, which is responsible for the synthesis of division septa, and the elongasome, which is responsible for cell elongation. The eukaryotic-like Ser/Thr protein kinase StkP and its cognate phosphatase PhpP are major regulators of the pneumococcal cell cycle. StkP is a transmembrane protein consisting of a conserved cytoplasmic N-terminal kinase domain connected via a hydrophobic linker to four so-called PASTA repeats. To our current knowledge, the StkP pathway mediates a signal about the cell wall status and, in response, phosphorylates many proteins involved in cell wall biosynthesis and cell division thus acting as a switch between the modes of PG septal and elongation synthesis. StkP has been found to interact and cooperate with the cell division protein GpsB. GpsB is a small hexameric adaptor protein that has been shown to be an important regulator of PG synthesis in rod-shaped G+ species. Our phosphoproteomic analysis revealed that GpsB is phosphorylated by StkP at several Thr and Ser residues under standard cultivation conditions and during cell wall stress induced by β-lactam antibiotics. We confirmed the direct phosphorylation of GpsB by StkP and showed that mutation of the phosphoacceptor residues negatively affects the kinase activity of StkP and the interaction of the two proteins. As a result, *S. pneumoniae* cell division is severely impaired. The regulation of the PASTA -kinase PrkC by GpsB via a negative feedback loop was previously described in *B. subtilis*. We conclude that the activity of GpsB and StkP in pneumococci are also intertwined and the phosphorylation status of GpsB most likely influences the assembly of the cell division protein complex.

Keywords: *Streptococcus pneumoniae*, cell division, phosphorylation, cell signaling

*P-26

Catalyzed Disulfide Bond Formation in *Bacillus subtilis*

Tobias Schilling[1], Jan Maarten van Dijl[1]

[1] *University of Groningen, University Medical Center Groningen (Netherlands)*

Biotherapeutics and enzymes represent a huge global market, and disulfide-bonded proteins form a significant portion of that. While bacteria, such as *Bacillus subtilis*, are commonly used workhorses for the secretion of enzymes in large industrially relevant quantities, their expression and secretion capacity for complex biopharmaceuticals with multiple disulfide bonds is limited. For constructing a *B. subtilis* strain capable of producing such proteins, it is essential to understand and modulate the cells' thiol-disulfide oxidoreductases (TDORs), responsible for proper protein oxidation and folding. Also, it may be relevant to explore the integration of recombinant TDORs from other micro-organisms or mammals in *B. subtilis*. Moreover, strains from the genome-reduced midi*Bacillus* strainline were shown to have a high potential for the production of difficult-to-express target proteins. Besides serving industrial requirements as being depleted from prophages, extracellular proteases and genes for sporulation, these genome-minimized strains simplify the identification of important key factors for protein production. Lastly, testing different promoter systems, signal peptides to direct secretion, and gene copy numbers can facilitate a further optimization of the yields and quality of the produced proteins.

Here, we present our results on the expression of *Gaussia* luciferase, a model protein with five disulfide bonds, which was used for benchmarking the performance of disulfide bond formation in different midi*Bacillus* strains. Importantly, these genome-minimized strains achieved significantly higher titers of the disulfdide-bonded and active enzyme than the parental strain 168. Intriguingly, the improved productivity relates only partly to the deletion of all extracellular proteases. Altogether, our present observations show that genome-minimized strains of *B. subtilis* represent promising chassis for the production of proteins with multiple disulfide bonds.

Keywords: *B. subtilis*, secretion, recombinant protein expression, disulfide bond formation

P-27

New phosphosites of the KhpB protein and the effect of its phosphorylation on growth and morphology in *S. pneumoniae*

Václava Stauberová[1,2], **Aleš Ulrych**[1], Linda Doubravová[1], Karolina Buriánková[1], Pavel Branny[1]

[1] *Institute of Microbiology of the CAS (Czech Republic)*
[2] *Charles University, Faculty of Science, Prague (Czech Republic)*

KhpB is the cell division and elongation protein of the important human pathogen *S. pneumoniae*. KhpB was first described as a substrate of the StkP-protein kinase and PhpP-phosphatase signalling pair, which controls important cellular processes (virulence, competence) and regulates cell division. The KhpB protein consists of 3 parts - the Jag_N domain, which is important for its septal localisation, the interdomain region where the major StkP phosphorylation site T89 is located, and two RNA-binding domains (KH and R3H) at the C-terminus. In addition to T89, KhpB is phosphorylated by StkP kinase at at least one previously undescribed site detected by anti-P-Thr antibody. We purified His-Jag-T89A from *E. coli* and performed an in vitro kinase assay. Subsequent analysis by mass spectrometry identified several potential phosphorylation sites. Two of the identified sites were confirmed in vivo by phosphoproteomic analysis. By generating phosphoablative mutations at the newly identified sites and subsequent immunodetection with anti-PThr antibody, we identified the second phosphorylation site detected by anti-PThr antibody. Here we present data showing how phosphorylation of the newly identified phosphorylation sites affects T89 phosphorylation. We also show the effects of single/multiple phosphoablative and phosphomimetic mutations on *S. pneumoniae* growth and morphology.

Keywords: *Streptococcus pneumoniae*, phosphorylation, cell division

*P-28

SubtiWiki: a look into the future

Christoph Elfmann[1], Tiago Pedreira[1], Stülke Jörg[1]

[1] *University of Göttingen, Dept. General Microbiology, Götting (Germany)*

The popular SubtiWiki database (http://subtiwiki.uni-goettingen.de) provides the research community with high-quality curated data on *Bacillus subtilis*. It not only includes up-to-date information on genes and their products, but also features intuitive tools to investigate their genomic context, expression levels under different conditions, biological networks and pathways. Genes are annotated with functional categories, operons and regulons. All of this data is presented in a comprehensive, user-friendly way, making SubtiWiki a valuable tool in the research of the *Bacillus subtilis* community. Starting out in 2009, it has grown to become a powerful, full-fledged relational database, enabling the dynamic representation of complex data. Recently, homology analyses and COG data have been added. Moreover, we have included structure predictions for each protein from the AlphaFold Protein Structure Database, and now directly provide an interactive structure viewer. Together, these new features have increased the value of SubtiWiki even further. However, with the rise of new studies with more complex data, the development of SubtiWiki is far from being finished. Here, we evaluate the current state of SubtiWiki and shed light on future directions to our database. Among these, we highlight a more versatile interaction browser that focuses on more complex interaction types involving metabolites and RNAs. To integrate these novel features and to provide a better platform, we are now reworking SubtiWiki's codebase for more flexibility and stability. With this, we aim to keep providing the community with their favorite platform with stronger features than ever.

Keywords: SubtiWiki, database, genome annotation

P-29

A dual role of FtsA in *Streptococcus pneumoniae* cell wall synthesis?

Karolína Buriánková[1], Andrea Mura[1], Federico Corona[2], Ana I. Rico[3], Linda Doubravová[1], Pavel Branny[1], William Margolin[4], Orietta Massidda[5]

[1] *Institute of Microbiology, v.v.i., Prague (Czech Republic)*
[2] *EMBL, Heidelberg (Germany)*
[3] *Centro Nacionale de Biotecnología, CSIC, Madrid (Spain)*
[4] *University of Texas, McGovern Medical School, Houston (United States of America)*
[5] *CIBIO, University of Trento, Trento (Italy)*

Streptococcus pneumoniae is an ovoid-shaped Gram-positive pathogen that like the model rods, *Escherichia coli* and *Bacillus subtilis*, grows by alternating cycles of peripheral (side-wall) and septal peptidoglycan (PG) synthesis, which may overlap at least partially during the cell cycle. While in the model rods these processes are organized, respectively, by the actin-like proteins MreB and FtsA, it is unknown how peripheral elongation occurs in pneumococci, which have FtsA but lack MreB, albeit maintaining all the other components that constitute the elongasome.

We have previously shown that *S. pneumoniae* FtsA (SpFtsA) is essential and its inactivation results in cell lysis rather than cell elongation and also in delocalization of both cell elongation and division proteins as well as dispersion of PG synthesis, suggesting an additional role for SpFtsA in coordinating peripheral PG synthesis.

Here, we follow up this idea and report the characterization of different *S. pneumoniae* ftsA thermosensitive (Ts) mutants, in which the original multiple allelic variants located in different domains of the FtsA structure, thought to be involved in FtsA polymerization, interactions with FtsZ and/or with other cell division proteins, were characterized individually at the native locus. Furthermore, we show that MreB from the closely related lactic acid species *Leuconostoc* mesenteroides significantly corrects the phenotype resulting from inactivation of SpFtsA in the most severe of the *S. pneumoniae* ftsA Ts mutants, further supporting the idea that SpFtsA may have both FtsA-like and MreB-like roles.

Keywords: FtsA, *Streptococcus pneumoniae*, cell division

P-30

Metabolic characterization of *Bacillus subtilis* sporulation and spore revival

Iqra Kasu[1], **Alan I Derman**[1], Nadja Hüttmann[1], Alejandro D. Bonive-Boscan[1], Javier Lopez-Garrido[1]

[1] *Max Planck Institute for Evolutionary Biology, Plön (Germany)*

Sporulation in *Bacillus subtilis* has been studied extensively as a developmental program, but the metabolic processes that are required for spore maturation and how these processes are apportioned within the sporangium between the mother cell and forespore have only begun to be elucidated. Likewise, the metabolic requirements for spore germination and outgrowth are not well understood. Many of the relevant enzymes are essential and strains lacking the corresponding genes are not viable. We have circumvented this problem with a strategy in which we induce proteolytic degradation of a protein of interest in the mother cell or in the developing forespore in order to determine whether the protein is required in one or both of these compartments during spore development. In order to assess the importance of the protein for revival, we induce the protein's degradation in the forespore late in its development so that the protein is absent from the mature spore, and we follow germination and outgrowth of the handicapped spores. We are systematically applying this strategy to major metabolic pathways, including central metabolism and the pathways involved in the synthesis of essential cellular components. Here we detail our experimental strategy and provide an overview of our recent results.

Keywords: *Bacillus subtilis*, sporulation, germination, metabolism

P-31

Optimization of intracellular delivery of proteins in *Bacillus*: delivery of Cas9 ribonucleoprotein complexes for CRISPR/Cas9 genome editing

João Costa-Filho[1], Arthur ZN Fernandes[1], Frederico J Gueiros-Filho[1]

[1] *Universidade de São Paulo, São Paulo (Brazil)*

CRISPR/Cas9 genome editing of bacterial cells usually employs plasmid-based expression of Cas9 and gRNAs. However, a plasmid free system for genome editing would be beneficial specially for hard to transform recalcitrant bacteria. Here, we explored different strategies to introduce proteins and ribonucleoprotein complexes (RNPs) into *B. velezensis* FZB42 cells as a step towards a plasmid-free genome editing procedure. We initially tested the ability of fusogenic liposomes to deliver GFP to FZB42 protoplasts. Treatment with liposomes made with DOPE/DODAC (1/1 mol/mol) permitted the delivery of GFP in 22% of cells. However, protoplast regeneration was very inefficient, therefore, using this strategy for delivery of RNPs was not pursued further. We next tested electroporation using GFP and the restriction enzyme EcoRI as test proteins. The efficiency of EcoRI electroporation to cells was quantified by the drop in viability (CFU/mL). Our results showed a higher percentage of fluorescent cells when the parameters of 25 µF / 200 Ω / 5 kV.cm-1 (41%), 50 µF / 5 kV.cm-1 (41%), and 75 µF / 3 kV.cm-1 (72%) were used. The application of 25 µF / 200 Ω / 6 kV.cm-1 parameters killed 13% of the cells and allowed the introduction of EcoRI in 98% of the cells that survived electroporation. When higher voltage (8 and 10 kV.cm-1) were used, the cells also received EcoRI (93 and 80%), but increased the mortality (92 and 97%). Viability was also used to quantify RNP delivery, which once inside of the cells will cleave the chromosome. When the parameters 25 µF / 200 Ω / 6 kV.cm-1, 25 µF / 8 kV.cm-1, 50 µF / 6 kV.cm-1, and 75 µF / 3 kV.cm-1 were used, RNP delivery efficiency to the cells were 24, 21, 19, and 13%. Considering the characteristics of RNP complexes, we hypothesize that the delivery efficiency was lower when compared with EcoRI because RNPs are larger and target a single site. We are currently investigating ways to optimize our protocol of RNP delivery to make it efficient enough to directly select for genome modifications.

Keywords: CRISPR/Cas9, *Bacillus velezensis* FZB42, electroporation, RNP delivery

*P-32

The effect of subunit composition of RNA polymerase on sporulation in *B. subtilis*

Debora Kálalová[1], Jiří Pospíšil[1], Hana Šanderová[1], Klára Mikesková[1], Libor Krásný[1]

[1] *Institute of Microbiology, Czech Academy of Sciences, Prague (Czech Republic)*

Some bacterial species can survive in hostile and even extremely adverse conditions due to their ability to sporulate. This process depends on precisely temporally and spatially regulated gene expression, which is mediated by RNA polymerase (RNAP), the central enzyme of transcription, and a cascade of alternative sigma factors. Here we describe the effects of small, non-essential subunits of RNAP, delta and omega, on sporulation in the model Gram-positive bacterium *Bacillus subtilis*. Experiments revealed a synergistic interplay between these subunits, the RNAP core, sigma factors, and gene expression and provided insights into their role in sporulation. A model of their involvement in the sporulation cascade will be presented and discussed, including implications for biotechnological applications.

Keywords: Gram-positive bacteria, *Bacillus subtilis*, sporulation, small subunits, RNA polymerase

P-33

HelD regulates RNA polymerase level in *Mycobacterium smegmatis*

Dilip Kumar[1], Viola V. Hausnerová[1], Mahmoud M. Shoman[1], Libor Krásný[1], Jarmila Hnilicová[1]

[1] *Institute of Microbiology, AS CR, 1083 Vídeňská, Prague 4 (Czech Republic)*

HelD binds RNA polymerase (RNAP) in *Bacillus subtilis* and *Mycobacterium smegmatis*, where it dissociates stalled transcription complexes. We show that overexpression of HelD in *M. smegmatis* decreases the total amount of RNAP protein and the level of Ms1 RNA (RNAP-associated small RNA) in stationary phase of growth. The two main subunits of RNAP, beta and beta´, are encoded by rpoB-rpoC genes which are located in one operon. Using chromatin immunoprecipitation coupled with next generation sequencing (ChIP-seq), we found that HelD protein is highly enriched at the rpoB-rpoC promoter. rpoB-rpoC mRNA has an unusually long and structured 5´UTR of unknown function that could be a target of HelD regulation.

In addition, we identified a complete set of genes that are occupied by HelD protein by ChIP-seq. HelD associates mainly with the genes encoding structured RNAs – rRNAs and tRNAs. HelD might help to RNAP to transcribe genes encoding highly structured RNAs, or, help to release stalled RNAP from these genes. We generated ΔhelD strain to compare the RNAP profiles with the wild type strain by ChIP-seq. We assume that the genome-wide occupancy of RNAP will differ in ΔhelD strain compared to the wild type.

Keywords: RNA polymerase, *Mycobacterium smegmatis*, HelD regulation

P-34

Role of the Epipeptide Toxin EPE in Multicellular Differentiation of *Bacillus subtilis*

Nathalie S. Becker[1], Philipp F. Popp[2], Thorsten Mascher[1]

[1] *Institute of Microbiology, TU Dresden, Dresden (Germany)*
[2] *Institute for Biology, Humboldt-Universität, Berlin (Germany)*

Cannibalism is a bacterial programmed cell death-like process that links multicellular differentiation to endospore formation in *Bacillus subtilis*. The differentiation strategy enhances biofilm formation, prolongs or potentially even prevents full commitment to endospore formation under starvation conditions, and protects cells within the biofilm against competing species. Cells suffering starvation form cannibalism toxins and the autoimmunity at low activation levels of the sporulation master regulator, Spo0A, consequently killing surrounding cells, that have not started the sporulation process. The nutrients released by lysed cells can be used by the toxin producers, thereby delaying the full activation of Spo0A. Originally, two toxin loci have been associated with cannibalism: the sporulation killing factor SKF and the sporulation delaying protein SDP. Recently, a third cannibalism toxin has been described: the epipeptide EPE, which is genetically determined by the epeXEPAB locus. The epeX gene encodes the pre-pro-peptide, which is modified by the epimerase EpeE. Subsequently, the transmembrane protease EpeP processes and exports the mature antimicrobial peptide EPE to the environment, while the ABC transporter EpeAB provides intrinsic auto-immunity. EPE causes severe membrane perturbations and induces the cell envelope stress of *B. subtilis*. Mutants of the undomesticated *B. subtilis* strain NCIB3610 lacking epeX or epeAB show severely altered colony morphologies, indicating that EPE is involved in structuring and functionalizing *Bacillus* colonies. Its physiological impact on the spatiotemporal organization of differentiated *B. subtilis* colonies is currently under investigation. Understanding the mechanism of cannibalism and its role as a multicellular survival and/ or differentiation strategy will deepen the insight into the biofilm formation process of *B. subtilis*.

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Keywords: *Bacillus subtilis*, cannibalism, multicellularity, epipeptide

*P-35

Does an updated annotation of the *B. subtilis* genome lead to better sRNA target predictions?

Andreas Lawaetz[1], Emma Denham[1]

[1] *University of Bath, Bath (United Kingdom)*

Bacteria need to respond accurately to environmental changes, whether profound or subtle. Small non-coding RNAs (sRNAs) form important parts of gene regulatory networks and offer means of fine-tuning gene expression. The playbook for studying sRNAs entails detection of transcripts followed by the detection of their RNA targets. In vivo target identification typically relies on pull-down assays using tagged proteins or RNAs. In Gram-negative bacteria such as *E. coli* and *Salmonella* hundreds of sRNAs and their mRNA targets have been reported through immunoprecipitation studies of the global RNA chaperone Hfq. In Gram-positive species, Hfq homologues are dispensable for most sRNA activity and no equivalent global RNA binding protein (RBP) has been identified. Circumventing lab-based methods, computational predictions offer an alternative in silico pipeline. Studies using CopraRNA and IntaRNA report accuracies comparable to experimental detection by protein-based RNA pull-down assays such as RIL-seq, but depends on precise annotations of transcripts for accurate predictions. In the model system *Bacillus subtilis*, several hundred sRNAs have been described, however only a handful have been characterised. A challenge regarding the study of sRNAs in *B. subtilis*, is the accurate annotation to single nucleotide resolution of this genome. Single nucleotide resolution RNA sequence data are publicly available but have so far only been used to analyse annotated coding sequences. We have therefore created a pipeline that converts Rend-seq data to a genome wide annotation file. This resolves transcript boundaries, identifies sRNA isoforms, and detects RNase processing events. Assuming one of the limiting factors in computing accurate target predictions is the precise annotation of 5’ and 3’ ends of sRNAs and mRNAs, our annotation offers a leap forward in describing the function of sRNAs in *B. subtilis*. We expect that a sharpened annotation file updated to the single nucleotide will provide a useful tool for understanding non-coding RNA function within the *B. subtilis* community.

Keywords: *Bacillus subtilis*, sRNA, Rend-seq, in silico target predictions

*P-36

Characterization of MoaB2 from *Mycobacterium smegmatis*: a novel binding factor of mycobacterial SigmaA

Barbora Brezovská[1,2], Michaela Šiková[1], Jarmila Hnilicová[1], Hana Šanderová[1], Subhash Narasimhan[3,4], Lukáš Žídek[3,4], Libor Krásný[1]

[1] *Institute of Microbiology, Czech Academy of Sciences, Prague (Czech Republic)*
[2] *Faculty of Science, Charles University, Prague (Czech Republic)*
[3] *Central European Institute of Technology (CEITEC) (Czech Republic)*
[4] *Faculty of Science, Masaryk University (Czech Republic)*

Mycobacterium is a genus of medicinally important bacteria with a number of human pathogens. Understanding the mycobacterial gene expression is necessary to develop new antibacterial approaches to fight diseases such as tuberculosis and leprosy. The central enzyme of bacterial gene expression is RNA polymerase (RNAP) whose core is catalytically active but to initiate transcription it needs a sigma factor. Factor A is the primary sigma factor (SigA) responsible for transcription of housekeeping genes but it is not known how exactly SigA levels are regulated in the mycobacterial cell. In stationary phase of growth, we identified a new protein associated with SigA. This protein, MoaB2 (17.9 kDa), is predicted to be involved in the biosynthesis of molybdopterin, an essential cofactor of a diverse group of redox enzymes. However, by several approaches, we demonstrated that MoaB2 binds to SigA, and pilot experiments suggest that MoaB2 affects the biological half-life of SigA, thereby regulating its intracellular level. Finally, we solved the 3D structure of *Mycobacterium smegmatis* MoaB2 by crystallography, providing a basis for its further studies. Taken together, MoaB2 represents a new binding partner of SigA in mycobacteria, and extends thus our knowledge of the architecture of the transcriptional machinery.

This work is supported by grant No. 19/12956S from the Czech Science Foundation

Keywords: MoaB2, SigA, RNA polymerase, Mycobacteria

*P-37

Analysis of the global control by the two 6S RNAs in an undomesticated *Bacillus subtilis* wild-type strain

M. Amri C. Schlueter[1], Marietta Thuering[1], Sweetha Ganapathy[1], Roland K. Hartmann[1]

[1] *Philipps-University, Marburg (Germany)*

Bacterial 6S RNA directly interferes with gene expression by binding to the active site of RNA polymerase holoenzymes. *Bacillus subtilis* encodes two 6S RNA paralogs, 6S-1 and 6S-2 RNA, that differ in their expression profiles.

As the wild-type (WT) ancestor strain NCIB 3610 of the well-studied laboratory strains (B. sub. 168 & PY79) retains functions that are impaired or lost in the derived laboratory strains, we chose this strain to construct 6S-1/2 RNA single & double knockouts. We revealed strong phenotypes of the Δ6S-2 strain (derepressed biofilm formation, retarded swarming activity & earlier spore formation) and the Δ6S-1+2 strain (prolonged lag phase of growth & reduced growth rate in rich media & under oxidative, high salt & alkaline stress conditions; also reduced spore formation).

To investigate the regulatory impact of the two 6S RNAs on a global level, we are performing transcriptomic and proteomic analyses of the single and double knockouts in rich medium as well as in specialized media that enhance certain genetic programs like sporulation.

Transcriptome analysis under standard LB conditions clearly conformed with the observed phenotype, as major biofilm genes are significantly upregulated in the 6S-2 RNA knockout strain (tapA operon, eps operon), while motility genes (σD-dependent) are downregulated. Results for the 6S double knockout strain are also in line with the phenotypic studies (sporulation genes strongly downregulated).

As *B. subtilis* NCIB 3610 is able to induce the formation of subpopulations under certain conditions, the impact of 6S RNAs on the distribution of such heterogeneous cell types is explored by individual fluorescence-reporter gene assays (measured as single-cell distribution by flow cytometry) to further deepen our understanding of the regulatory influence 6S RNAs exert on gene expression.

Keywords: *Bacillus subtilis* NCIB 3610, 6S RNA, biofilm, motility, sporulation, transcriptomics, proteomics, subpopulation

*P-38

Genomic modification of non-competent *Bacillus* sp. by using a novel all-in-one plasmid

Sarah Miercke[1], Tomislav Vološen[1], Karen Stetter[1], Diana Wolf[1]

[1] *TU Dresden, Faculty Biology, General Microbiology (Germany)*

Natural competence describes the ability of bacteria to take up foreign DNA and its use as a nutrient or to compete with other microorganisms, e. g. via antibiotic resistance. Additionally, natural competent microorganisms are of considerable advantage for the use as model organisms under laboratory conditions in terms of simple insertion of genome modifications. *B. subtilis* W168 is such a model strain, which belongs to the genus *Bacillus*. Other strongly related *Bacillus* species, e. g. *B. subtilis* subsp. spizizenii ATCC 6633, *B. thuringiensis* 407, *B. cereus* ATCC 14579 or *B. licheniformis* ATCC 10716 are not naturally competent. DNA implications in these strains can be done so far by conjugation only.

In the last few decades, methods for genetic engineering of non-transformable *Bacillus* sp. strains have been improved and became of interest for biotechnological applications such as protein production and biosensor molecule detection.

In this work, we demonstrate how a novel conjugation plasmid helps to introduce genetic rearrangements in non-competent *Bacillus* strains. Furthermore, the plasmid contains a tunable origin of replication, and thereby the plasmid copy number can be controlled. In addition, by cultivation under non-permissive conditions and plating the cells on selective media, the genomic integration of the plasmid can be forced successfully and leads to, e. g. markerless deletion of a gene of interest.

Altogether, we established an efficient protocol for genomic modifications in naturally non-competent and so far poorly transformable *Bacillus* sp. that can be further applied to a broad range of biotechnologically relevant strains (e. g. *B. pumilus* and *B. velezensis*) as well as to strains of special interest like B. anthracis.

Keywords: *Bacillus* sp., genomic modification, all-in-one plasmid, tunable origin of replication, markerless integration

*P-39

HelD mediated rifampicin resistance in *Bacillus subtilis*

Tamara Balgová[1], Petra Sudzinová[1], Dragana Vítovská [1], Libor Krásný [1]

[1] *Institute of Microbiology, Czech Academy of Sciences, Prague (Czech Republic)*

Nowadays, antibiotic resistance is a serious global problem. Rifampicin is a clinically important antibiotic that acts against bacterial infections. Rifampicin binds to RNA polymerase (RNAP) and stops the synthesis of RNA in an early phase, at the stage of only several nucleotides transcribed. Recent studies in actinobacteria reported that HelD, an interaction partner of RNAP, increases its resistance to rifampicin by removing rifampicin-stalled RNAP from DNA and causing dissociation of rifampicin from RNAP. Intriguingly, one of these studies reported that HelD from *Bacillus subtilis* does not provide this rifampicin-protective effect. Here, we re-investigated the possibility of whether *B. subtilis* HelD plays a role in rifampicin resistance. First, contrary to the published results, we showed that the presence of the helD gene increases the minimal inhibitory concentration to rifampicin. Second, we demonstrated that rifampicin induces expression of the helD gene. Next, we identified a region in the 5' untranslated region of the helD gene, which is responsible for this induction, and showed that the regulation is at the transcriptional and not translational level. Results of experiments addressing the mechanism of regulation of helD gene expression and resistance of *B. subtilis* to rifampicin will be presented and discussed.

Keywords: HelD, rifampicin, RNA polymerase, *B. subtilis*

P-40

Functional profiling of CHAP domain-containing peptidoglycan hydrolases of *Staphylococcus aureus* USA300 uncovers potential targets for antistaphylococcal therapies

Min Wang[1], **Xiaofang Li**[1], Girbe Buist[1], Jan Maarten van Dijl[1]

[1] *University of Groningen, UMCG, Groningen. (Netherlands)*

Staphylococcus aureus is a Gram-positive bacterium responsible for many hospital- and community- acquired infections worldwide. *Staphylococcus aureus* employs a thick cell wall for protection against physical and chemical insults. This cell wall plays important roles in maintaining barrier integrity and in bacterial growth and division. The main cell wall component is peptidoglycan. Accordingly, the bacteria produce so-called peptidoglycan hydrolases (PGHs) that cleave glycan strands to facilitate growth, cell wall remodeling, separation of divided cells and release of exported proteins into the extracellular milieu. A special class of PGHs contains so-called 'cysteine, histidine-dependent amidohydrolase/peptidase' (CHAP) domains.

In the present study, we profiled the roles of 11 CHAP PGHs encoded by the core genome of *S. aureus* USA300 LAC. Mutant strains lacking individual CHAP PGHs were analyzed for growth, cell morphology, autolysis, and invasion and replication inside human lung epithelial cells. The results showed that several investigated CHAP PGHs contribute to different extents to extracellular and intracellular growth and replication of *S. aureus*, septation of dividing cells, daughter cell separation and autolysis. In particular, the CHAP PGHs Sle1 and SAUSA_2253 control intracellular staphylococcal replication and the resistance to β-lactam antibiotics including oxacillin. This makes the *S. aureus* PGHs in general, and the Sle1 and SAUSA300_2253 proteins in particular, attractive targets for future prophylactic or therapeutic anti-staphylococcal interventions.

Next, we will investigate the cell wall composition and structure of the USA300 wild-type strain and derivative CHAP PGHs mutant strains. We will also investigate the host-pathogen interactions of CHAP PGHs mutant strains in lung epithelial cells in our further research.

Keywords: *Staphylococcus aureus* USA300, cell wall, CHAP-domain containing peptidoglycan hydrolase

*P-41

Metabolic rewiring compensates for the loss of glutamate biosynthesis in *Bacillus subtilis*

Saba Mardoukhi[1], Fabian M Commichau[1]

[1] *Universität Hohenheim, Stuttgart (Germany)*

Glutamate, the major cellular amino group donor, is either taken up from the environment or newly synthesized. The Gram-positive model bacterium *Bacillus subtilis* exclusively relies on the ATP-dependent glutamine synthetase (GS) and the glutamate synthase (GOGAT) for de novo synthesis of glutamate. The GDHs of *B. subtilis* are strictly devoted to glutamate degradation (Gunka and Commichau, 2012). Previously, it has been shown that the growth of a *B. subtilis* mutant lacking the GOGAT encoding gltAB genes depends on extracellular glutamate. Serendipitously, we found that the gltAB mutant rapidly acquires suppressor mutations in medium containing ammonium as the sole source of nitrogen. Genome sequencing analyses revealed that two loss-of-function mutations enable the gltAB suppressor mutants to synthesise glutamate via a previously unknown route. By constructing a series of mutants with disruptions in genes encoding TCA cycle enzymes in combination with metabolome analyses, we could confirm that the enzymes of aspartate metabolism, namely aspartase and aspartate transaminase, functionally replace the GOGAT. Furthermore, we found that the overexpression of the aspartase compensates for the loss of the aspartate transaminase. A suppressor analysis with an *Escherichia coli* mutant lacking the GOGAT and the GDH revealed that the aspartase/aspartate transaminase-dependent glutamate biosynthetic pathway may also evolve in phylogenetically distantly related bacteria.

Keywords: Glutamate, *Bacillus subtilis*, aspartate, aspartase, GOGAT

*P-42

The role of c-di-AMP in osmoadaptation and fosfomycin uptake in *Listeria monocytogenes*

Inge Schwedt[1], Mengyi Wang[1], Fabian M Commichau[1]

[1] *Universität Hohenheim, 70599 Stuttgart (Germany)*

c-di-AMP controls osmolyte homeostasis in several bacteria, including *Listeria monocytogenes*. c-di-AMP also indirectly stimulates the DNA-binding activity of CodY, which negatively controls the expression of genes required for adaptation to nutrient limitation. In *L. monocytogenes*, c-di-AMP is synthesized by the diadenylate cyclase CdaA and degraded by the phosphodiesterases GdpP and PgpH. c-di-AMP is essential for growth because it prevents uncontrolled uptake of osmolytes. Elevated cellular c-di-AMP concentrations are also often associated with increased resistance of bacteria to cell wall-targeting antibiotics. To get further insights into the cellular role of c-di-AMP in *L. monocytogenes*, we studied the phenotypes of cdaA and gdpP pgpH mutants and characterized suppressor mutants derived from them. We identified cdaA suppressor mutants that can be assigned to two different classes. In the first class of mutants carrying mutations in the relA (p)ppGpp synthase gene, the CodY regulon was affected. These mutants turned out to be sensitive to fosfomycin, which inhibits peptidoglycan biosynthesis. In the second class of mutants, the opp oligopeptide transporter genes were inactivated, resulting in a fosfomycin-resistant phenotype. Thus, the suppressor analysis identified a major route for fosfomycin uptake. We also observed that casamino acids and isoleucine are toxic for the cdaA mutant. A subsequent suppressor screen revealed that isoleucine toxicity is readily relieved by mutations in the codY gene. The encoded CodY variants are less responsive to isoleucine and have reduced DNA binding activity. Thus, a c-di-AMP-free strain shows increased uptake of isoleucine, which in turns leads to CodY hyperactivity. The characterization of the gdpP pgpH mutant revealed that the bacteria are osmosensitive, a phenotype that is invariably suppressed by the acquisition of loss-of-function mutations in the cdaA diadenylate cyclase gene. In the future, we aim to elucidate how the diadenylate cyclases sense the environmental osmolarity.

Keywords: second messenger, c-di-AMP, osmoregulation

P-43

Characterization of the conjugation protein ConB of *Bacillus subtilis*

Adelyn E Ragucci[1], Sirui Chen[1], Michelle López[1], Kathy Nguyen[1], Haley B Dame[1], Sabrina K Phanor[1], Omar R Pinkhasov[1], **Melanie B Berkmen**[1]

[1] *Suffolk University, Boston, MA (United States of America)*

Conjugation, or mating, is a major contributor to horizontal gene transfer and the spread of antibiotic resistance. During conjugation, DNA is transferred from a donor to a recipient cell through a DNA translocation channel classified as a Type IV Secretion System (T4SS). Our research is focused on characterizing the T4SS of ICEBs1, a conjugative DNA element found in *Bacillus subtilis*. ConB is a VirB8-like bitopic membrane protein of the ICEBs1 T4SS. Previously, we showed that ConB is necessary for conjugation as well as for localization of another T4SS protein, ConE, to the membrane. ConB has four predicted domains: an N-terminal cytoplasmic tail, a single-pass transmembrane segment, followed by two extracellular NTF2 domains. Here, we confirm that ConB interacts with itself and ConE, but we find no evidence of interaction with other T4SS proteins. We discovered that ConB fused to mCherry localizes to the membrane, predominantly at the cell poles, similar to its interacting partner ConE. While ConE requires ConB for localization, ConB is able to localize independently of other ICEBs1 T4SS proteins. We next delineated which domains of ConB are required for mating, oligomerization, interaction with ConE, and ConE localization. We found that all four of ConB's domains are required for mating, but that only its transmembrane domain is required for oligomerization. ConB's cytoplasmic N-terminus, transmembrane domain, and central NTF2 domain are required for interaction with and localization of ConE. Our research sheds new light on a key protein mediating horizontal gene transfer.

Keywords: conjugation, mating, *Bacillus subtilis*, membrane, localization, secretion, horizontal gene transfer

P-44

Pulcherrimin is an iron reserve and inhibitor of ROS production during an interspecies interaction

Vincent Charron-Lamoureux[1], Maude Pomerleau[1], Lounes Haroune[1], Jean-Philippe Bellenger[1], **Pascale B Beauregard**[1]

[1] *Université de Sherbrooke, Sherrbrooke (Canada)*

Iron (Fe) is an essential micronutrient mostly insoluble in the environment that can cause deleterious oxidative stress via the Fenton reaction. For Fe homeostasis, *Bacillus* and *Pseudomonas* use a variety of siderophores that bind and solubilize Fe3+. *B. subtilis* also secretes pulcherriminic acid (PA), which sequester Fe by forming the insoluble pulcherrimin complex.

To determine the impact of pulcherrimin on interspecies Fe competition, we grew *B. subtilis* and *P. protegens* PF-5 as side-by-side colonies on Murashige-Skoog (MS) agar. In contrast to what was observed on MSgg, no red pulcherrimin halo formed around *B. subtilis* grown alone. However, the presence of PF-5 led to pulcherrimin accumulation around *B. subtilis*. Using a fluorescent bioreporter (PyvmC-YFP), we determined that PF-5 volatiles and 2,4-DAPG both stimulated PA production. ICP-MS analysis revealed that pulcherrimin did not interfere with Fe accumulation because *B. subtilis* biofilms formed by WT or PA-deficient mutant accumulated more Fe when in presence of PF-5 than alone. This suggests that pulcherrimin can act as a Fe source for *B. subtilis*, which was confirmed by growth of WT cells in a medium with purified pulcherrimin as sole iron source. *B. subtilis* siderophore bacillibactin is key in this process, since a ΔdhbF mutant showed a severe growth defect in the same medium. Also, bacillibactin can transcomplex Fe from pure pulcherrimin. Importantly, PA-deficient mutant showed an important biofilm defect when facing PF-5, suggesting a role for PA secretion in this interaction. Since oxidative stress was shown to reduce biofilm formation by *B. subtilis*, we examined if pulcherrimin could act as a Fenton reaction inhibitor. Quantification of reactive oxidative species (ROS) revealed that pulcherrimin inhibited accumulation of ROS triggered by PF-5, thereby preventing inhibition of biofilm formation. This study reveals that pulcherrimin is an insoluble Fe immobilizer that can act as a local source of Fe and a naturally occurring Fenton reaction quencher, limiting oxidative stress.

Keywords: biofilm, pulcherrimin, interspecies interaction, iron, reactive oxygen species

*P-45

THE LIPOIC ACID SALVAGE PATHWAY IN *STAPHYLOCOCCUS AUREUS* IS A PROMISING TARGET FOR ANTIBACTERIAL DRUGS

Albertina Scattolini[1], Konstantinos Grammatoglou[2], Björn Windshügel[3], Aigars Jirgensons[2], María C Mansilla[1]

[1] *Instituto de Biología Molecular y Celular de Rosario (Argentina)*
[2] *Latvian Institute of Organic Synthesis (Latvia)*
[3] *Fraunhofer Institute for Molecular Biology and Applied Ecolo (Germany)*

Infections with methicillin resistant *Staphylococcus aureus* are a global problem. The increasing emergence of multidrug resistant strains urgently requires novel therapeutic approaches in order to keep the drug discovery pipeline filled. Lipoic acid (LA) is a universally conserved sulfur-containing cofactor involved in one-carbon and oxidative metabolism. In *Escherichia coli* LA can be acquired by a salvage pathway, in which it is attached to lipoyl domains of GcvH, the H subunit of the glycine cleavage system, or the E2 subunits of dehydrogenase complexes by a lipoate ligase, LplA. Lipoate can also be de novo synthesized by a pathway requiring an octanoyltransferase and a lipoate synthase. We have characterized a more complex pathway in the model Gram-positive bacterium *Bacillus subtilis*, referred to as "lipoyl-relay", that requires two additional proteins: GcvH and LipL, an amidotransferase. *S. aureus* contains two additional enzymes with presumptive roles in LA salvage during infection, LplA2 and GcvH-L. Due to its essentiality to cell viability and virulence, interfering with LA synthesis represents a promising approach for treating *S. aureus* infections. In this work, we performed a phenotypic screen of different molecules that were identified by a virtual screen against several *S. aureus* enzymes involved in LA salvage. One of the compounds caused a marked inhibition of the growth of the WT strain. This effect was lower in ΔlplA1 or ΔlplA2 single mutants, however the double mutant ΔlplA1 ΔlplA2 was able to grow in presence of the compound. Using protein extracts of different mutants of *S. aureus*, deficient in LA synthesis and uptake, we determined that lpl-004 is bound to E2s and recognized by anti-LA antibodies. These results indicate that, by the sequential action of lipoate ligases and amidotransferase, lpl-004 is accepted as a substrate and transferred to E2s, eventually impairing dehydrogenase activity. This compound would be useful for further drug development against this pathogenic bacterium.

Keywords: Lipoic acid, *Staphylococcus aureus*

*P-46

Systematic Mutational Analysis of the Leader and N- and C-terminal Residues of the Core Peptide of a Novel *Bacillus*-derived Circular Bacteriocin

Fangfang Liu[1], Auke J. van Heel[1], Oscar P. Kuipers[1]

[1] *University of Groningen, Groningen (Netherlands)*

Circular bacteriocins, also known as bacterial head-to-tail cyclized peptides, are a subgroup of ribosomally synthesized and post-translationally modified peptides (RiPPs). Compared with their conventional linear counterparts, circular bacteriocins are highly stable over a broad temperature and pH range, and circularization also decreases proteolytic degradation by exopeptidases. These features render them great potential to withstand strident conditions when used as scaffold candidates in food- and pharmaceutical applications. However, much is still unknown about the mechanism of biosynthesis of circular bacteriocins, for example, the requirements for leader removal, the circularization mechanism, as well as the biosynthetic tolerance for the mutant processing. In this study, we investigated the biosynthesis of a novel *Bacillus* circular bacteriocin using mini*Bacillus* PG10 as the expression host, with the main focuses on the mutational analysis of the 32-aa leader peptide and the terminal residues of the core peptide. Our results suggest that the leader peptide still retains its biosynthetic function even when being trimmed to one amino acid residue. Conversely, the N- and C-terminal residues of the core peptide are well conserved and mutations at these positions lead to a dramatic decrease in antimicrobial production. Overall, these studies provide excellent insights into the biosynthetic role of the leader peptide and the importance of terminal residues on the maturation of circular bacteriocins, contributing to a better understanding of the biosynthesis of circular bacteriocins.

Keywords: *Bacillus*, Circular Bacteriocin, Mutational Analysis, Biosynthesis, Antimicrobial activity

P-47

The positioning of sporulation septum in *Bacillus subtilis*

Katarína Muchová[1], Evelína Kalocsaiová[1], Jiří Pospíšil[2], Zuzana Chromiková[1], Silvia Žarnovičanová[1], Libor Krásný[2], Imrich Barák[1]

[1] *Institute of Molecular Biology, SAS (Slovakia)*
[2] *Institute of Microbiology, CAS (Czech Republic)*

Bacillus subtilis is a rod-shaped bacterium that divides precisely at mid-cell during vegetative growth. In conditions that are not rich enough to support vegetative growth, the most important *B. subtilis* survival strategy is sporulation. During this process, *B. subtilis* switches its site of division from the middle of the cell to a position close to the cell pole. After the axial filament has formed, the midcell Z-ring is converted into a spiral-like structure that travels towards each cell pole [1]. The spiral then splits into two polar spirals and redeployment of FtsZ results in formation of two separate Z-rings near the two poles. This redeployment of the Z-ring requires SpoIIE and increased expression of ftsAZ from a sporulation-specific promoter. Finally, one of two polar Z-rings becomes the site of asymmetric division. The asymmetric septum forms around 1/6 of a cell length from one of the cell poles with high precision [2]. Several proteins have been implicated to play an important role in correct sporulation septum positioning such as SpoIIE, MinCD and RefZ. Here using bacterial two-hybrid system and pull down assay we identified RefZ putative interaction partners among proteins present at this stage of sporulation. In addition, we follow localization and co-localization of these proteins by fluorescence microscopy and SIM.

Keywords: sporulation, asymmetric division, RefZ

P-48

Implantation of *Bacillus pseudomycoides* chromate efflux pump increases chromate tolerance in *Bacillus subtilis*

Zuzana Chromiková[1], Romana Kalianková-Chovanová[1], Dragana Tamindźija[2], Barbora Bártová[3], Dragan Radnović[2], Rizlan Bernier-Latmani[3], Imrich Barák[1]

[1] *Institute of Molecular Biology, SAS, Bratislava (Slovakia)*
[2] *University of Novi Sad, Faculty of Sciences, Novi Sad (Serbia)*
[3] *Ecole Polytechnique Fédérale de Lausanne, Lausanne (Switzerland)*

Toxic Cr(VI) is soluble and highly mobile in the environment. Due to structural similarity to sulfate, Cr(VI) is actively transported into the cell. During intracellular reduction, the toxicity of Cr(VI) is unleashed by induction of massive oxidation stress. Although all microorganisms transform Cr(VI) to nontoxic Cr(III), this process is for most of them lethal, especially in the nutritionally poor environments. However, some bacterial species are able to sustain both the vegetative growth and the reduction of Cr(VI) even at harsh conditions. The mechanisms governing their survival include the chromate specific strategies such as the activation of chromate efflux pumps. Since Cr-resistance determinants are often difficult to study in the original species, introduction of these genes into bacterial model organisms enables resolving their mode of action. In our study, we introduced a gene encoding chromate efflux pump, chrA, from highly Cr resistant environmental strain of *B. pseudomycoides* into moderately Cr tolerant strain of *B. subtilis* laboratory strain PY79. We observed the increase of ChrA expression with ascending concentration of Cr(VI) as well as the beneficial effect it had on survival of *B. subtilis* in the presence of chromate.

This work was supported by VEGA – Grant No. 2/0001/21 from the Slovak Academy of Sciences and a Grant from the Slovak Research and Development Agency under contract APVV-18-0104.

Keywords: chromate tolerance, chromate efflux pump, *B. subtilis*, B. pseudomycoides

*P-49

Perception and detoxification of the polyketide tartrolon B by *Listeria monocytogenes*.

Tim Engelgeh[1], Jennifer Herrmann[2,3], Rolf Müller[2,3], Sven Halbedel[1]

[1] *Robert Koch Insitute, Wernigerode (Germany)*
[2] *Helmholtz Institute for Pharmaceutical Research, Saarbrücken (Germany)*
[3] *German Centre for Infection Research, Braunschweig (Germany)*

The gram-positive bacterium *Listeria monocytogenes* is a member of the *Bacilli*. It occurs ubiquitously in the environment and can cause severe invasive diseases in humans upon ingestion. Like most bacteria, *L. monocytogenes* can deal with harmful substances, secreted by natural competitors such as other bacteria or plants by induction of gene expression of specific ATP binding cassette (ABC) transporters. Transporters for such natural compounds are usually highly specific. We recently identified the lieAB operon as essential for resistance against aurantimycin, a compound secreted by Streptomyces aurantiacus to fight competitors. After screening of various promoters of genes encoding potential multiple drug resistance transporters (MDR transporters) fused to lacZ, we identified a promoter (PtimABR) that is being induced upon contact with tartrolon B, a secondary metabolite produced by myxobacterium Sorangium cellulosum. We here show, that a mutant lacking the corresponding transporter genes timAB shows a 24-fold lower minimal inhibitory concentration (MIC) to tartrolon B than the wild type. The MIC of tartrolon B has already been described for *Bacillus subtilis* (3). In good agreement with our observations, we confirmed that expression of timAB is sufficient to confer tartrolon B resistance to *B. subtilis*. Measurement of β-galactosidase activity of a strain carrying PtimABR-lacZ in the absence of the repressor protein led us to observe a stronger promoter activity than in the wild type, being a good indication that the repressor binds to its own promoter. This binding was also confirmed by electrophoretic mobility shift assay (EMSA) where a TimR-PtimABR interaction was only observed when tartrolon B was not present. Our results provide insights into the function of a not yet characterized ABC transporter TimAB and its repressor TimR in *L. monocytogenes* and helps to further understand the survival mechanisms of this important human pathogen to survive within its environmental reservoir.

Keywords: ABC transporter, detoxification, macrolide, polyketide

*P-50

Metabolic engineering of *Lactiplantibacillus plantarum* as an aroma-adding microorganism in beverage fermentation

Xiangang Li[1], Pascal Schönberg[2], Tabea Wucherpfennig[3], Kristina Fleischer[3], Thomas Henle[3], Thorsten Mascher[1]

[1] *Institute of microbiology, TU Dresden, Dresden (Germany)*
[2] *Medical faculty, TU Dresden, Dresden (Germany)*
[3] *Department of food chemistry, TU Dresden, Dresden (Germany)*

Lactiplantibacillus plantarum (formerly *Lactobacillus plantarum*) is a Gram-positive lactic acid bacterium (LAB) that produces lactic acid as the main product of fermentation. Due to their generally recognized as safe (GRAS) status, LAB are widely used in food industry and production of pharmaceuticals. In beverage production, *L. plantarum* is used for sour beer fermentation. Previous studies found that the major compounds that contribute to the flavor of beer are two monoterpenoids linalool and geraniol present in hops. These compounds can also be found in lavender, rose and other aromatic plants, but both the raw material and the extraction process are costly. In this study, we aim to genetically engineer *L. plantarum* WCFS1 as an efficient aroma producer. First, we developed a modular and standardized Golden Gate Assembly-based toolbox for the de novo assembly of shuttle vectors from *Escherichia coli* to LAB. The toolbox consists of a collection of the most relevant genetic parts for LAB, including different origins of replication, resistance cassettes for selection and a range of promoters with different strengths. Each genetic part of a plasmid can be exchanged conveniently due to their standardized fusion sites. Next, we introduced four plant-derived linalool and geraniol synthase genes that were expressed under the control of inducible promoters. The production of these two volatiles was verified by headspace solid-phase microextraction gas chromatography mass spectrometry (HS-SPME-GC-MS). To boost the supply of monoterpenoid precursors, genes of two rate-limiting enzymes from the MVA pathway of *Saccharomyces cerevisiae* were integrated into the toolbox and co-expressed with linalool/geraniol synthases driven by constitutive promoters. In summary, we have developed a highly efficient and flexible cloning toolbox for engineering LAB as promising probiotics and biofactories. By fine-tuning their metabolism such as the MVA pathway in *L. plantarum* WCFS1, we hope to engineer LAB as flavor-adding microorganisms in fermented beverages on a laboratory and pilot scale.

Keywords: lactic acid bacteria, fermentation, synthetic biology, terpenoid

P-51

Introducing non-canonical Amino Acids in Lantibiotics and Application of Click Chemistry Using *Lactococcus lactis* as Expression Host

Longcheng Guo[1], Jaap Broos[1], Oscar P Kuipers[1]

[1] *Molecular Genetics Dept, University of Groningen, Groningen (Netherlands)*

Lantibiotics are antimicrobial peptides that contain unusual post-translationally modified amino acid residues. Nisin is produced by *Lactococcus lactis* and is the first discovered and the best studied lanthipeptide to date. The noncanonical amino acids (ncAAs) in lantibiotics play an important role in their structural stability and biological activity. Other ncAAs offer a further highly diverse pool of building blocks that can introduce unique physicochemical properties. Among ncAAs, the methionine analogues are of particular interest. For instance, azidohomoalanine possesses unique reactive groups which can serve as chemical handles. By incorporating ncAAs with functional group, we can dramatically expand the functional space of lantibiotic structures and enable the design of novel lantibiotics.

Various expression hosts have been developed for the incorporation of ncAAs and although the incorporations were successful, the yields were not satisfying, being about 0.1-0.2 mg per liter purification. Recently, a new incorporation system was developed by us. We tested the new system with different methionine analogues (azidohomoalanine, norleucine, ethionine) incorporation. Tricine-PAGE and mass results showed all the analogues can be incorporated at different positions (1, 17, 21, and 35) into nisin and peaks of peptides containing methionine were undetectable, which means the incorporation efficiency was above 99.5%, making it easier for purification. Agar well diffusion assays showed that nisin with the ethionine incorporation at the first position looks an interesting one with great activity improvement. The diameter increased from 17.5 mm to 20.0 mm with the first position methionine incorporation as control. Minimal inhibitory concentration suggested with the azidohomoalanine incorporation, nisin derivatives retained higher activity. At the same time, the nisin variants yield improved from 10 to 15 times compared with the previous one, making it easier to conjugate with fluorophores, glycans, lipids, peptide moieties through click chemistry.

Keywords: *Lactococcus lactis*, nisin, noncanonical amino acids, methionine analogues, click chemistry

P-52

Role of NADH peroxidase (Npr) in extracellular electron transfer in *Enterococcus faecalis*

Lars Hederstedt[1]

[1] *The Microbiology Group, Dept of Biology, Lund University (Sweden)*

Enterococcus faecalis cells can transfer electrons generated in catabolism directly, or indirectly via a mediator, to an electrode (1) or to Fe(3+) ions in the growth medium (2). Electron transfer to Fe(3+) depends on the NADH:menaquinone oxidoreductase (Ndh3), the EetA protein, and menaquinone (2). Fe(3+) reductase activity is attenuated when the cytochrome bd oxygen reductase is active, i.e., when cells are grown aerobically in the presence of heme (2).

In the present work a library of transposon-insertion mutants were screened for increased Fe(3+) reductase activity when grown in the presence of heme. Two npr mutants were found. As a spin-off result, an eetB mutant was found defective in electron transfer to Fe(3+). The findings can be used to maximize extracellular electron transfer by *E. faecalis* cells. For example, this is important for optimization of electric current production in microbial fuel cells.

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Keywords: *E. faecalis* , extracellular electron transfer, NADH peroxidase, eetB

*P-53

Discovery and characterization of Balucin, a novel lanthipeptide co-produced with Subtilin in *Bacillus subtilis* EH11

Yuxin Fu[1], Lu Zhou[1], Oscar P Kuipers[1]

[1] *University of Groningen, Groningen (Netherlands)*

Bacillus subtilis EH11, an antimicrobial substance producing strain was isolated during the screening of potential plant growth-promoting rhizobateria from tomato rhizosphere soil. MALDI mass spectrometry analysis of HPLC purified fractions revealed *B. subtilis* EH11 yielded two antimicrobial peptides that differed in activity, with masses as 3724 Da and 3349 Da, respectively. In silico analysis for secondary metabolites unraveled the presence of two class I lanthipeptides biosynthetic gene clusters (BGCs), involved in the biosythesis of a subtilin variant with a mass of 3349 Da and a novel lanthipeptide with a mass of 3724 Da, that we named Balucin, which was highly acive against Gram-positive bacteria. The unusual structural features of Balucin were identified by tandom mass spectrometry (ESI-MS/MS) , showing the negative charged aspartic acid flanking the C-terminal of the lanthionine rings, which has been proved not acceptable to the lanthionine biosynthetic enzymes of the well-studied class I lanthipeptide Nisin. To get more insight into the biosynthetic potential of balucin in lanthipeptide engineering, the heterologous expression of balucin in a standardized expression system was performed, and the proteolytic removal of the leader peptide was performed in vitro. In such a system, the balucin biosynthetic enzymes may be employed to create novel peptides which not acceptable to the other lanthionine biosynthetic enzymes. Our study explored the novel lanthipeptide identified from strain B subtilis EH11and its biosynthetic potential in engineering, highlighting the great biosynthetic potential of genus *Bacillus* for production of novel lantibiotics.

Keywords: *Bacillus subtilis*, antimicrobial activity, novel lanthipeptides, biosynthetic potential

P-54

Construction of plasmid- and genome-based inducible CRISPRi platforms and their utilization in studying the role of Rnase YbeY in *Lactococcus lactis*

Meishan Liu[1]

[1] *University of Groningen, Groningen (Netherlands)*

A plasmid- and a genome-based inducible CRISPRi system were developed for *Lactococcus lactis*. The nisin-inducible promoter PnisA was used to drive the expression of dCas9 from Streptococcus pyogenes, while the strong constitutive *L. lactis* promoter Pusp45 was used to express single-guide RNA (sgRNA). In addition, a superfolder fluorescence protein (sfGFP) was fused to the C-terminal of dCas9 to visualize the induction level of the fusion protein. Both systems successfully inhibited the expression of the major autolysin AcmA of *L. lactis* and the known morphological change (formation of long chains of unseparated cells) was observed. However, the plasmid-based system was leaky and mutation phenotypes were already observed without adding the inducer nisin. A functional CRISPRi system was obtained by lowering the copy number of the dcas9 gene variants by inserting them in the *L. lactis* chromosome. The knock-down level can be relatively regulated by controlling the nisin induction time. The chromosomal CRISPRi system was utilized to knock down the essential gene ybeY in *L. lactis*. Knock-down of YbeY caused severe growth defects. The knock-down caused growth defection can be recovered by inserting a YbeY copy with 2 amino acid synonymous mutations at the sgRNA target site. We subsequently studied the effect of YbeY absence in *L. lactis* by whole-genome RNA-sequencing.

Keywords: CRISPRi, YbeY, *Lactococcus lactis*

P-55

Proteomic detection of protein-protein interaction interfaces in *Bacillus subtilis*

Christian Forbrig[1], Francis O'Reilly[1], Andrea Graziadei[1], Rica Bremenkamp[2], Kristine Charles[1], Swantje Lenz[1], Lutz Fischer[1], Jörg Stülke[2], Juri Rappsilber[1,3]

[1] *Technische Universität Berlin, Chair of Bioanalytics (Germany)*
[2] *Georg-August-University Göttingen (Germany)*
[3] *University of Edinburgh, Wellcome Centre for Cell Biology (United Kingdom)*

Knowing protein-protein interactions (PPIs) is crucial in understanding cellular processes. We present a detection approach for PPI interfaces using multiple techniques, each of them alone having their limitations, but together confidently identify complexes.

B. subtilis (strain 168) was crosslinked using disuccinimidyl sulfoxide (DSSO) while growing in log phase, thereby stabilising protein complexes in their native environment. The soluble complexes were then extracted and analysed after tryptic digestion via high resolution mass spectrometry (MS) to yield a network of 311 protein-protein interactions based on detected crosslinks. An additional co-fractionation MS analysis of the soluble complexes resulted in 637 candidate PPIs derived from correlation analysis of elution profiles in size exclusion chromatography with or without in-cell crosslinking. We analysed all experimental PPIs together with PPIs accessible on the subtiwiki homepage via Alphafold multimer to generate interface models that were in full agreement with our crosslink-based restraints. Our final list of PPI interfaces contains a high proportion of novel interactions and also interactions involving uncharacterised proteins, some of them verified via subsequent bacterial-2-hybrid experiments in this study.

Keywords: protein-protein interaction (ppi) prediction, mass spectrometry, co-fractionation, alphafold multimer

*P-56

Listeria monocytogenes gene essentiality under standard laboratory growth conditions and during macrophage infection

Martin Fischer[1], Tim Engelgeh[1], **Patricia Rothe**[1], Stephan Fuchs[2], Andrea Thürmer[2], Sven Halbedel[1]

[1] *Robert Koch Institute, Wernigerode (Germany)*
[2] *Robert Koch Institute, Berlin (Germany)*

The Gram-positive bacterium *Listeria monocytogenes* occurs widespread in the environment and infects humans when ingested along with contaminated food. Such infections are particularly dangerous for risk group patients, for whom they represent a life-threatening disease. To invent novel strategies to control contamination and disease, it is important to identify those cellular processes that maintain pathogen growth in- and outside the host. We here have applied transposon insertion sequencing (Tn-Seq) to *L. monocytogenes* for the identification of such processes on a genome-wide scale [1]. Our approach classified 394 open reading frames as essential for growth under standard laboratory conditions and identified 42 further genes, which become additionally essential during intracellular growth in macrophages. Most essential genes encode components of the translation machinery, act in chromosome-related processes, cell division and biosynthesis of the cellular envelope. Several cofactor biosynthesis pathways and 29 genes with unknown functions were also essential, opening novel options for the development of antilisterial drugs. Among the genes specifically required during intracellular growth were known virulence factors, genes compensating intracellular auxotrophies and several cell division genes. Our experiments also highlight the importance of PASTA kinase signalling, glycine metabolism and chromosome segregation for efficient intracellular growth of *L. monocytogenes*.

[1] Fischer, M., Engelgeh, T., Rothe, P., Fuchs, S., Thürmer, A. and Halbedel, S., *Listeria monocytogenes* gene essentiality under standard laboratory growth conditions and during macrophage infection. biorxiv. doi: https://doi.org/10.1101/2022.03.04.482958

Keywords: cozEb, ftsK, parA, prkA, whiA

*P-57

Bacterial wars: Identification of new toxins in *Bacillus subtilis*

Jiří Pospíšil[1], Hana Šanderová[1], Martin Hubálek[2], Libor Krásný[1]

[1] *Institute of Microbiology of the CAS, v. v. i., Prague (Czech Republic)*
[2] *Institute of Organic Chemistry and Biochemistry, CAS, Prague (Czech Republic)*

Different bacterial species use extracellular toxins to fight against each other. The main aim of this project is to identify and characterize so far unknown extracellular toxic proteins/lipids produced by gram-positive *Bacillus subtilis* 168. First, genes for known toxins such as Sublancin 168 or WapA were deleted. Second, using culture filtrates of the ΔsunA ΔwapA strain, we observed that the strain was still able to kill *Bacillus* megaterium and the unknown toxins did not require direct cell-to-cell contact of the toxin producer with the target cell. Next, by a systematical analysis (molecular weight separation and mass spectrometry) of culture filtrates, we localized genes of those toxins to prophage regions. Subsequently, we revealed that the toxin(s) was a protein susceptible to proteinase K treatment and inactivated by high temperature (100° C). The molecular weight was >100 kDa; alternatively, it formed complexes of this size. The identity of the toxins will be discussed. This project thus reveals new weapons used by *B. subtilis* in the interspecies war.

Keywords: *Bacillus*, toxins, cell-cell interaction

*P-58

Analysis of the bacteriome and resistome of legume sprouts by nanopore sequencing

Milada Šolcová[1], Katerina Demnerova[1], Sabina Purkrtova[1]

[1] *University of Chemistry and Technology, Prague (Czech Republic)*

The spread of antibiotic resistance (AR) is a global public health problem, currently. Many localities in the environment are exposed to excessive doses of antibiotics and evolutionary changes, which can lead to the spread of AR. Antibiotic resistance genes (ARG) and antibiotic-resistant bacteria (ARB) from different localities can then circulate and accumulate in the water, soil, and air from where they can spread further into the food chain. Sprouts are known for their high loads of microorganisms and because they are consumed raw, they can serve as a transmission route for antibiotic resistance bacteria (ARB) and antibiotic resistance genes (ARG) to the gastrointestinal microbiome.

Nanopore sequencing (NS) is a portable next-generation sequencing technique that is based on the detection of changes in electrical conductivity that occur as DNA passes through a nanopore located in the flow cell. These changes are specific to each base on the basis of their different electrical resistance.

Our goal was to compare the diversity of the bacteriome and resistome in 3 different samples of legume sprouts. We performed culture and DNA analyses of the bacteriome of these legume sprout samples (after incubation 48 h, 30 °C). Isolated culturable microorganisms were identified by MALDI-TOF MS. Isolated DNA (Food DNA isolation kit, Norgen Biotek Corp., Canada) was used for NS performed by barcoding and ligation-sequencing kit (ONT, Oxford, UK) and the sequencing data were analyzed using EPI2ME software. This analysis revealed that of the Gram-positive bacteria in legume sprouts, the most abundant are lactic acid bacteria of the genera *Leuconostoc*, *Lactococcus*, and *Enterococcus* followed by the genera Staphylococcus and *Bacillus*. From Gram-negative bacteria, the genera of the Enterobacteriaceae family (*Escherichia*, *Klebsiella*, *Citrobacter*, and also *Salmonella*) were the most present. The presence of ARG was analyzed with the usage of the CARD database. This work was supported from the grant of Specific university research – grant No. A2_FPBT_2022_047.

Keywords: spread of antibiotic resistance, legume sprouts, food safety, nanopore sequencing

P-59

Use of *Bacillus subtilis* spores displaying RBD domain of SARS-Cov-2 spike protein as a potential oral vaccine

Romana Kalianková Chovanová[1], **Andrea Gúcka**[1], Radoslava Rechtoríková[1], Imrich Barák[1]

[1] *Institute of Molecular Biology, SAS, Bratislava (Slovakia)*

The novel coronavirus SARS-CoV-2 that began to spread in Wuhan in December 2019, rapidly grew into a global pandemic. Recently, the most effective mean of controlling SARS-CoV-2 is by the administration of COVID-19 vaccines. The current pandemic situation requires the development of vaccines in large quantities with an easy route of administration. The genome of SARS-CoV-2 encodes 29 proteins, of which four are the major structural proteins, namely the spike (S) protein, the envelope (E) protein, membrane (M) and nucleocapsid (N) proteins. The receptor binding domain (RBD) of S1, the spike protein subunit, is an attractive target for SARS-CoV-2 vaccines because it elicits high quality, functionally relevant antibodies. Recombinant spores of bacterium *Bacillus subtilis* could be employed as an effective vaccine delivery system. The advantages of spores include safety, stability and easy preparation. Additionally, their low cost contributes even further to the benefits of spores as antigen vehicles. *B. subtilis* spores are surrounded by a structurally complex protein shell known as the coat. Six proteins, CotV, CotX, CotY, CotZ, and CgeA are morphogenetic proteins of the spore crust - the outermost layer of spore coat. CotY seems to be the most abundant, closely followed by CotZ. These attributes suggest that CotZ and CotY might be good anchor proteins for the spore surface display. Herein, we displayed the SARS-CoV-2 spike glycoprotein RBD on the surface of *B. subtilis* spores using CotZ or CotY as possible candidates for an oral vaccine against the SARS-CoV-2 virus. In addition, we placed a flexible linker peptide between the anchor and target proteins while focusing on the construction of fusion proteins of both types, the SARS-CoV-2 spike glycoprotein RBD to the N-terminus and C-terminus of the spore coat protein CotZ or CotY and detected them on the surface of the spores by fluorescence microscopy.

This work was supported by VEGA – Grant No. 2/0001/21 and a Grant from the Slovak Research and Development Agency under contract APVV-18-0104.

Keywords: SARS-CoV-2 RBD, *Bacillus subtilis*, CotZ, CotY, oral vaccination, spores

*P-60

Absolute Protein Quantification of the secretome in industrially relevant microbes

Borja Ferrero Bordera[1], Jürgen Bartel[1], Sandra Maaß[1], Dörte Becher[1]

[1] *Institute Microbiology, Universität Greifswald, Greifswald (Germany)*

Proteomics is one of the most promising methodological fields in life science. Hence, mass spectrometry-based approaches have been developed to deeply characterize the key players in life, the proteins. Some of these approaches aim at absolute quantification of proteins in order to provide data suitable for modelling cellular processes in the context of systems biology or to obtain insights into protein stoichiometry in a global manner. Nevertheless, at the moment it is only feasible to absolutely quantify cytosolic and membrane proteins, leaving behind the extracellular protein fraction. However, this sub-proteome is of great interest because of its implications in industrial protein production and pathogenesis. Thus, it is of remarkable need to develop a method that allows to absolutely quantify what is called the secretome, all proteins secreted to the extracellular media. Such novel approach combined with already established workflows would permit to determine protein concentrations for a large fraction of proteins expressed in Gram-positive bacteria such as *Bacillus subtilis* and hence would provide comprehensive data for integration in biomathematical models.

Keywords: *Bacillus subtilis*, Proteomics, Stress, Absolute Quantification, Secretion, mass spectrometry

*P-61

Identification of novel interacting partners of RNA polymerase in *Mycobacterium smegmatis*

Nabajyoti Borah[1], Hana Šanderová[1], Tamara Balgová[1], Alena Křenková[2], Martin Hubálek[2], Libor Krásný[1]

[1] *Institute of Microbiology CAS , Prague (Czech Republic)*
[2] *Institute of Organic Chemistry and Biochemistry CAS, Prague (Czech Republic)*

Transcription is a key process of gene expression that requires coordinated interaction of DNA-dependent RNA polymerase (RNAP) with several other transcription factors. Our knowledge of the bacterial transcriptional machinery largely comes from the established bacterial model systems such as *Escherichia coli* and *Bacillus subtilis*. However, studies have shown that different bacterial species deploy discrete transcription factors to optimally regulate gene expression. In this regard, *Mycobacterium smegmatis* has evolved as another model organism to study the transcription factors in prokaryotes. Discoveries of two essential mycobacterial transcription factors- CarD and RbpA, absent in *E. coli*, have encouraged researchers to search for novel transcription factors that would help understand the complexity and versatility of bacterial gene regulation. In this study, an attempt was made to identify novel transcription factors by pulling down FLAG-tagged RNAP in *M. smegmatis*. In the experimental design for pull down assay, two sets of cell lysates were prepared – one set was treated with endonuclease benzonase to remove any nucleic acids bound to RNAP while the other set was untreated. Several proteins were selected for further analysis and four previously uncharacterized proteins were also able to reciprocally pull down RNAP when used as baits. These proteins are now being extensively characterized and their effect on RNAP and transcription is being investigated. The results will be presented, extending our knowledge of the architecture and functioning of the bacterial transcription machinery.

Keywords: Transcription factor; Uncharacterized protein; RNA polymerase; Mycobacteria

*P-62

Elucidating genetic factors associated with *Staphylococcus aureus* bovine mastitis

Marita T Mårli[1], Xue Liu[2], Vincent de Bakker[2], Maria V Heggenhougen[1], Anne O Nordraak[1], Davide Porcellato[1], Jan-Willem Veening[2], Morten Kjos[1]

[1] *Norwegian University of Life sciences, Ås (Norway)*
[2] *University of Lausanne, Lausanne (Switzerland)*

Staphylococcus aureus is the major pathogen associated with bovine mastitis, an inflammation of the mammary gland. The disease is one of the most prevalent and costly diseases in the dairy industry, with detrimental effects on animal welfare and milk production. Despite many years of research and widespread implementation of control strategies, *S. aureus*-associated mastitis remains difficult to eradicate. Knowledge about which factors that contribute to survival in the farm environment, proliferation in milk, biofilm formation, interaction with host cells and with host microbiota, can contribute to finding new anti-microbial or anti-infective targets to combat mastitis. To elucidate the genetics of how *S. aureus* cause bovine mastitis, we have constructed a pooled, genome-wide, tetracycline inducible CRISPR interference (CRISPRi) knockdown library in *S. aureus*. The functionality of the tetracycline inducible system was tested and verified. By growing the pooled CRISPRi library under different conditions, followed by CRISPRi sequencing, we can determine the fitness of each gene and identify genes that are conditionally costly or essential. Using this approach, we have identified genes that are conditionally essential or costly for proliferation in bovine milk. Furthermore, by exposing the library to sub-lethal levels of penicillin G and trimethoprim-sulfamethoxasole, we have identified potential targets which can sensitize *S. aureus* to these antibiotics, which are commonly used to treat bovine mastitis.

Keywords: *Staphylococcus aureus*, bovine mastitis, CRISPRi

*P-63

Identification of a novel septally-localized protein involved in pneumococcal cell division

Jessica Burnier[1], Clement Gallay[1], Jan-Willem Veening[1]

[1] *University of Lausanne, Lausanne (Switzerland)*

Accurate synchronization between cell elongation and division is crucial for bacteria to maintain a proper cell shape. The human pathogen *Streptococcus pneumoniae* differs from well described rod-shaped model bacteria as it has an ovoid-shape and its lateral and septal peptidoglycan (PG) biosynthesis are coupled together. Both the elongasome and divisome are located at the middle of the cell, suggesting an interconnection between these machineries. However, the link between these two mechanisms is still poorly understood in this organism as it lacks the actin homolog MreB, known to control the PG elongation machinery in other organisms. Here, we characterized a small protein of unknown function that we tentatively called DpuF for division protein of unknown function. DpuF is conserved in Lactococci and possesses a trans-membrane spanning domain coupled to an extracellular LysM domain, which is a widely distributed protein motif in peptidoglycan-binding proteins. Interestingly, we showed that DpuF is localized at mid-cell. While DpuF is not essential on its own, we identified several genetic interactions in division and elongation genes using a genome-wide CRISPRi-seq-based synthetic lethality screen. Indeed, we show that DpuF becomes essential when DivIC, DivIB, MreD, MurE and RodZ are depleted. Given that these proteins are involved in peptidoglycan biosynthesis and / or cell division processes, we hypothesize that DpuF could be also involved in one of these processes. The characterization of this unknown peptidoglycan-related protein can broaden our understanding of pneumococcal growth and division.

Keywords: *Streptococcus pneumoniae*, peptidoglycan, CRISPRi-seq, synthetic lethality, cell division, septal protein

P-64

Antagonistic action of *Bacillus subtilis* PS-216 towards the food pathogen *Campylobacter jejuni* is governed by environmental conditions

Katarina Šimunović[1,2], Polonca Štefanič[1], Anja Klančnik[2], Sonja Smole Možina[2], Ines Mandić Mulec[1]

[1] *Department of Microbiology, BF-UL, Ljubljana (Slovenia)*
[2] *Department of Food Science and Technology, BF-UL, Ljubljana (Slovenia)*

Campylobacter jejuni is a major cause of the most commonly reported bacterial gastroenteritis, a serious food safety hazard that spreads mainly via contaminated poultry meat. Current control strategies have to be improved to increase the safety of food and probiotic bacteria are being considered as a control strategy.

Bacterial interactions are complex and the interaction of *Bacillus subtilis* and pathogenic bacteria as competition and antagonism is an interesting and potentially lucrative topic, as some *B. subtilis* strains are being used as probiotics in animals and humans.

Although it is very relevant for the characterization of these interactions there is little known of how environmental parameters affect these interactions (T, O2, medium). In this study, we evaluated PS-216 as an antagonist of *C. jejuni* during co-cultivation at different temperatures (42 °C, 37 °C, 20 °C), atmospheres (aerobic, microaerophilic), and in different growth media (Mueller-Hinton, chicken litter medium, chicken intestinal content medium). This interaction was confirmed in vivo in the chicken host.

Under microaerophilic conditions, PS-216 effectively inhibited the growth of *C. jejuni* at 42 °C (Δlog 4.19) and at 37 °C (Δlog 1.63) and lost at 20 °C. Under oxygen-replete conditions, *B. subtilis* supported *C. jejuni* survival. PS-216 grew in non-sterile intestinal content and inhibited the growth of *C. jejuni* in sterile chicken litter and intestinal content, showing potential as a chicken probiotic that can be integrated into the chicken intestinal microbiota. This was confirmed when *B. subtilis* PS-216 spores were added to chicken drinking water for 21 days, as this spore addition reduced the numbers of *C. jejuni* in the chicken intestine by 1.3 logCFU/g cecum. Interestingly, the spore treatment also resulted in a significant weight gain in broilers.

Although our results show that environmental conditions play a vital role in these interactions, we also show that PS-216 has a strong anti-Campylobacter effect in chicken intestinal content and most importantly, in the chicken gut.

Keywords: *Bacillus subtilis* PS-216, *Campylobacter jejuni*, interaction, antagonism, probiotic, broiler chicken

*P-65

ChIP-seq analysis for the leading mycobacterial transcriptional factors: CarD, RbpA and a novel transcriptional regulator; CrsL.

Mahmoud Shoman[1], Viola Vankova Hausnerova[1], Jitka Jirát Matějčková[1], Dilip Kumar[1], Libor Krásný[1], Jarmila Hnilicová[1]

[1] *Institute of Microbiology of the CAS, (Czech Republic)*

The mycobacterial transcriptional machinery comprises two global transcription regulators binds to RNAP- CarD, RbpA. Both CarD and RbpA stabilize the open complex during transcription and are important for the sensitivity of RNAP to rifampicin; the first-line drug against tuberculosis. We discovered a new transcription factor that binds to CarD-RNAP complex and named it CrsL. Our data shows that CrsL regulates CarD level in the stationary phase and reveals a new mechanism of transcriptional regulation in mycobacteria.

We performed Chromatin Immunoprecipitation followed by next-generation sequencing (ChIP-seq) for CarD and RbpA transcriptional regulators in *Mycobacterium smegmatis*. In addition, we did this experiment for CrsL to see if it associates with the same promoters as CarD/RbpA.

Furthermore, we developed a webpage for visualizing our ChIP-seq data in addition to other sequencing data (RNA-seq) which we previously published. This webpage will be useful to visualize the expression profile of different genes compared to the presence of CarD/RbpA at these genes within *M. smegmatis* genome. Therefore, it will provide novel insights into the control of mycobacterial transcriptional regulation in order to develop new specific anti-tuberculosis drugs.

Keywords: ChIP-seq, Mycobacteria, transcription, gene expression, tuberculosis

P-66

Two small proteins, SR1P and SR7P, modulate the degradosome-like network (DLN) of *Bacillus subtilis*

Inam Ul Haq[1], Matthias Gimpel[2], Peter Müller [1], Sabine Brantl[1]

[1] *Friedrich-Schiller-Universität Jena (Germany)*
[2] *Technische Universität Berlin (Germany)*

The *Bacillus subtilis* degradosome-like network (DLN) comprises the major endoribonuclease RNase Y, 3'-5' exoribonuclease PnpA, the endo/5'-3' exoribonucleases J1/J2 and helicase CshA. Furthermore, three metabolic enzymes, phosphofructokinase (PfkA), enolase (Eno) and glyceraldehyde-3-phosphosphate dehydrogenase A (GapA) moonlight as scaffolding components of the DLN. So far, we have identified two small proteins that bind to scaffolding components of the degradosome: SR1P encoded by the dual-function sRNA SR1 binds GapA, promotes the GapA-RNase J1 interaction and increases the RNase J1 activity. Recently, we found that SR1P additionally increases the GapA-RNase Y interaction and enhances the RNase Y activity. SR7P encoded by the dual-function antisense RNA SR7 binds to enolase thereby enhancing the enzymatic activity of enolase-bound RNase Y. SR1P is expressed under gluconeogenic and sporulation conditions whereas SR7P is induced under ethanol, salt, heat and acid stress conditions. We discuss the role of SR1P and SR7P in modulating the activity of GapA and enolase, respectively, under different physiological conditions. Currently, it is not clear if SR1P and SR7P have overlapping or alternating function with regard to their modulation of RNase Y activity by two different scaffolding components of the DLN. Future research will reveal if the degradosomes or DLNs of other bacteria comprise glyceraldehyde-3-phosphosphate dehydrogenase and if they are attuned by small proteins, too.

Keywords: *Bacillus subtilis*, SR1P, SR7P, degradosome, RNA degradation

*P-67

Exploiting the enzymatic power of engineered *Bacillus subtilis* for agri-food waste valorization

Valeria Bontà[1], Stefania Colombini[2], Marco Battelli[2], Gianluca Galassi[2], Cinzia Calvio[1]

[1] *University of Pavia (Italy)*
[2] *University of Milan (Italy)*

Bacillus subtilis is a widely exploited host for the production of several bio-commodities. Moreover, being a soil bacterium, it has evolutionarily acquired a wide range of hydrolytic enzymes necessary to thrive on decaying vegetable biomass. The intrinsic capability of the bacterium to secrete degradative enzymes has been genetically optimized, thus boosting the deconstruction of the raw biomass. Exploiting the ability of *B. subtilis* to autonomously saccharify vegetable biomasses, this work explores the feasibility of using low-cost vegetable waste as a fermentation feedstock for *B. subtilis*, bypassing any pre-saccharification step.

Together with rice straw (RS), a widespread agro-waste from rice harvest, low lignin-fruits and vegetable wastes were used as substrates for bacterial growth either alone or in different combinations. Surprisingly, *B. subtilis* could grow on such waste-based buffered media. The main challenges faced during this work arose from the uncontrolled origin of the experimental material, conferring it an intrinsic variability, and from the presence of particulate fibers in the medium, impairing the measurement of bacterial growth.

The RS-grown cultures of *B. subtilis* were also exploited as feed additives. The digestibility of several dairy cow feed ingredients was measured both as the amount of gas produced and as Neutral Detergent Fiber Digestibility during several in vitro ruminal fermentation with RS-grown cultures. With respect to controls, both parameters improved in the presence of *B. subtilis*, suggesting that the hydrolytic enzymes released in the growth medium increased the degradation rate of the traditional feeds, thereby improving the quality of the feed itself. Thus, the effect of *B. subtilis* grown on waste substrate may translate into feed additives that boosts the ruminant performances in terms of feed use efficiency.

Keywords: Engineered *Bacillus subtilis*, enzymatic degradation, agri-food waste

*P-68

Prokaryotic molybdenum cofactor biosynthesis protein (MoaB2) may play a role in RNA Transcription

Subhash Narasimhan[1], Martin Černý [1], Barbora Brezovská[3], Hana Šanderová[3], Libor Krásný[3], Lukáš Žídek[1,2]

[1] *Central European Institute of Technology(CEITEC)-MU, Brno (Czech Republic)*
[2] *National Centre for Biomolecular Research (NCBR) - MU, Brno (Czech Republic)*
[3] *Institute of Microbiology of the CAS, Prague (Czech Republic)*

Molybdoenzymes (MOEs) catalyze complex multistep oxygen transfer reactions to incorporate the trace element molybdenum, essential in almost all forms of life. Molybdenum cofactor biosynthesis protein B (MoaB2) enzyme, involved in adenylation of molybdenum cofactor (Moco) intermediate, was shown to be catalytically inactive in prokaryotes. A gene upstream of the operon, mogA is instead involved in the Moco adenylation in prokaryotes sharing a similar fold and tertiary structure. Unexpectedly, an immunoprecipitation study with the housekeeping transcription sigma factor A (σA) revealed its association with moaB2 in *Mycobacterium smegmatis* (see poster by B. Brezovska). This interaction was subsequently confirmed by size-exclusion electrophoretic studies and mass spectrometry analyses. To better understand the structural aspects of the MoaB2-σA interaction, the crystal structure of MoaB2 from *M. smegmatis* was determined by molecular replacement at a resolution of 2.6 Å and refined to R factor of 20.4% (Rfree = 24.6%). The data collection was performed at the DESY Beamline, Hamburg. The obtained structure was compared with already known structures of proteins with homologous sequences. The analysis revealed that MoaB2 shares structural similarity with the orthologous proteins of molybdenum cofactor biosynthesis. Our data document that the moaB gene may encode proteins playing different roles in different organisms and reveal so-far unknown details of bacterial transcription.

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Keywords: Molybdoenzymes, MoaB2, prokaryotes, transcription, X-ray crystallography

P-69

Running the Gauntlet of the Bacterial Cell Wall

Amirah Alofi[1], Richard Daniel

[1] *Newcastle University, Newcastle Upon Tyne (United Kingdom)*

In nature, bacteria have verity shapes from simple cocci and rods to more complicated spiral or appendage structures. Under normal conditions, they need a strong exoskeleton that can maintain their shape during growth and protect them from changes in environmental conditions. This rigid structure is generally provided through the assembly of a cell wall that encases the cell membranes. This cell wall is a complex and dynamic structure containing multiple layers of a proteoglycan polymer, peptidoglycan, and components. In *Bacillus subtilis*, there are several cell wall hydrolysing enzymes that play critical roles in remodelling the bacterial cell wall. These enzymes are synthesized in the cytosol of the cell and secreted across the cell membrane to carry out their functions in a controlled way. The expression of these enzymes is highly regulated, and they carry out their biological functions either at the membrane surface, within the cell wall or from the outside surface of the cell. The mechanisms by which these proteins are localized correctly in the cell wall and how their activity is directed are poorly understood.

Recent results point toward the idea that the autolytic enzymes do not act on newly synthesized peptidoglycan unless it is specifically modified either through altered precursors incorporated into the polymer or by post polymerisation maturation. Results obtained in this lab, and others clearly indicate the role for the modification of PG mediated by AsnB at the precursor level in combination with another post synthesis modification. These modifications can be related to effects on the essential roles of CwlO and LytE in cell wall metabolism. Our results build on previous work to provide a model for the integration of cell wall synthesis and degradation in rod-shaped Gram-positive bacteria.

P-70

Bacillus-Salmonella interactions in mixed-species biofilm are affected by the pathogen's extracellular matrix components

Eva Kovačec[1], Eli Podnar[1], Tjaša Danevčič[1], Bram Lories[2], Hans Steenackers[2], Ines Mandic-Mulec[1]

[1] *Biotechnical faculty, University of Ljubljana (Slovenia)*
[2] *KU Leuven (Belgium)*

Probiotics represent an alternative approach to conventional antibiotic therapy, mainly because of emerging multi-resistant pathogens and the spread of antibiotic resistance. Probiotic bacteria can combat gastrointestinal pathogens through antagonism by niche exclusion guided by spatial and nutritive competition, modulation of the host's immune system, and production of inhibitory compounds. Probiotic supplements containing *Bacillus* sp. are gaining in popularity. They show potential to combat enteropathogens (e.g. *Salmonella*) and as growth promotors for improving the growth of poultry, piglets, ruminants, and shrimps. Although *Bacillus* probiotics are already in use, the knowledge on mechanisms driving mixed-species interactions in biofilms is scarce.

Moreover, information on how extracellular matrix components modulate interspecies interactions is limited. Our study examined the effects o the *Bacillus subtilis* PS-216, a potential probiotic strain, on an enteropathogenic *Salmonella* enterica. We compared the effect on the wild type pathogen and its mutants defective in the biofilm matrix, namely the ΔcsgB mutant incapable of producing curli and the ΔbcsA mutant incapable of producing cellulose. All used strains produced fluorescent proteins and carried antibiotic markers, which monitored their fitness and spatial distribution in biofilms. Results show that the distribution of both species in biofilm remains similar, regardless of the presence or absence of a specific matrix component. However, the wild type and two *Salmonella* mutants had lower adhesion to surfaces in mixed-biofilms with *B. subtilis* than their monoculture. PS - 216 also decreased biofilm thickness of wild type and *Salmonella*'s mutants. As expected, *B. subtilis* PS-216 negatively affected *Salmonella*'s overall fitness in coculture. However, the best competitor with *B. subtilis* was the strain of *Salmonella* incapable of producing cellulose. Our results suggest that *B. subtilis* PS-216 has a biocontrol potential against a critical pathogen and thus may be of potential interest as a probiotic strain.

Keywords: probiotic, *Bacillus subtilis*, *Salmonella* enterica, biofilm

*P-71

Novel Extremophilic Metalloproteases for Consumer Product Applications

Ben M Astles[1]

[1] *The University of Edinburgh, Edinburgh (United Kingdom)*
[2] *Unilever R&D, Port Sunlight (United Kingdom)*

Proteases are of great commercial importance, with applications in the detergents, tanning and dairy industries. This project aims to develop novel metalloproteases for the detergents industry with industrially-desirable characteristics. This will be achieved through screening of metagenomic libraries for likely protease candidates and synthesis of candidate DNA, codon-optimising for expression in a heterologous host: *Bacillus subtilis*. The activity of these proteases will be assessed through high-throughput screening (HTS), followed by directed evolution. The proteases will then be purified and assessed in wash-studies. The final protease will ideally have improved washing performance in industrially relevant conditions when compared with current standards.

*P-72

pTricolor: a plasmid-based system for the characterization of heterogeneous populations in *Bacillus subtilis*

David Núñez Nepomuceno[1], Hannes Wolfgramm, Marco Harms, Petra Hildebrandt, Ulrike Mäder, Alexander Reder , Uwe Völker

[1] *University Medicine Greifswald, Greifswald (Germany)*

The Gram-positive bacterium *Bacillus subtilis* lives in the upper layers of the soil and has to face and adapt to various environmental conditions. A very sophisticated strategy that *B. subtilis* employs is the differentiation into distinct subpopulations to increase the chances of survival. Multiple approaches have been used to study this heterogeneity. One of the most popular methodologies is the usage of florescent reporter systems. Transcriptional fusions with fluorescent proteins in combination with flow cytometry allow a time-resolved monitoring of physiological responses and the identification of subpopulations in the culture. Furthermore, if two transcriptional fusions are combined, it will permit the study of the interactions between the two physiological responses.

Currently there is no plasmid based-system that allows the simultaneous monitoring of two different cellular responses and contains an internal control for the plasmid copy number. In this work, we have developed the pTricolor to fill this gap. As a proof of concept, we designed a pTricolor-variant that is able to monitor the SigB-dependent general stress response and the PerR-regulated oxidative stress response. Several improvements of the different genetic elements have been integrated in the plasmid to improve sensitivity and dynamic range of the monitoring. These include optimized promoter and Shine-Dalgarno sequences, codon optimization of the reporter genes and optimization of terminators.

Our aim is to use pTricolor to characterize stress responses during recombinant protein production under industrial settings. From a biotechnological point of view, it is important to study these stress responses because this knowledge can be the base for the improvement of industrial *Bacillus* strains.

Keywords: *Bacillus subtilis*, heterogeneity, stress, fluorescence, plasmid, flow cytometry

*P-73

Role of the Stickland pathways on *C. difficile* virulence in vivo.

Auriane Monestier[1,2], Aidan Pavao[3], Madeline Graham[3], Mary Delaney[3,4], Johann Peltier[2,1], Bruno Dupuy[1], Lynn Bry[3,4]

[1] *LPBA, Institut Pasteur, Paris (France)*
[2] *ARNclo, I2BC, CNRS Gif-sur-Yvette (France)*
[3] *Brigham & Women's Hospital, Harvard Medical School, Boston (United States of America)*
[4] *Clinical Microbiology Laboratory, Brigham & Women's Hospital (United States of America)*

Clostridioides difficile, a Gram-positive, strictly anaerobic gut bacterium, is the primary cause of infectious nosocomial diarrhea in adults. Commensals provide primary protection against *C. difficile* infection though the underlying mechanisms of action remain ill-defined. *C. difficile* must compete against the commensal bacteria for nutrients but disruption of the microbiota by antimicrobials enriches carbon sources, including carbohydrates and fermentable amino acids, for the pathogen. *C. difficile* uses Stickland fermentations to extract energy from amino-acids and evidence highlight that these pathways confer a fitness advantage to the bacterium. Three Stickland pathways have been identified in *C. difficile*: proline reductase, glycine reductase, and the reductive leucine pathway.

In this study, we aim to define the importance of the different *C. difficile* Stickland pathways on its virulence. We created single deletion mutants of the prdB, grdAB and hadA genes to disrupt each pathway in *C. difficile* ATCC 43255, a strain that causes symptomatic infections in mice. In vitro analyses revealed a growth defect of the prd and hadA mutants in a glucose-free rich medium, while the growth of the grd mutant was not affected. Intriguingly, addition of glucose in the medium rescued the growth defect of the hadA but not of the prdB mutant. We also found that deletion of hadA or prdB increased toxin production and that a strain lacking any of the Stickland pathways was strongly impaired in sporulation. In-vivo studies in germ-free mice revealed a pronounced impact of the hadA mutant on host survival compared to the wild type strain, and a slight effect on extension of survival with the prdB mutant. In contrast, deletion of grd had no impact on mouse survival. Work is in progress to assess the effects of *C. difficile* mutants on host outcomes in mice co-colonized with protective versus disease-promoting microbiota to assess functions of *C. difficile*'s Stickland pathways with the commensal microbiota.

Keywords: *Clostridioides difficile*, nosocomial disease, gut colonization, Sticklands pathways, commensals

P-74

Lacticaseibacillus rhamnosus Impedes Growth of *Listeria* spp. in Cottage Cheese through Manganese Limitation

Lieke A. van Gijtenbeek[1], Quinn Singer[2], Louise E. Steffensen[1], Shannon Neuens[1], Helle S. Guldager[1], Susanne Bidstrup[1], Tina Høgholm[1], Mikkel G. Madsen[1], Kathleen Glass[2], Solvej Siedler[1]

[1] *Chr. Hansen A/S, Hørsholm (Denmark)*
[2] *FRI, University of Madison-Wisconsin, Madison (United States of America)*

Acidification and nutrient depletion by dairy starter cultures is often sufficient to prevent outgrowth of pathogens during post-processing of cultured dairy products. In the case of cottage cheese, however, the addition of cream dressing to the curd and subsequent cooling procedures can create environments that may be hospitable for the growth of *Listeria monocytogenes*. We identified on a *Lacticaseibacillus rhamnosus* strain that severely limits the growth potential of *L. monocytogenes* in creamed cottage cheese by depletion of manganese (Mn), thus through competitive exclusion of a trace element essential for the growth of many microorganisms. Growth of *Streptococcus thermophilus* and *Lactococcus lactis* that constitute the starter culture, on the other hand, were not influenced by reduced Mn levels, unraveling a different demand for Mn availability by various Gram-positive bacteria.

Keywords: *Listeria* inhibition, lactic acid bacteria, *L. rhamnosus*, cottage cheese, manganese

P-75

Toward the Molecular Details of Electrical Signaling in *Bacillus subtilis* Biofilms

Celina Thiel[1], Michael F. Fuss[1], Igor Tascón[1], Inga Hänelt[1]

[1] *Goethe University, Frankfurt (Germany)*
[2] *Goethe University, Frankfurt (Germany)*
[3] *Instituto Biofisika, Bilbao (Spain)*
[4] *Goethe University, Frankfurt (Germany)*

Electrical signaling is well known as a way of long-range neuronal communication. However, also prokaryotes appear to make use of this elegant communication process. In *Bacillus subtilis* biofilms, channel-mediated efflux of potassium has been shown to serve the long-range communication of metabolic status within a biofilm, the attraction of motile bacteria to a biofilm and the synchronisation of distant biofilms. Upon glutamate starvation of the interior cells in a biofilm, the potassium channel YugO opens allowing efflux of potassium ions. This causes a depolarisation of neighbouring cells which results in decreased glutamate uptake. In a chain reaction this causes the potassium-mediated signal to propagate through the biofilm causing a limited glutamate consumption by the peripheral cells. Glutamate can therefore diffuse deeper into the biofilm where it is taken up by the interior, hyperpolarised cells, which in turn supply the peripheral cells with ammonium.

Until now, the molecular details of the processes leading to potassium efflux through YugO remain unclear. In bacterial cells, the membrane potential is established to around -110 mV by the electron transport chain, allowing the accumulation of potassium to approximately 300 mM. Therefore, a prior depolarisation of the cell would be necessary to allow substantial potassium efflux upon YugO opening. To monitor and potentially manipulate the membrane potential and observe influences on the signaling process, we aim to establish the expression of bacterial rhodopsins such as PROPS, KR2 or Xenorhodopsin in *B. subtilis* cells growing in a biofilm. Further, preliminary structural and functional data point towards ammonium, a metabolic product of glutamine synthesis, as a direct ligand of YugO's RCK domain. We hypothesise that binding of ammonium to the RCK domain could keep the channel closed, while upon glutamate starvation and subsequent depletion of ammonium the channel opens, enabling potassium efflux if the membrane potential is favorable.

Keywords: biofilm, rhodopsin, electrical signaling, potassium, membrane transport

P-76

Molecular mechanisms of *Bacillus subtilis*-induced protection against α-synuclein aggregation in *Caenorhabditis elegans*

Deep Prakash[1], Maria E Goya[1,2], Samanta P Recio[1], Feng Xue[1], Sofia Arnaouteli[3], Nicola R Stanley-Wall[3], Maria Doitsidou[1]

[1] *CDBS, University of Edinburgh, Edinburgh (United Kingdom)*
[2] *ERIBA, UMCG, University of Groningen (Netherlands)*
[3] *School of Life Sciences, University of Dundee, Dundee (United Kingdom)*

Parkinson's disease (PD) is the second most common neurodegenerative condition. Accumulation of α-synuclein (α -syn) protein within Lewy bodies is one of the hallmarks of disease and central to the pathogenesis. Growing evidence suggests that gut microbiota play an important role in progression and severity of the condition. However, how gut bacteria affect PD pathology remains unclear.

We used a well-characterized C. elegans model that expresses human α -syn fused to yellow fluorescent protein in muscle cells. When this transgenic strain is fed with a laboratory diet of *E. coli* OP50, α -syn aggregates are formed, which can be quantified. In a screen testing the effect of different bacterial diets on α -syn aggregation, we previously showed that *B. subtilis* PXN21, isolated from a commercially available probiotic product, inhibits, delays, and reverses α-syn aggregation. (Goya et al, 2020) Both *B. subtilis* vegetative cells and spores protect against aggregation, and biofilm formation in gut plays a role in maintaining reduced levels of aggregation. Additionally, feeding *C. elegans* with *E. coli* OP50 supplemented with a crude extract from *B. subtilis* cultures, leads to a significant reduction in α -syn aggregation. The efficacy of the crude extract has a dose-dependent effect, suggesting the involvement of one or more stable and extractable bacterial metabolite(s). However, the identity of these compounds and their mechanism of action remains elusive.

To identify the genetic regulators of *B. subtilis* necessary for its effect on α -syn aggregation we are screening a genome reduction library of non-essential genes of *B. subtilis* 168 (Tanaka et al, 2013). Our results so far suggest the involvement of the TCA cycle and other bacterial pathways in modulating α -syn aggregation. Current efforts are directed toward validating the role of individual bacterial genes. In a complementary approach, we use comparative metabolomics to identify potentially protective metabolites. Overall, our study can reveal compounds with disease-modifying potential for PD.

Keywords: Parkinson's disease, *Bacillus subtilis, Caenorhabditis elegans*, protection against α-synuclein aggregation, metabolomics

P-77

Molecular elements underlying *Bacillus tequilensis* unrestrained loss of social traits

Camilo García-Botero[1], Tatiana Z. Cuellar-Gaviria[2], Valeska Villegas-Escobar[1]

[1] *Universidad EAFIT, Medellín (Colombia)*
[2] *Ohio State University, Columbus (United States of America)*

Bacillus tequilensis EA-CB0015, isolated from the phyllosphere of a banana plant, has been previously studied given its potential to control different plant pathogens. Recently, EA-CB0015 has been of interest due to a rapid and recurrent emergence of morphological variants in different culture conditions. Variants were characterized by showing smoother rather than wrinkly colonies as in the WT. Four of these variants were isolated and all of them lacked or displayed affected multicellular traits such as sporulation, biofilm, and lipopeptides production. Differential gene expression experiments between EA-CB0015 and phenotypical variant, have suggested a role of two important regulatory forces on the emergence of variants: the restriction-modification system (RM system) and prophage excision and lytic activity.

Here we showed that when comparing SMRT-sequenced genomes of EA-CB0015 variants, they showed SNPs common only in IS3 elements and 16S rRNA genes and suggested limited phenotypical effect. A potential novel type-I RM system was predicted in WT genome and epigenomic analyses from SMRT reads of variants, showed an increase of m4C and m6A from variants in contrast to WT, yet the type I RM do not appear responsible for that change. Finally, computational prediction on variants genomes revealed several prophages regions from which one classified as an SPβ prophage seems active. Overall, these findings lead us to consider that variants emergence, as an unrestraint loss of mechanism, could be mediated by the methylome fluctuation and therefore account for differential gene expression profiles observed previously. Moreover, methylation downstream of a prophage repressor gene and prophage experiments lead us to believe there is a potential role of a new phage during *B. tequilensis* EA-CB0015 lifecycle and variants emergence.

Keywords: *Bacillus*, *Bacillus tequilensis*, genomics, epigenomics, SMRT, prophages

P-78

BSGatlas: A Unified *Bacillus Subtilis* Genome and Transcriptome Annotation Atlas with Enhanced Information Access

Adrian Sven Geissler[1], Christian Anthon[1], Ferhat Alkan[1], Enrique González-Tortuero[1,2], Line Dahl Poulsen[3], Thomas Beuchert Kallehauge[4], Anne Breüner[4], Stefan Ernst Seemann[1], Jeppe Vinther[3] and Jan Gorodkin[1]

[1]*Center for Non-coding RNA in Technology and Health, Department of Veterinary and Animal Sciences, University of Copenhagen (Denmark)*
[2]*Division of Oncogenomics, Netherlands Cancer Institute (The Netherlands)*
[3]*Section for Computational and RNA Biology, Department of Biology, University of Copenhagen, Copenhagen (Denmark)*
[4]*Novozymes, Bagsværd (Denmark)*

A large part of our current understanding of gene regulation in Gram-positive bacteria is based on *Bacillus subtilis*, as it is one of the most well studied bacterial model systems. The rapid growth in data concerning its molecular and genomic biology is distributed across multiple annotation resources. Consequently, the interpretation of data from further *B. subtilis* experiments becomes increasingly challenging in both low- and large-scale analyses. Additionally, *B. subtilis* annotation of structured RNA and non-coding RNA (ncRNA), as well as the operon structure, is still lagging behind the annotation of the coding sequences. To address these challenges, we created the *B. subtilis* genome atlas, BSGatlas, which integrates and unifies multiple existing annotation resources. Compared to any of the individual resources, the BSGatlas contains twice as many ncRNAs, while improving the positional annotation for 70% of the ncRNAs. Furthermore, we combined known transcription start and termination sites with lists of known co-transcribed gene sets to create a comprehensive transcript map. The combination with transcription start/termination site annotations resulted in 717 new sets of co-transcribed genes and 5335 untranslated regions (UTRs). In comparison to existing resources, the number of 5' and 3' UTRs increased nearly fivefold, and the number of internal UTRs doubled. The transcript map is organized in 2266 operons, which provides transcriptional annotation for 92% of all genes in the genome compared to the at most 82% by previous resources. We predicted an off-target-aware genome-wide library of CRISPR–Cas9 guide RNAs, which we also linked to polycistronic operons. We provide the BSGatlas in multiple forms: as a website (https://rth.dk/resources/bsgatlas/), an annotation hub for display in the UCSC genome browser, supplementary tables and standardized GFF3 format, which can be used in large scale -omics studies. By complementing existing resources, the BSGatlas supports analyses of the *B. subtilis* genome and its molecular biology with respect to not only non-coding genes but also genome-wide transcriptional relationships of all genes.

Keywords: genome annotation; non-coding and structured RNAs; operons

P-79

sRNA-based regulation of sporulation in *Bacillus subtilis*

Barbara Cardoso-Domingues[1], Elodie Foulquier[1], Regine Lebrun[2], Anne Galinier[1], **Clementine Delan-Forino**[1]

[1] *Laboratoire de Chimie Bactérienne, Marseille (France)*
[2] *Proteomic platform, IMM, Marseille (France)*

Bacteria needs to constantly adapt their physiology to environmental changes and stress. *Bacillus subtilis* is a powerful Gram-positive model bacterium. Notably, it accurately regulates its growth rate and its size according to the carbon sources and nutriments available in the medium. In presence of non-preferential carbon source such as pyruvate, its size can be twice smaller than in glucose media. In response to starvation, *B. subtilis* is able to differentiate in a dormant spore able to survive in extreme environmental conditions through complex regulation mechanisms.

Bacterial small non-coding RNAs (sRNAs) have emerged as the main class of post-transcriptional regulators in bacteria. sRNA synthesis itself is tightly controlled and allows fast and fine tuning of physiology and metabolism. In *B. subtilis*, the sRNA-based regulation network is surprisingly poorly characterized. Among more than 150 detected putative sRNAs only five have identified targets. sRNAs interact with specific proteins and RNA-chaperones, to promote and sRNA-target RNA interactions. In contrast to Gram-negative bacteria relying on two general RNA chaperones, *B. subtilis* seems to employ several RNA-binding proteins depending not only on the corresponding sRNA but the specific sRNA/target RNA system, suggesting the existence of numerous, still unknown, sRNA partners.

Our project aims to bring to light the sRNA-based network in *B. subtilis* regulating cellular processes (growth rate, morphogenesis) or sporulation depending on the nutriment availability. We found sRNAs involved in sporulation and are currently exploring its mechanism of action through global and targeted approaches. Overall, our work will enable identification of key sRNAs, their cofactor and their targets leading to the characterization of new regulation mechanisms coordinating nutriment availability and cellular processes in *B. subtilis*.

Keywords: sRNA, *Bacillus subtilis*, sporulation, proteomics