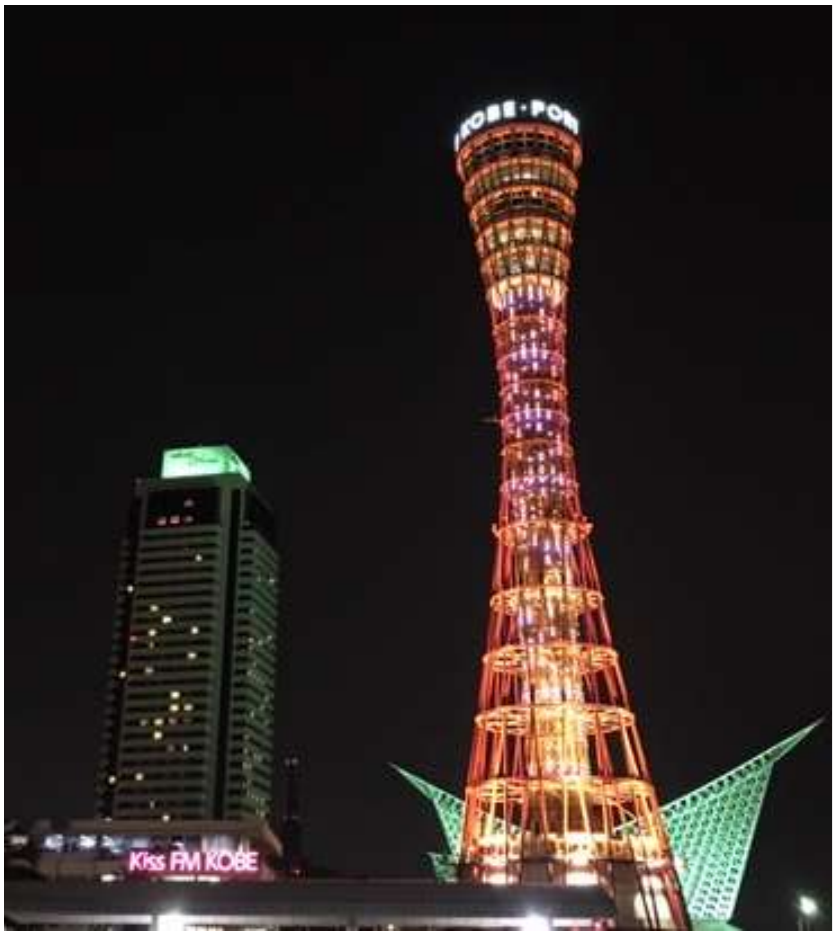
BACELL2020

2020 年度グラム陽性菌ゲノム機能会議



ABSTRACT BOOK

The Kobe Chamber of Commerce and Industry and Ariston Hotel

Kobe, Japan

19-22 April 2020

--- Sadly BACELL2020 had to be cancelled…

Since the *Bacillus subtilis* genome sequencing project meeting in Paris in 1990, the series of European *Bacillus* meetings, presently defined as “BACELL”, has been held annually as an international conference to discuss academic, industrial, and technological development of studies on *B. subtilis* and its related Gram-positive microorganisms.

BACELL2020 was originally scheduled as the first Euro-Japan BACELL meeting on 19-22 April 2020 in Kobe, Japan. In March 2020, however, due to the epidemic of the new coronavirus, various events in Kobe gathering a large number of people became cancelled or postponed, also the same in Europe. Thus, the idea to hold BACELL2020 had to be re-considered.

The BACELL2020 local organizing committee had discussed how we could make the event take place with representatives of the conference venue, the Kobe Chamber of Commerce and Industry and Ariston Hotel. We could have arranged both the lecture and poster halls spacy enough to avoid deep contact among participants, lunches served in boxes, drinks disposable for single use, and banquet prepared on seated tables instead of buffet. In this way, we could have taken the most careful measures to prevent the spread of the virus infection. Thus, our original intention was to hold BACELL2020 on schedule if possible.

However, a number of participants reported one after another that their trips to Kobe had to be banned. In addition, we had an increasing fear that our Universities, Kobe City, or our governments might prohibit any large international events such as ours. Moreover, possibly major flights to Japan would have been suspended. In light of the above situation, although it was a total pity, discussions at the local organizing committee came to a painful conclusion that our only choice was to cancel BACELL2020.

As an alternative to holding the actual conference, this abstract book was published in pdf version and posted on the website, and at least the research announcements and the sponsoring company's advertisements can be made in such a way.

On the other hand, many participants from overseas seemed to have already arranged air tickets and accommodations, and this could have caused considerable losses. Therefore, the BACELL2020 local organizing committee decided to refund all paid registration fee, although it was ashamed that the other additional costs were unable to be covered.

Again it was an intolerable pity. The BACELL2020 local organizing committee hoped everybody would kindly understand and cooperate with this inevitable decision. Everybody wished that this world-wide calamity of the new coronavirus would end as soon as possible.

The BACELL2020 local organizing committee

Chair: Ken-ichi Yoshida, Kobe Univ

Co-chair (General affairs): Kei Asai, Tokyo Univ Agri
Accountants: Kenji Tsuge / Shu Ishikawa (auditor), Kobe Univ
External affairs: Hiromu Takamatsu, Setsunan Univ
Public relations: Nobuhiko Nomura, Tsukuba Univ

March 2020

BACELL2020 was organized by the local organizing committee and **National Bio-Resource Project**, and co-organized by the **Institute of Oceanic Research and Development, Tokai University**, and **Graduate School of Science, Technology and Innovation, Kobe University**.

BACELL2020 was financially supported by companies as follows:

Platinum supporter:

Toa Biopharma Co. Ltd.

Gold supporter:

Synplogen Co., Ltd.

Silver supporters:

Kao Corporation

Amano Enzyme Inc.

Fujicco Co., Ltd.

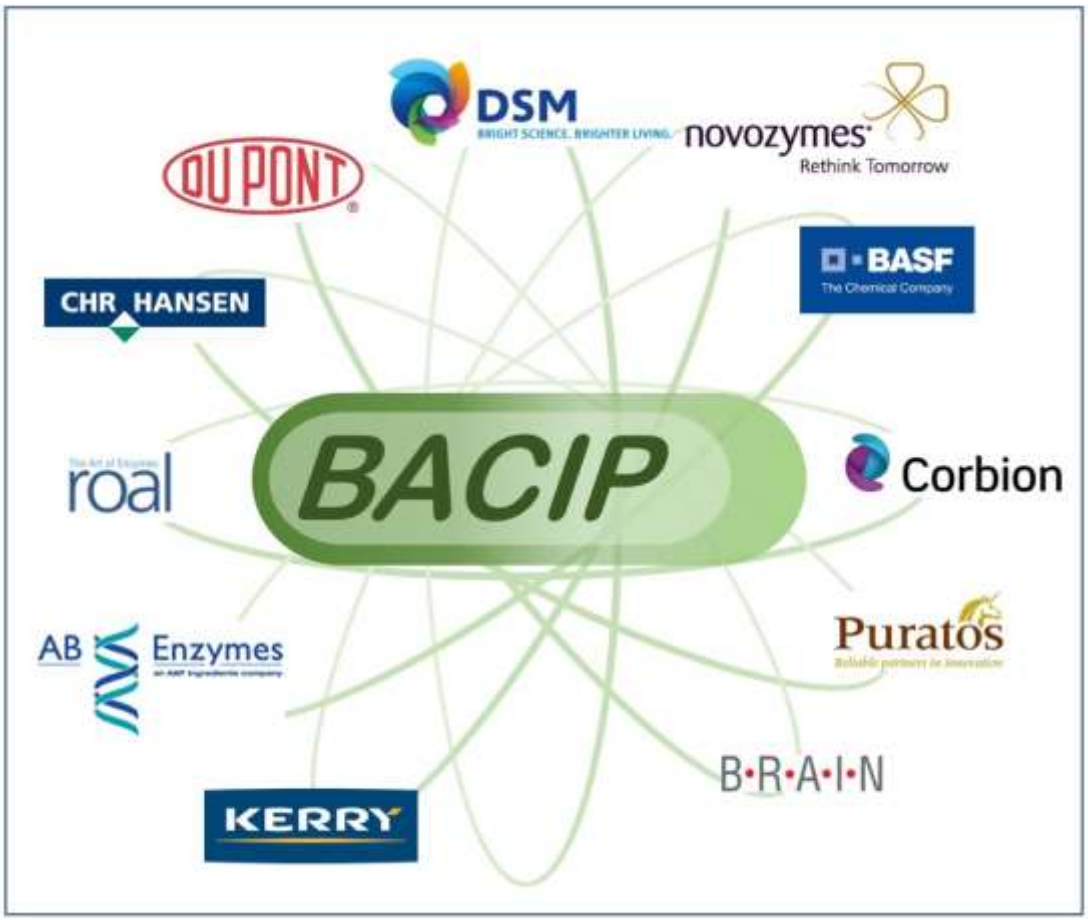
TOMY Digital Biology Co.,Ltd.

KANEKA Corporation

BACIP European student travel award

Thanking to the generous support of Bacillus Industrial Platform (BACIP), the 10 European students listed below have been selected to enjoy the travel award. The selection was done strictly based on the quality of the submitted abstracts:

Sema Ejder (Newcastle University, United Kingdom)
Timothy Hoffmann (University of Bath, United Kingdom)
Peter Klausmann (University of Hohenheim, Germany)
Patrick Lenz (Heinrich Heine University Düsseldorf, Germany)
Luiza Morawska (University of Groningen, Netherlands)
Mathilde Nordgaard Christensen (Technical University of Denmark, Denmark)
Karen Stetter (Technical University of Dresden, Germany)
Iñigo Urrutia-Irazabal (University of Bristol, United Kingdom)
Valeria Verrone (Newcastle University, United Kingdom)
Sabrina Wamp (Robert Koch Institute, Germany)



BACELL2020 Scientific Program

Monday 20th April:

Session 1: Biotechnology 1 (9:00-10:15) for 5 talks (15 min for each)

O1 Kenji Tsuge (Kobe University, JP)
"Long-chain DNA biofoundry at Kobe University"

O2 Patrick Lenz (Heinrich Heine University Düsseldorf, DE)
"Assays for online monitoring of protein production and secretion stress in *Bacillus subtilis*"

O3 Karen Stetter (TU Dresden, DE)
"A novel, fast and host-independent plasmid-based strategy for rapid, efficient and simple genetic modification in *Bacillus* sp."

O4 Philippe Jacques (University of Liège, BE)
Evolution of Primary Metabolites and Surfactin Production In Leucine
"Overproducing Mutant of *Bacillus subtilis* 168"

O5 Michael Dolberg Rasmussen (Novozymes, DK)
"Screening for increased heterologous enzyme activity in a library of 23 PrsA homologs in *Bacillus subtilis*"

Break (10:15-10:45)

Session 2; Biotechnology 2 (10:45-12:00) for 5 talks

O6 Hannah Brück (University of Liège, BE)
"Molecular strategies to adapt *B. subtilis* 168 to biofilm cultivation systems"

O7 Christopher Scheidler (Ludwig-Maximilians University Munich, DE)
"Establishing a genetic code expansion system in *Bacillus subtilis*"

O8 Adrian Geissler (University of Copenhagen, DK)
"BSGatlas: A comprehensive gene and transcript annotation for the *Bacillus subtilis* genome"

O9 Grace Goldsmit (University of Newcastle upon Tyne, UK)
"Compartmentalisation of genetic circuits for industrial application"

O10 Etienne Dervyn (INRA Micalis, FR)
"*Bacillus subtilis* genome size reduction by random deletions"

Lunch (12:00-13:00)

Rapid fire session (13:00-14:00) for 40 talks (90 sec for each)

Session 3: Biotechnology 3/Antimicrobials and toxins (14:00-15:00) for 4 talks

O11 Jan Maarten van Dijl (University of Groningen, NL)
"The SECRETERS PhD training network"
O12 Jolanda Neef (University of Groningen, NL)
"A Tale of Two Cell Factories Heterologous protein secretion in *Bacillus subtilis* and *Lactococcus lactis*"
O13 Monika Ehling-Schulz (Institute of Microbiology, Vetmeduni Vienna, AT)
"The ABC transporter CesCD is an essential component of the cereulide toxin biosynthetic NRPS complex"
O14 Moshe Shemesh (ARO Volcani Center, IS)
"Mitigating biofilm formation by magnesium ions provides a novel concept for developing healthier and safer food"

Break (15:00-15:30)

Session 4: Cell division (15:30-17:00) for 6 talks

O15 Richard Daniel (University of Newcastle upon Tyne, UK)
"Functional redundancy of PBPs in *Bacillus subtilis*"
O16 Leendert W. Hamoen (University of Amsterdam, NL)
"Cell division mechanism for cell size homeostasis in *Bacillus subtilis*"
O17 Koichi Yano (National Institute of Genetics, JP)
"Profiling single-strand DNA segments within an rRNA gene, which is a loading site for bacterial condensing"
O18 Yoshikazu Kawai (University of Newcastle upon Tyne, UK)
"Cell wall inhibition in L-forms or via β-lactam antibiotics induces ROS-mediated killing through increased glycolytic flux"
O19 Dirk-Jan Scheffers (University of Groningen, NL)
"Membrane fluidity controls peptidoglycan synthesis and MreB movement"
O20 Sabrina Wamp (Robert Koch Institute, DE)
"ReoM and ReoY connect cell wall integrity surveillance with peptidoglycan biosynthesis via an essential protein phosphorylation event"

Poster session 1 (17:00-18:00) for odd numbers

Tuesday 21st April:

Session 5: Regulation 1 (9:00-10:15) for 5 talks

O21 Colin Harwood (University of Newcastle upon Tyne, UK)
"Analysis of the *Bacillus subtilis* Signal Recognition Particle (SRP)"
O22 Emma Denham (University of Bath, UK)
"SpoVG – not really a sporulation protein?"
O23 Sylvain Durand (Institut de Biologie Physico-Chimique, FR)
"Identification of sRNA targets in *B. subtilis* by pulsed-SILAC"
O24 Shinobu Chiba (Kyoto Sangyo University, JP)
"Ribosome rescue mechanism in *Bacillus subtilis*"
O25 Ciarán Condon (Institut de Biologie Physico-Chimique, FR)
"Transfer RNA maturation defects lead to inhibition of ribosomal RNA processing via synthesis of pppGpp"

Break (10:15-10:45)

Session 6: Regulation 2 (10:45-12:00) for 5 talks (15 min for each)

O26 Zhihui Xu (Nanjing Agricultural University, CN)
"Lipopeptide bacillomycin D regulates biofilm development and root colonization through the iron acquisition pathway"
O27 Harald Putzer (Institut de Biologie Physico-Chimique, FR)
"Compartmentalization of mRNA decay in *Bacillus subtilis*"
O28 Matthieu Jules (INRA Micalis, FR)
"tRNA aminoacylation levels in bacteria are actively controlled via a (p)ppGpp-mediated translation inhibition"
O29 Ulrike Mäder (University Medicine Greifswald, DE)
"Antisense transcription in *Bacillus subtilis*: General features and physiological functions"
O30 Satoshi Matsuoka (Saitama University, JP)
"Glucolipids affect the activities of SigI, an alternative sigma factor, and WalKR, an essential two component system, in *Bacillus subtilis*"

Lunch (12:00-13:00)

Poster session 2 (13:00-14:00) for even numbers

Session 7: Evolution and mobile genetic elements (14:00-15:15) for 5 talks

O31 Nicolas Mirouze (Université Paris-Sud, FR)
"Antibiotic sensitivity reveals that wall teichoic acids mediate DNA binding during competence in *Bacillus subtilis*"

O32 Briana Burton (University of Wisconsin, USA)
"Genus- and genome- wide analysis of natural transformation events"

O33 Pauline Hinnekens (UCLouvain, BE)
"Insight into the conjugative transfer locus of pXO16 from *Bacillus thuringiensis* sv. *Israelensis*"

O34 Mathilde Nordgaard Christensen (Technical University of Denmark, DK)
"Evolution of *Bacilli* on plants roots reveals novel adaptation strategies"

O35 Grace Joyce (University of Warwick, UK)
"The impact of a horizontally acquired virulence plasmid on *Bacillus cereus* G9241, the causative agent of an anthrax-like illness"

Break (15:15-15:45)

Session 8: Microbial interaction/Sporulation and development/Stress response (15:45-17:00) for 5 talks

O36 Libor Krásný (Iinstitute of Microbiology of the Czech Academy of Sciences, CZ)
"Bacterial nanotubes: Genetic determinants, formation dynamics & biological role"

O37 Heiko T. Kiesewalter (Technical University of Denmark, DK)
"Small, but mighty: the ecology of *B. subtilis* secondary metabolites"

O38 Adriano O. Henriques (Universidade Nova de Lisboa, PT)
"Role of a *Bacillus subtilis* transglutaminase in the macromolecular assembly of the spore surface layers"

O39 Sandra Maaß (University of Greifswald, DE)
"Global absolute quantification of membrane proteins in *Bacillus subtilis*"

O40 Vasili Hauryliuk (Umeå University, SE)
"Molecular mechanisms overcoming ribosomal stalling"

Banquet (18:00-20:00)

Social program

Optional excursion (Himeji Castle Day Trip)

Date: April 22 (Wed) 10:30-16:30

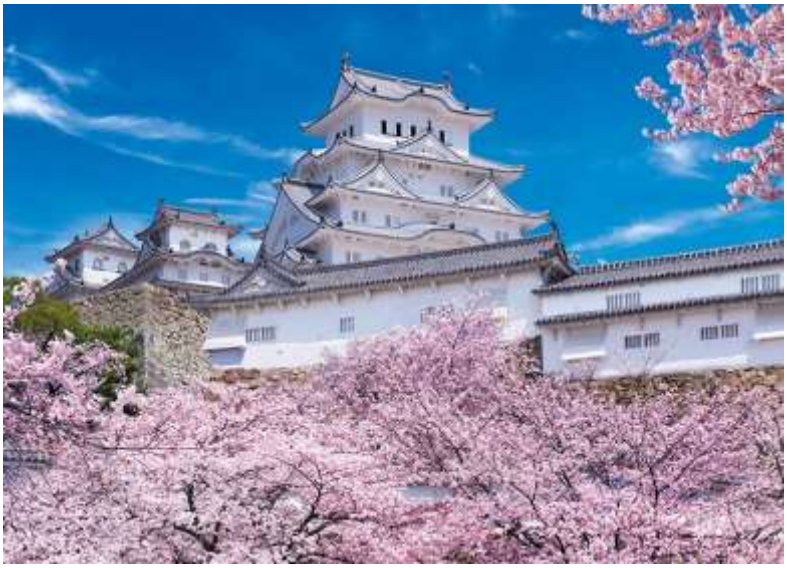
Price: JPY10,000

Includes: Transfer, English-Speaking Guide, Lunch Box, Admission fees and taxes as indicated in the itinerary. Due to the limited transport capacity, only 90 people can participate, and your earlier registration is strongly recommended.

＜ Schedule ＞

Kobe Chamber of Commerce and Industry ➔ Himeji Castle ➔ Sannomiya

Himeji Castle Himeji Castle is nicknamed "White Heron" Castle because of its white walls which are covered with white plaster. The reason for using white plaster is that it is fireproof. Like other surviving Japanese castles, Himeji Castle is built of wood, not stones, and so fire prevention is very important. As well as the fact that white plaster has fireproofing capabilities, it is also considered to be a strong reinforcement. Therefore it covers both walls and almost every outside structure from eaves to pillars. The castle is built on the top of a hill called Himeyama, which is 45.6 meters above sea level. The main tower, which is the center and the symbol of the castle, is 46.4 meters high; namely, it stands 92 meters above sea level. Himeji Castle is famous for not only this huge main tower but also the highly effective and complicated defensive design, which is just like a maze. Even today, with the route clearly marked, many visitors are easily lost. You, too, may be lost in the labyrinth.



Abstracts for Oral presentations

O1

Long-chain DNA biofoundry at Kobe University

Kenji Tsuge, and Akihiko Kondo

Kobe University, Kobe, Japan

As developing synthetic biology era, technology for synthesis long-chain DNA with arbitrary sequence at low cost is desired. Development of such kind of technology, however, requires many of improvements through the whole process. For this purpose, we have begun to develop a long-chain DNA synthesis foundry in Kobe University, where all of automation system are equipped. Our core long-chain DNA synthesis technology is OGAB method, which uses *Bacillus subtilis* plasmid transformation system. Due to trait of *B. subtilis*, OGAB method intendedly requires long tandem repeat ligation products of material DNA blocks and assembly vector in vitro. However, long tandem repeat ligation product is obtained only after precise equimolar adjustment of the blocks, to realize this, we developed unique automation system for equimolar mixing by collaboration with Precision System Science, Co. Moreover, we specially developed chemical DNA synthesizer of long DNA synthesis by collaboration with Nihon Techno Service, Co. This synthesizer can synthesize up to 200 nt of single strand DNA at low cost. Together with specially developed PCR-based single strand DNA assembly condition, we can synthesize almost all of material DNA fragment of OGAB that are including DNAs refused by commercial synthesis services. By using this total system, now we can construct long-chain DNAs up to 100 kb in size from as much as 50 of DNA blocks.

O2

Assays for online monitoring of protein production and secretion stress in *Bacillus subtilis*

Patrick Lenz[1], Kristina Volkenborn[1], Lana Groetschel[1], Marzena Malek[1], Andreas Knapp[1], Roland Freudl[2], and Karl-Erich Jaeger[1,2]

[1]*Institute of Molecular Enzyme Technology, Heinrich Heine University Düsseldorf, Germany,*
[2]*Institute of Bio- and Geoscience IBG-1: Biotechnology, Forschungszentrum Jülich GmbH, Germany.*

Bacillus subtilis is an industrially well-established host for the production and secretion of recombinant proteins. Hence, it is highly desirable to develop assays for simultaneous online monitoring of protein production and secretion stress. We therefore aim to combine the split GFP assay reporting protein production with a biosensor indicating secretion stress to construct an integrated online monitoring system for *B. subtilis*.

For the split GFP assay, the eleventh β-sheet of superfolder GFP (sfGFP) is fused to a target protein and can complement a truncated detector protein (sfGFP1-10) to form fluorescent holo-GFP. As proof of concept, we have successfully monitored the intracellular production of an *E. coli* β-glucuronidase and the secretion of a *Fusarium solani pisi* cutinase by *B. subtilis*.

For online monitoring of product formation, sfGFP1-10 must be produced by *B. subtilis* intra- or extracellularly, respectively. The intracellular detector production has already been established and β-glucuronidase can be detected qualitatively *in vivo*. For detection of extracellular proteins, the best signal peptide will initially be identified for sfGFP secretion by increased extracellular fluorescence, and subsequently transferred to sfGFP1-10.

The secretion stress biosensor senses misfolded secreted proteins at the membrane-peptidoglycan interface by the CssRS system. It was constructed by integration of a fluorescence reporter controlled by a *htrA* promoter into the *B. subtilis* genome using CRISPR/Cas9. Currently, we are evaluating the sensitivity of this biosensor with a *B. subtilis* lipase, amylase and xylanase. Next, we will combine all monitoring systems to allow for online detection of protein production and secretion.

O3

A novel, fast and host-independent plasmid-based strategy for rapid, efficient and simple genetic modification in *Bacillus sp.*

Stetter, K.[1], M. Felle[2], T. Mascher[1], and D. Wolf[1]

[1]*TU Dresden, Dresden, Germany*
[2]*BASF SE, Ludwigshafen, Germany*

(Abstract is not available.)

O4

Evolution of Primary Metabolites and Surfactin Production In Leucine Overproducing Mutant of *Bacillus subtilis* 168

Debarun Dhali[1], François Coutte[1], Michael Lalk[2], Joachim Niehren[3,4], Cristian Versari[4], Ariane Theatre[5] and **Philippe Jacques**[1,5]

[1]Université Lille, ProBioGEM team, EA 7394-ICV Institut Charles Viollette, F-59000 Lille, France; [2]University of Greifswald, Institute of Biochemistry, Greifswald 17487, Germany; [3]Université Lille, BioComputing team, CRIStAL Lab (CNRS UMR9189), Villeneuve d'Ascq, France; [4]Inria Lille, Villeneuve d'Ascq, France; [5]Microbial Processes and Interactions, TERRA Teaching and Research Centre, Gembloux Agro-Bio Tech, University of Liege, Gembloux, Belgium

Surfactin is a lipopeptide produced by *Bacillus subtilis* with biosurfactant and anti-*Legionella* activity and ability to induce systemic resistance in plant. This molecule consists of a cyclic heptapeptide containing four leucine residues linked to a β-hydroxy fatty acid chain. The isomery and the length of the fatty acid chain have a strong influence on the surfactin activities. In this study, a set of knock-out mutants were generated to increase leucine intracellular concentration and surfactin production. Genes to interrupt were chosen on the basis of a branched amino acid metabolism modelling. Three types of genes were selected: those involved in the regulation of this metabolic pathway, those encoding proteins responsible for the degradation or the exportation of the amino acid residues. The mutants were cultivated in Landy medium with $(NH_4)_2SO_4$ and at various time points the surfactin production was quantified both quantitatively and qualitatively using RP-HPLC and LC-MS/MS. The results obtained with these mutants showed a significant enhancement of specific surfactin production. Based on the mutation, pyruvate is transformed through various pathways to numerous metabolites, which are effluxed into the extracellular medium. The analysis of these metabolites was carried out using ^{1}H-NMR spectroscopy. Fluxes of 3-phosphoglycerate, Krebs cycle's metabolites and amino acid residues involved in the biosynthesis of branched chain fatty acid were particularly impacted. Finally, a co-relation between primary metabolism and surfactin production was proposed.

O5

Screening for increased heterologous enzyme activity in a library of 23 PrsA homologs in *Bacillus subtilis*

Ane Quesada-Ganuza[1,3], Karen F. Appel[1], Thomas K. Kallehauge[1], Lars Hjort Bjerre[1], Astrid Mørkeberg Krogh[1], Michael Lees[1], Morten Gjermansen[1], Peter F. Hallin[1], Mogens Kilstrup[2], **Michael D. Rasmussen[1]**, and Allan K. Nielsen[1]

[1] *Research &Technology, Novozymes A/S, Krogshøjvej 36, DK-2880 Bagsværd, Denmark*
[2] *Department of Biotechnology and Biomedicine, Technical University of Denmark (DTU), Søltofts Plads, building 221, DK-2800 Kgs. Lyngby, Denmark.*
[3] *CIC Nanogune research center in San Sebastian, at the Nanobiomechanics group.*

In microbial cell factories, the yield of heterologous protein products varies from one product to another even when industrial and biological settings are kept constant. Two closely related proteins with few amino acid differences can show very different yields in isogenic strains. In many cases the secretion rate is the major bottleneck as a consequence of a change in folding kinetics of the individual protein. PrsA foldases, when co-expressed with heterologous amylases and various other enzymes, often enhances yield by supporting post-translocational folding and proper secretion of the product. Nature offers a wide range of both industrial interesting enzymes and PrsA chaperones and choosing the right match may increase the frequency of productive interactions between the enzyme and the foldase. In this study we set out to co-express six heterologous enzymes with each of 23 heterologous PrsAs, using B. subtilis as a host. We constructed a full matrix of strains containing all combinations of the six enzymes genes and the 23 heterologous PrsAs and studied the importance of choice of PrsA for the final yield of the product. We show how the different heterologous PrsAs exhibit a diversity of specificity against different enzymes and demonstrate how a good individual match can increase the productivity.

O6

Molecular strategies to adapt *B. subtilis* 168 to biofilm cultivation systems

Hannah Brück[1,2], François Coutte[2], Frank Delvigne[1], Pascal Dhulster[2], and Philippe Jacques[1,2]

[1]*MiPI, TERRA Teaching and Research Centre, Gembloux Agro-Bio Tech, University of Liège, Avenue de la Faculté, 2B, B-5030, Gembloux, Belgium;*

[2]*Univ. Lille, INRA, ISA, Univ. Artois, Univ. Littoral Côte d'Opale, EA 7394 - ICV - Institut Charles Viollette, F-59000 Lille, France*

Biofilm cultivation is a promising alternative for the production of biosurfactants since it provides process stability through cell immobilization while foam formation is avoided. The widely used *B. subtilis* 168 strain is a potential producer of a very powerful biosurfactant called surfactin, but exhibits only poor biofilm formation capacities. In this work, filamentous *B. subtilis* 168 strains with restored exopolysaccharide production were designed to increase their ability to grow in biofilm-based processes. EPS restoration (*epsC+*) has been considered for improving the natural immobilization step of the bacteria. The strains contain also a functional *sfp+* gene required for surfactin production. Additionally, a gene involved in the cell division process (*sepF*) has been deleted to induce filamentous cell growth. At first, cell adhesion and biofilm formation capacities have been analyzed based on a low shear stress cultivation system, i.e. drip-flow reactor. Based on this device, *epsC+* mutants showed the best biofilm formation performances, whereas filamentation had a minor impact on the process. Then, cultivations in a trickle-bed biofilm reactor containing a structured metal packing with a high specific surface area for cell colonization have been performed. Even in this device with substantially higher shear stress, the *epsC+* mutants showed significantly increased adhesion capacities. In this case, cell filamentation provided a better cell cohesion and led to reduced cell detachment. The surfactin production in a continuous process was increased compared to a batch cultivation. These results point out the importance of the environmental constraints when designing new microbial strains for a specific process.

O7

Establishing a genetic code expansion system in *Bacillus subtilis*

Christopher Scheidler, and Sabine Schneider

Ludwig-Maximilians University Munich

Functionalizing proteins with new reactive groups, fluorophores and altering their physicochemical propensities has become more and more important for biotechnical and pharmaceutical applications. For biotechnological applications, the modifications can lead to a better characterization of the protein and its interaction partners. In pharmaceutical research, the solubility and the biological half-life are the two main parameters, which have to be considered when using proteins as therapeutic agents. Furthermore, protein – protein/drug – conjugates, especially antibody-drug conjugates are very desirable due to broader therapy possibilities.

However, due to the limited number of 22 canonical amino acids the number of possible modifications is low. This challenge can be overcome by Amber codon suppression, a site-specific method to modify a protein. By introducing a bioorthogonal reactive group a broad field of continuative reactions is opened. This versatile method has been established in *Escherichia coli*, *Saccharomyces cerevisiae* and in recent years in human cell lines – but not in the biotechnological highly used gram-positive prokaryote *Bacillus subtilis.*

In summary, we were able to establish an efficient Amber suppression system in *B. subtilis* using the aminoacyl–tRNA synthetase/tRNA pair from *Methanosarcina mazei.* Through a modular expression setup, every part of this system can be exchanged, allowing the production of a broad range of target proteins. With incorporation rates up to 30% and yields in the mg/l range, we are able to secrete our bioorthogonally-modified protein directly into the supernatant from where it is easily purified in its active confirmation for further tasks.

O8

BSGatlas: A comprehensive gene and transcript annotation for the *Bacillus subtilis* genome

Adrian Sven Geissler[1], Christian Anthon[1], Enrique González-Tortuero[1], Line Dahl Poulsen[2], Thomas Beuchert Kallehauge[3], Stefan Ernst Seemann[1], Jeppe Vinther[2], 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]Section for Computational and RNA Biology, Department of Biology, University of Copenhagen, Denmark;[3]Novozymes A/S, Bagsværd, Denmark

**Corresponding author, gorodkin@rth.dk*

Bacillus subtilis is a highly used model organism for which interpretation of experiments heavily rely on its genomic annotation. However, the current annotations are stored across multiple resources providing different types of content, and the information is therefore not readily available in a single combined resource. Furthermore, the quality and abundance of annotations for structured RNAs and non-coding RNA genes (ncRNA) are still substantially lower than that of coding genes. We address these challenges in the *B. subtilis* genome atlas (BSGatlas) by integrating and unifying (1) existing annotation resources, (2) including annotations for structured RNAs and ncRNAs and (3) including transcription start and termination sites (TSS and TTS) and known transcriptional units (TU). Compared to the individual resources, the BSGatlas improved annotations in several directions: The number of structured RNA and ncRNA annotations has doubled, and the positional annotation for 70% of the ncRNAs in the existing resources has been improved. The combined collection of TSSs, TTSs, and TUs implied new isoform transcripts, which allowed the deduction of 815 new TUs, 5593 untranslated regions (UTRs), and 2309 operons. Compared to any single existing resource, our integration annotates five-times more annotated UTRs and provides transcript annotations for 93% of all coding and non-coding genes, which is an increase of 13.8%. We compiled the BSGatlas according to the GFF3 standard for use in high-throughput analyses, and additionally provide an online browser for instantaneous visualization (https://rth.dk/resources/bsgatlas/).

O9

Compartmentalisation of genetic circuits for industrial application.

Grace G. Goldsmith, Aurelie Guyet, and Richard Daniel

University of Newcastle upon Tyne, Biosciences Institute, United Kingdom

Metabolic burden is imposed on the host when a portion of the essential resources such as energy and carbon are diverted to an engineered pathway used for bio-production of compounds. This project aims to isolate biosynthetic pathways or proteins that are incompatible or induce unwanted responses into anucleated cells in order to increase product yield and maintain host fitness. The first model proposed is to compartmentalise self-regulating genetic circuits into minicells, using a Δ*minC* strain. Minicells are known to contain contents from the mother cell cytoplasm at the time of division which includes ribosomes, RNA, and plasmids (Frazer and Curtiss, 1975). As an alternate approach we have developed a "maxicell" where the anucleate compartments are larger. Historically maxicells were made in DNA repair pathway mutants, DNA is removed by irradiation under UV light in order to damage the DNA and not allow repair (East and Errington, 1989) or chemical inhibition of replication. However, we have utilized a synthetic biology chassis that generates anucleated cells continuously without induction. Both studies has used a GFP reporter to show controlled compartmentalised expression using microscopy, flow cytometry and enzyme activity assays. We will present data showing that the compartmentalisation of genetic circuits is possible, and show how autonomous regulation and efficiency of plasmid segregation may be possible to optimise the system. The results obtained provide a novel way to investigate the properties of the cells cytoplasm and cellular processes in the absence of *de novo* synthesis of its components.

O10

Bacillus subtilis genome size reduction by random deletions

Dervyn, E. [1#], A.G. Planson[1#], K. Tanaka[2#], K.I. Yoshida[2], P. Noirot[1,3], and M. Jules[1]

[1]Université Paris-Saclay, INRAE, AgroParisTech, Micalis Institute, 78350, Jouy-en-Josas, France . [2]Departement Biofunc. Chem., Kobe Univ., Kobe 657-8501 Japan. [3]Biosciences Division, Argonne National Laboratory, Argonne, IL 60439 USA.

[#] contributed equally to this work

Based on our repertoire of the *Bacillus subtilis* chromosomal regions dispensable for growth in rich medium (1), we combined 28 large interval deletions by iterative marker-free deletion method. Hence, we reduced the *B. subtilis* genome down to 2.73 Mb, which corresponds to an overall reduction of 35% in comparison with the reference genome (2).

Characterization of strains with the most reduced genomes showed a strongly affected transcriptional profile and displayed significantly diminished growth. We performed continuous evolution experiments to select for better fitness. We were unable to select reduced-genome strains with a significant fitness increase, although we obtained several evolved parental strains with a significant fitness increase. Genome sequencing of clones from the parental evolved strains identified several mutations. The most promising mutations, re-introduced in the genome-reduced strains, allowed for a significant increase in fitness. Further analysis revealed that genome-reduced strains exhibit a much lower mutation rate than the parental strain, as well as a decrease in mitomycin sensitivity. In genome-reduced strains, increasing the mutation rate, allowed for the selection of reduced-genome strains with better fitness. Altogether, our results suggest that reduced-genome strains were unable to accumulate mutations as they fell in a dead-end evolutionary status. Beyond its relevance in basic science, we will discuss the implication of such conclusion in the field of the synthetic biology.

1- Tanaka K, *et al*.. Nucleic Acids Res, 2013. **41**:687-99.

2- BaSynthec consortium (FP7-244093 EU project)

O11

The SECRETERS PhD training network

Jan Maarten van Dijl

University Medical Center Groningen-University of Groningen, Groningen, the Netherlands.

The 'SECRETERS' partnership aims to develop new concepts for the production of recombinant proteins, particularly Biotherapeutics and Industrial Enzymes. Both are critical products for the biotechnology sector, with combined markets in excess of $140 billion p.a.; the former are essential for treatment of major diseases, whereas the latter permeate every aspect of our daily life. Many proteins in these categories pose severe problems in production, especially disulphide-bonded proteins and new format 'difficult-to-express' proteins. SECRETERS has started training a team of 15 Early Stage Researchers to develop a new generation of microbial production hosts, including *Bacillus* species, *Escherichia coli* and the yeast *Pichia pastoris*. The project capitalises on a series of recent innovations and involves close collaboration between 5 academic partners with expertise in redox chemistry, synthetic biology and protein expression, and 5 non-academic partners, who include biotherapeutic and industrial enzyme companies. The developed concepts may enable production of challenging medicines at lower costs, resulting in new drugs and wider patient access, and allow the enzymes industry to deliver a range of new products.

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O12

A Tale of Two Cell Factories
Heterologous protein secretion in *Bacillus subtilis* and *Lactococcus lactis*

Jolanda Neef, Girbe Buist*, and Jan Maarten van Dijl*,

Department of Medical Microbiology, University of Groningen, University Medical Center Groningen, Hanzeplein 1, P.O. Box 30001, 9700 RB Groningen, the Netherlands.
**These authors contributed equally*

Secreted recombinant proteins are of great significance for industry, healthcare and a sustainable bio-based economy. Consequently, there is an ever-increasing need for efficient production platforms to deliver such proteins in high amounts and high quality. Gram-positive bacteria, particularly bacilli such as *Bacillus subtilis*, are favoured for the production of secreted industrial enzymes. Nevertheless, protein production in the *B. subtilis* cell factory can be very challenging due to bottlenecks in the general (Sec) secretion pathway as well as this bacterium's intrinsic capability to secrete a cocktail of highly potent proteases. This has placed another Gram-positive bacterium, *Lactococcus lactis*, in the focus of attention as an alternative, non-proteolytic, cell factory for secreted proteins. We present our current understanding of the pro's and con's of the general secretion machinery employed by *B. subtilis* and *L. lactis* to guide proteins from their site of synthesis, the cytoplasm, into the fermentation broth. In addition, we address species-specific signatures of signal peptide that direct proteins into the respective secretory pathways. An advantage of this cell factory comparison is that it identifies new opportunities for protein secretion pathway engineering to remove or bypass current production bottlenecks. Altogether, it is foreseen that engineered lactococci will find future applications in the production of high-quality proteins at the relatively small pilot scale, while engineered bacilli will remain a favoured choice for protein production in bulk.

O13

The ABC transporter CesCD is an essential component of the cereulide toxin biosynthetic NRPS complex

Gacek-Matthews Agnieszka[1], Z. Chromikova [1,2], Stelli Stancheva[1], Timo Stark[3], Genia Lücking[4], Imrich Barak[2], and **Monika Ehling-Schulz**[1]

[1]*Institute of Microbiology, Vetmeduni Vienna, Vienna, Austria,*
[2]*Institute of Molecular Biology, Slovak Academy of Sciences, Bratislava, Slovakia,*
[3]*Chair of Food Chemistry and Molecular Sensory Science, Technische Universität München, Freising, Germany*
[4]*ZIEL Institute for Food and Health, Technische Universität München, Freising, Germany*

Bacillus cereus is an opportunistic human pathogen, which provokes panoply of local and systemic diseases [1]. The emetic strains, harbouring a virulence mega plasmid similar to *B. anthracis* pOX1, produce the extremely thermo- and pH- resistant toxin, cereulide. This non-ribosomal peptide, pre-formed in food contaminated by *B. cereus*, is responsible for the emetic form of *B. cereus* foodborne disease and occasionally leads to rhabdomyolysis, acute liver failure and death. The cereulide biosynthetic genes are organized within the *ces* operon, including the structural non-ribosomal peptide synthetase genes *cesA* and *cesB* as well as the putative ABC drug efflux transporter *cesC* and *cesD*. Disruption of this putative ABC drug efflux pump results in a cereulide negative phenotype, despite the wild type level of CesAB. These results suggest a scaffolding function of CesCD as component of toxin biosynthetic machinery. Indeed, as revealed by a bacterial two hybrid screen and a pull-down assay, CesA/B interacts with CesC in vitro. To confirm these *in vitro* interactions, *in vivo* co-localization studies were carried out using *Bacillus subtilis* as a heterologous host. Furthermore, site-directed mutagenesis studies were performed to identify the molecular interaction between CesA/B and CesC/D. In summary, our *in vitro* and *in vivo* studies identified the ABC transporter CesC/D as an essential component of the non-ribosomal peptide biosynthetic machinery for cereulide toxin. To our knowledge this is the first report of an ABC transporter directly involved in the non-ribosomal peptide biosynthetic machinery.

[1] Ehling-Schulz et al., 2019. Microbiol. Spectrum 6(1): GPP3-0032-2018.

O14

Mitigating biofilm formation by magnesium ions provides a novel concept for developing healthier and safer food

Keren Demishtein, and **Moshe Shemesh**

Department of Food Sciences, Institute for Postharvest Technology and Food Sciences, Agricultural Research Organization (ARO) The Volcani Center, Israel.

Biofilm is the predominant form of bacterial living in natural and industrial settings. Biofilm formation depends on the synthesis of an extracellular matrix that holds constituent cells together. In the model bacterium *Bacillus subtilis*, expression of the matrix genes is induced by environmental cues via a signal transduction pathway. Biofilm triggering molecules presumably interact with components of the cytoplasmic membrane. Our results suggest that cardiolipin (CL), an anionic membrane phospholipid, plays a key role in inducing biofilm formation by *B. subtilis*. Moreover, we find that the CL-induced biofilm formation is mitigated by magnesium ions. Our further findings indicate about high sensitivity of bacterial cells, in the presence of Mg^{2+} ions, to heat pasteurization undertaken during food processing. The downregulation in the expression of genes responsible for the production of the biofilm matrix could explain the increased sensitivity of bacterial cells to heat treatments. Besides, we find an improvement in the technological properties of food products such as soft cheeses following the fortification of milk with magnesium. Thus, we believe that the enrichment of food with magnesium provides an opportunity for developing healthier, safer, and qualitative food products.

O15

Functional redundancy of PBPs in *Bacillus subtilis*

Sassine, J., G. Goldsmith, A. Guyet, and **R. Daniel**

Centre for Bacterial Cell Biology, Newcastle University Biosciences Institute, Newcastle, UK.

The genome of Bacillus subtilis encoded multiple penicillin binding protein of which some have been assigned functions in either vegetative growth or sporulation, but most seem to have redundant roles under normal culture conditions. During vegetative growth 8 PBPs are easily detected by penicillin as being expressed, but of those only 4 have functional roles attributed to them (PBP 2B, PBP 2A, PonA and DacA) and only 2 seem to have essential functions (PBP 2B and PBP 2A).

This functional redundancy complicates understanding the biological roles of individual PBPs and so we have constructed a systematic set of deletion strains that exhibit normal vegetative growth. In parallel, we have investigated the potential use of β-lactams and cephaolsporins as an alternative to gene deletion where the protein is still present, but in an inactive state. Through this work we have determined the minimal set PBPs and have characterised the accessory roles of apparently functionally redundant PBPs. This analysis has started to indicate interactions domains that are unique to the essential PBPs and provide an understanding of how they are integrated into specific complexes to direct cell wall assembly.

Here we will present our characterisation of specific PBPs and how they function in the cell during cell growth in *B. subtilis*, combined with other results form collaborators that suggest that the properties we have determined may be conserved in other bacterial species.

O16

Cell division mechanism for cell size homeostasis in *Bacillus subtilis*

Edward. A. de Koning, Norbert O. E. Vischer, Marten Postma, and **Leendert W. Hamoen**

Swammerdam Institute for Life Sciences, University of Amsterdam, Science Park 904, 1098XH Amsterdam, the Netherlands
Correspondence: l.w.hamoen@uva.nl, Tel: 0031–0205252193

In many bacteria cell size homeostasis is controlled by an adder model. How this is achieved is not yet known. We reasoned that the variation in timing in divisome assembly may be responsible for adder regulation. To investigate this, time lapse microscopy was done of single *Bacillus subtilis* cells expressing both fluorescently labeled early and late cell division proteins, ZapA and Pbp2B, respectively, to time the formation of Z–rings, recruitment of late proteins and septum synthesis. For WT, variation in birth size negatively correlates with divisome maturation, from Z–ring formation to constriction. Mutants affected either in Z–ring formation (ΔsepF and FtsZ over–expression) or late complex stability (ΔdivIB and ΔrasP) are generally born larger and the longer birth sizes negatively correlate to Z–ring initiation, with the largest cells already starting ring formation before birth. For all cells a continuous increase in both ZapA and Pbp2B intensity at mid–cell is observed, with reproducible average intensities at the subsequent division steps. ZapA intensity negatively correlates to division step progression, with high ZapA intensity preceding shorter step intervals. We propose that increasing divisome threshold levels incorporate growth dependent accumulation of cell division proteins, allowing for cell size homeostasis by directly connecting growth to cell division.

O17

Profiling single-strand DNA segments within an rRNA gene, which is a loading site for bacterial condensin

Koichi Yano[1], Hideki Noguchi[2], and Hironori Niki[1]

[1]*Microbial Physiology Laboratory (National Institute of Genetics, Mishima, Japan),* [2]*Center for Genome Informatics (Research Organization of Information and Systems, Mishima, Japan)*

Condensin is a crucial player in compaction and segregation of bacterial chromosomal DNA. We reported that the *Bacillus subtilis* SMC condensin complex is loaded onto rRNA genes near replication origin (Yano and Niki. *Cell Rep*. 2017). Since bacterial condensin topologically preferentially binds to ssDNA rather than double-stranded DNA, we reason that a single-stranded DNA (ssDNA) segment in a highly transcribed rRNA gene is a loading site for the SMC condensin complex. To confirm the involvement of the ssDNA segment in the loading of bacterial condensin, we analyzed C to T nucleotide-conversion efficiency in the rDNA gene after the treatment of cells with sodium bisulfite. Cytosine residues in the ssDNA segment are converted to uracil by sodium bisulfite, but not in the dsDNA segment. After PCR amplification, uracil residues are converted to thymine. Therefore, the C to T nucleotide-conversion efficiency helps identify the ssDNA segment in the rRNA gene in vivo. As a result of the sodium bisulfite DNA treatment, a 400-bp DNA segment, located just downstream of the rDNA promoter tended to be ssDNA due to R-loop formation. These results suggest that the ssDNA segment in R-loop is crucial for the loading of the SMC condensin complex on chromosomal DNA.

O18

Cell wall inhibition in L-forms or via β-lactam antibiotics induces ROS-mediated killing through increased glycolytic flux

Yoshikazu Kawai, and Jeff Errington

Biosciences Institute, Newcastle University, Newcastle upon Tyne, UK

L-forms are strange bacterial variants that lack the normally essential cell wall. They can proliferate in the presence of cell wall active antibiotics such as β-lactams under osmoprotective conditions, including host environments. Remarkably, L-forms do not require the FtsZ-based division machine, which is normally essential, for bacterial proliferation. Since their discovery back to the 1930s there have been many reports on the possible role of L-forms in chronic or recurrent infections, but the molecular events underlying switching to and from the cell wall-deficient L-form state were poorly understood.

Here we show that the abnormal generation of ROS in the L-form transition correlates with an increase in glycolytic activity in *B. subtilis* and probably other Gram-positive bacteria. This leads to the production of reactive oxygen species from the respiratory chain, which prevents L-form growth. Compensating for the metabolic imbalance by slowing down glycolysis, activating gluconeogenesis or depleting oxygen enables L-form growth.

Our results show how a metabolic diversion induced in L-forms or by β-lactams contributes to cell killing in *B. subtilis* and provide an understanding of the conditions that influence the ability of cells to grow in the L-form state.

O19

Membrane fluidity controls peptidoglycan synthesis and MreB movement

Aleksandra Zielińska[#,1], Abigail Savietto[#,2,7], Anabela de Sousa Borges[1], Joël R. Roelofsen[1], Alwin M. Hartman[3,4,5], Rinse de Boer[6], Ida J. van der Klei[6], Anna K. H. Hirsch[3,4,5], Birgit Habenstein[8], Marc Bramkamp[*,2,7], and **Dirk-Jan Scheffers**[*,1]

[1]Molecular Microbiology, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, the Netherlands; [2]Biozentrum, Ludwig-Maximilians-Universität München, Germany; [3] Department of Drug Design and Optimization (DDOP), Helmholtz-Institute for Pharmaceutical Research Saarland (HIPS) - Helmholtz Centre for Infection Research (HZI), Saarbrücken, Germany; [4]Department of Pharmacy, Saarland University, Saarbrücken, Germany; [5]Stratingh Institute for Chemistry, University of Groningen, The Netherlands; [6]Molecular Cell Biology, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, the Netherlands. [7]Institute for General Microbiology, Christian-Albrechts-University Kiel, Germany. [8]IECB Institut Europeen de Chimie et Biologie, 33607 Pessac, France

The bacterial plasma membrane is an important cellular compartment. In recent years it has become obvious that protein complexes and lipids are not uniformly distributed within membranes. Current hypotheses suggest that flotillin proteins are required for the formation of complexes of membrane proteins including cell-wall synthetic proteins. We show here that bacterial flotillins are important factors for membrane fluidity homeostasis. Loss of flotillins leads to a decrease in membrane fluidity that in turn leads to alterations in MreB dynamics and, as a consequence, in peptidoglycan synthesis. These alterations are reverted when membrane fluidity is restored by a chemical fluidizer. In vitro, the addition of a flotillin increases membrane fluidity of liposomes. Our data support a model in which flotillins are required for direct control of membrane fluidity rather than for the formation of protein complexes via direct protein-protein interactions.

O20

ReoM and ReoY connect cell wall integrity surveillance with peptidoglycan biosynthesis via an essential protein phosphorylation event

Sabrina Wamp[1], Zoe J.Rutter[2], Jeanine Rismondo[1,3], Claire E. Jennings[4], Lars Möller[5], Richard J. Lewis[2], and Sven Halbedel[1]

[1] *FG11 Division of Enteropathogenic bacteria and Legionella, Robert Koch Institute, Burgstrasse 37, 38855 Wernigerode, Germany;*
[2] *Institute for Cell and Molecular Biosciences, University of Newcastle, Newcastle upon Tyne, NE2 4HH, United Kingdom;*
[3] *Section of Microbiology and MRC Centre for Molecular Bacteriology and Infection, Imperial College London, London SW7 2AZ, United Kingdom;*
[4] *Newcastle Drug Discovery, Northern Institute for Cancer Research, Paul O'Gorman Building, Medical School, Framlington Place, Newcastle upon Tyne NE2 4HH, UK;*
[5] *ZBS 4 - Advanced Light and Electron Microscopy, Robert Koch Institute, Berlin, Germany.*

In *Listeria monocytogenes* and its close relative *Bacillus subtilis*, the PASTA-domain containing serine/threonine protein kinase PrkA/PrkC is important for the regulation of many cellular processes including cell wall homeostasis by sensing peptidoglycan (PG) fragments (1,2).
We identified a novel PrkA substrate in *L. monocytogenes* that is also involved in ClpCP-dependent degradation of MurA (Lmo2526), the first enzyme of peptidoglycan biosynthesis that we therefore renamed ReoM (regulator of MurA degradation). Signal transmission from ReoM to ClpCP required ReoY, another novel factor that we identified here. ReoM and ReoY are conserved among the Bacilli and their corresponding homologues in *B. subtilis* are the unstudied YrzL and YpiB proteins, respectively. Regulated proteolysis of MurAA (the MurA homologue of *B. subtilis)* and other ClpCP substrates also required YrzL and YpiB in *B. subtilis*, indicating a conserved and general role for these proteins in ClpCP-dependent degradation. Biochemical and structural analyses of ReoM revealed Thr-7 as the phosphorylation site. Remarkably, the expression of a phospho-ablative T7A *reoM* allele was lethal. Analyzing the MurA levels in this mutant showed that the MurA amount is reduced compared to wild-type, and the same effect could be observed upon depletion of the PrkA kinase. These results show that the phosphorylation of ReoM by PrkA is essential for viability of *L. monocytogenes*, since it controls the degradation of another essential enzyme, MurA, and thereby provides a novel link between peptidoglycan biosynthesis and cell wall integrity sensing by PASTA-domain containing serine/threonine protein kinases.

1 - Pensinger et al., 2016. PLoS Pathog. 12(11):e1006001.
2 - Libby et al., 2016. PLoS Pathog. 11(6): e1005275.

O21

Analysis of the *Bacillus subtilis* Signal Recognition Particle (SRP)

Ismatul N.A. Ismail, Susanne Pohl, and Colin Harwood

Centre for Bacterial Cell Biology, Newcastle University, Newcastle upon Tyne, UK

The signal recognition particle is a ribonucleoprotein complex required for the secretion of membrane proteins. Much of what is currently understood about bacterial SRPs is derived from work on *Escherichia coli* with relatively few studies on Gram-positive bacteria. However, the structures of the SRPs in Gram-positive and Gram-negative bacteria are distinct, the former having two domains ("S" and "Alu"), the latter just one ("S"). This means that while Gram-positive and Gram-negative SRPs have similar roles, their modes of action are likely to be mechanistically distinct. Previous work indicated that the *Bacillus subtilis* SRP consisted of a 271-nucleotide small cytoplasmic RNA (scRNA) and two nucleotide binding proteins, the fifty-four-homologue protein (Ffh) and the histone-like protein (HBsu) We have revisited the composition of the *B. subtilis* SRP with the aim of gaining insights into the intracellular events associated with co-translational membrane protein secretion. Our data indicate that the previously reported binding of HBsu to scRNA is likely to be an artefact of its non-specific affinity for nucleic acids. In contrast YlxM and elongation factor Tu (EF-Tu) bind specifically and with high affinity to scRNA, in the region that links the Alu and S domains. While the role of YlxM is poorly understood, EF-Tu has a well-established role in relation to the entry of charged tRNA to the peptidyl transferase centre (PTC) of the ribosome. Our data suggests EF-Tu and YlxM have a role in stabilising the SRP/ribosome complex responsible for bringing about translation arrest, prior to its interaction with the translocation machinery.

O22

SpoVG – not really a sporulation protein?

Swati Mahapatra[1,3], Niki Christopoulou[2], Pedro Arede Rei[2] and, Juan H Fernaud[1], Tauqeer Alam[1], Chrystala Constantinidou[1], Sander Granneman[2], and **Emma L Denham[3]**

University of Warwick, Warwick, UK[1], University of Edinburgh, Edinburgh, UK[2], University of Bath, Bath, UK[3]

SpoVG was first identified in *Bacillus subtilis* as a stage V sporulation protein and was shown to mildly affect the process of spore development. When the Δ*spoVG* mutation is combined with other sporulation gene s*poIIB* the process of sporulation is blocked. 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 CLASH and RNA-Seq we have identified SpoVG as a global RNA-binding protein with affinity for specific RNA-binding motifs. It appears to be unlike any known RNA-binding protein in bacteria. Most of the RNAs highly bound by SpoVG encode proteins involved in central metabolism and antimicrobial compounds. We also carried out proteomics and RNA-Seq on growing and sporulating cultures to compare protein and RNA levels between the wild-type and a *spoVG* deletion strain. Several biological processes were significantly altered in these data sets including stress responses, motility, competence, secretion, iron-metabolism and biosynthesis of antimicrobial compounds. We also show that the *spoVG* deletion strain is perturbed in biofilm formation, swarming motility and growth with alternate carbon sources. 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.

O23

Identification of sRNA targets in *B. subtilis* by pulsed-SILAC

Delphine Allouche, Gergana Kostova, Marion Hamon, Christophe Marchand, Ciarán Condon, and **Sylvain Durand**

UMR8261 (CNRS, Université de Paris), Institut de Biologie Physico-Chimique, Paris, France.

Small non-coding RNAs (sRNA) play a key role in modulating genetic expression in prokaryotes. In most cases, sRNAs modify translation and/or degradation of their mRNA targets. Few sRNAs have been characterized in Gram-positive bacteria and identification of their mRNA targets is still challenging. In most studies, RNAseq is used to identify sRNAs targets. However, it is known that sRNAs can also affect mRNA translation without affecting mRNA stability, rendering RNAseq inefficient to identify these targets. Direct measurement of protein levels by label-free mass spectrometry does not completely solve this problem, since down-regulation of translation can be difficult to measure in short periods, because of intrinsic protein stability. To circumvent these biases, we performed a quantitative proteomic experiment by pulsed-SILAC (Stable Isotope Labeling by amino ACids) allowing measurement of new protein synthesis after a short overproduction of the RoxS sRNA in *B. subtilis*. Medium or heavy lysine were added to cultures we wished to compare at the same time as the sRNA was induced. We measured individual protein levels by mass spectrometry in *ΔroxS* strains expressing or not RoxS under control of an inducible promoter. We confirmed the efficiency of this method by identifying numerous known and new targets of RoxS and compared it to more classical RNAseq experiments performed in parallel. Although our data suggest that this mechanism of regulation is not predominant in *B. subtilis*, we were able to identify a number of genes (for example *ycsA* and *moaC*) that are only affected at the translational level by RoxS.

O24

Ribosome rescue mechanism in *Bacillus subtilis*

Naomi Shimokawa-Chiba[1], Claudia Müller[2], Keigo Fujiwara[1], Bertrand Beckert[2], Koreaki Ito[1], Daniel N. Wilson[2], and **Shinobu Chiba**[1]

[1] *Faculty of Life Sciences and Institute for Protein Dynamics, Kyoto Sangyo University, Kyoto, Japan*

[2] *Institute for Biochemistry and Molecular Biology, University of Hamburg, Hamburg, Germany*

Translation termination and subsequent dissociation of the ribosomes from mRNA are crucial for the initiation of the next round of translation. Such ribosomal recycling is essential to maintain cellular translation capacity. However, translation of the mRNA that lacks the stop codon (non-stop mRNA) will stall the ribosomes on the mRNA until the ribosome rescue systems liberate the stalled ribosomes. While bacteria have a conserved ribosome rescue mechanism, called trans-translation, some bacterial species also possess alternative ribosome rescue systems. One of them is the release factor (RF)-dependent ribosome rescue mechanism, which enables an RF to catalyze stop codon-independent translation termination. The RF-dependent rescue factors have been previously reported only for Gram-negative bacteria, as exemplified by *Escherichia coli* ArfA. We now discover that the Gram-positive *Bacillus subtilis* has an evolutionarily distinct ribosome rescue factor named BrfA. Genetic analysis shows that *B. subtilis* requires the function of either trans-translation or BrfA for growth. Biochemical and cryo-EM characterization demonstrates that BrfA binds to non-stop stalled ribosomes, recruits RF2, but not RF1, and induces its transition into an open, active conformation. Although BrfA shares no detectable sequence similarity with *E. coli* ArfA, they use convergent strategies in terms of mode of ribosome rescue, recognition of stalled ribosomes, and expression regulation.

O25

Transfer RNA maturation defects lead to inhibition of ribosomal RNA processing via synthesis of pppGpp

Aude Trinquier, Jonathan E. Ulmer, Laetitia Gilet, Sabine Figaro, Philippe Hammann, Lauriane Kuhn, Frédérique Braun, and **Ciarán Condon**

UMR8261 (CNRS-Université Paris Diderot), Institut de Biologie Physico-Chimique, Paris, France

Ribosomal RNAs and transfer RNAs universally require processing from longer primary transcripts to become functional for translation. Here we describe an unsuspected link between tRNA maturation and the 3' processing of 16S rRNA, a key step in preparing the small ribosomal subunit for interaction with the Shine-Dalgarno sequence in prokaryotic translation initiation. We show that an accumulation of either 5' or 3' immature tRNAs triggers RelA-dependent production of the stringent response alarmone (p)ppGpp in the Gram-positive model organism *Bacillus subtilis*. The accumulation of (p)ppGpp and accompanying decrease in GTP levels specifically inhibit 16S rRNA 3' maturation. We suggest that cells can exploit this mechanism to sense potential slow-downs in tRNA maturation and adjust rRNA processing accordingly to maintain the appropriate functional balance between these two major components of the translation apparatus.

O26

Lipopeptide bacillomycin D regulates biofilm development and root colonization through the iron acquisition pathway

Zhihui Xu, Ines Mandic-Mulec, Yan Liu, Xinli Sun, Weibing Xun, Nan Zhang, Ruifu Zhang, and Qirong Shen

Nanjing Agricultural University, Nanjing, Peoples R China.

Bacillus spp. produce a wide range of secondary metabolites, including antibiotics, which have been well studied for their antibacterial properties but less so as signaling molecules. Previous results indicated that lipopeptide bacillomycin D is a signal that promotes biofilm development of *Bacillus velezensis* SQR9. However, a mechanism behind this signaling is still unknown. Here we showed that bacillomycin D promotes biofilm development by promoting the acquisition of iron. Bacillomycin D promotes the transcription of iron ABC transporter FeuABC by binding to its transcription factor Btr. These actions increase intracellular iron concentration, activate the KinB-Spo0A-SinI-SinR dependent synthesis of biofilm matrix components. We demonstrate that this strategy is beneficial for biofilm development and competition with the *Pseudomonas fluorescens* PF-5. Our results unravel an antibiotic dependent signaling mechanism that links iron acquisition to biofilm development and ecological competition.

O27

Compartmentalization of mRNA decay in *Bacillus subtilis*

Lina Hamouche[1,3], Cyrille Billaudeau[2], Arnaud Chastanet[2], Soumaya Laalami[1], and **Harald Putzer[1]**

[1] *CNRS, Université de Paris, Institut de Biologie Physico-Chimique, Paris, France.*
[2] *Micalis Institute, INRA, Université Paris-Saclay, Jouy-en-Josas, France.*
[3] *LMGM, CBI, CNRS Université de Toulouse, Toulouse, France*

Studies of prototypical gram-negative and gram-positive bacteria have revealed the major enzymes involved in the initiation of mRNA degradation, i.e. RNase E and RNase Y. RNase Y initiates global mRNA decay via an endonucleolytic cleavage, as shown in the Gram-positive model organism *B. subtilis.* This enzyme is tethered to the inner cell membrane, a pseudo-compartmentalization coherent with its task of initiating mRNA cleavage/maturation of mRNAs which are translated at the cell periphery. Here, we used total internal reflection fluorescence microscopy (TIRFm) and single particle tracking (SPT) to visualize RNase Y and analyze its distribution and dynamics in living cells. We find that RNase Y diffuses rapidly at the membrane in the form of dynamic short-lived foci. Unlike RNase E, the major decay initiating ribonuclease in *E.coli*, the formation of foci is not dependent on the presence of RNA substrates. On the contrary, RNase Y foci become more abundant and increase in size following transcription arrest, suggesting that they do not constitute the most active form of the nuclease. A biofilm related protein complex has previously been shown to act as a specificity factor for RNase Y activity *in vivo*. We demonstrate that in its absence the number and size of RNase Y foci at the membrane increases. Our data suggest that modulation of the assembly status of RNase Y can alter the specificity of this key enzyme towards certain substrates like polycistronic mRNAs.

O28

tRNA aminoacylation levels in bacteria are actively controlled *via* a (p)ppGpp-mediated translation inhibition

Baudier, C.[1,2], M. Calabre[1], S. Aymerich[1], V. Fromion[2,‡], and **M. Jules**[1,‡]

[1] *Université Paris-Saclay, INRAE, AgroParisTech, Micalis Institute, 78350, Jouy-en-Josas, France*; [2] MaIAGE, INRA, Université Paris-Saclay, Jouy-en-Josas, F78350, France.

The key mechanism involved in cell adaptation to nutritional downshifts is known as the stringent response and relies on the interaction between the RelA/SpoT proteins and two metabolites with antagonistic effects, GTP and (p)ppGpp. In *Bacillus subtilis*, the (p)ppGpp exerts a direct control over intracellular GTP by inhibiting the activity of enzymes involved in GTP biosynthesis, and reduces translation *via* IF2. We propose that (p)ppGpp accumulation prevents translational errors (TEs) by reducing the number of translating ribosomes while amino acids are being depleted.

We test this hypothesis using a GFP-based reporter system to detect TEs in *B. subtilis*. During steady-state growth in poor medium, we show that higher TE rates are observed for a strain unable to synthesize (p)ppGpp (called (p)ppGpp^0) as compared to the WT. TEs are prevented by reducing intracellular GTP (*i.e.* by modulating GTP synthesis or controlling the supply of exogenous guanosine). During growth in rich medium, WT and (p)ppGpp^0 cells show similar TE rates, but during the transition to stationary phase, TEs peak in the (p)ppGpp^0 strain. The burst of TEs is directly proportional to GTP abundance, suggesting that GTP is a key determinant, though a close-to-zero GTP abundance during the transition does not fully abolish TEs. However, TEs are fully abolished in (p)ppGpp^0 cells upon the addition of drugs that reduce translation initiation during the transition. Altogether our results suggest that (p)ppGpp acts as the safeguard against TEs during growth adaptation in *B. subtilis* by inhibiting translation initiation, hence controlling tRNA aminoacylation levels.

O29

Antisense transcription in *Bacillus subtilis*: General features and physiological functions

Hermann Rath[1], Alexander Reder[1], Tamara Hoffmann[2], Erhard Bremer[2], Uwe Völker[1], and **Ulrike Mäder**[1]

[1] *Interfaculty Institute for Genetics and Functional Genomics, University Medicine Greifswald, Greifswald, Germany;* [2] *Laboratory for Microbiology, Department of Biology, Philipps-University Marburg, Marburg, Germany*

In a large-scale transcriptome study of *Bacillus subtilis* exposed to a wide range of nutritional and environmental conditions, antisense RNAs (asRNAs) were detected for 13 % of all protein-coding genes [1]. asRNAs have the potential to regulate their sense mRNA counterparts by various mechanisms, which rely on either base-pairing interactions or transcription interference. Many asRNAs are 3'-extensions of mRNAs resulting from lack of a termination site or read-through transcription, hence generated by imperfect control of transcription termination. In *B. subtilis*, another large fraction of asRNAs appear to be byproducts of spurious promoter recognition by condition-specific alternative RNA polymerase sigma factors. This data will be discussed in the context of the ongoing debate on the biological function of pervasive transcription, with particular focus on comparative analyses of different Gram-positive bacteria [2]. So far, only few of the identified asRNAs have been functionally characterized. One group of regulatory asRNAs is synthesized as part of the SigB-controlled general stress regulon of *B. subtilis* and their physiological roles within the general stress response are of particular interest [e.g. 3]. We discovered that transient SigB-dependent expression of asRNA S1290 is directly responsible for time-delayed osmotic induction of the operon encoding the choline-specific osmostress protectant uptake system OpuB. This is of ecophysiological relevance because the closely related OpuB and OpuC transporters thus not only possess different substrate specificities, but also different regulatory patterns. In response to acute osmotic stress, cells initially rely on the transport activity of the promiscuous OpuC system and only subsequently fully induce *opuB*.

[1] Nicolas *et al.* (2012) *Science*; [2] Mäder *et al.* (2016) *PLoS Genet*; [3] Mars *et al.* (2015) *Biochim Biophys Acta*

O30

Glucolipids affect the activities of SigI, an alternative sigma factor, and WalKR, an essential two component system, in *Bacillus subtilis*

Satoshi Matsuoka[1], Yoko Shimizu[1], Kaori Nobe[1], Kouji Matsumoto[1], Kei Asai[2], and Hiroshi Hara[1]

[1]*Saitama University, Saitama, Japan*
[2]*Tokyo University of Agriculture, Setagaya-Ku, Japan*

In a *Bacillus subtilis ugtP* mutant lacking glucolipids, SigI was activated in the log phase and the activation of SigI in the mutant was suppressed by expression of inherent *ugtP*, but not by *Acholeplasma laidlawii* heterologous glucolipid synthase. In the absence of RsgI, the SigI cognate membrane anti-sigma protein, no further activation of SigI in the *ugtP* mutant was observed. A series of mutation analyses of the *sigI* promoter revealed that both WalR binding sites regulate the *sigI* promoter positively as reported by Huang *et al*., and the effects of both binding sites and SigI were enhanced in the *ugtP* mutant. We found that not only SigI but also WalKR, the essential two component system in *B. subtilis*, was activated by lack of glucolipids. Phosphorylation of WalR was accelerated in the *ugtP* mutant. By contrast, WalR shifted to the de-phosphorylated form when *ugtP* was induced at *amyE* locus in the *ugtP* mutant cells. walK mutants exhibited morphology similar to that of the *ugtP* mutant, and the morphology of the mutants were suppressed by addition of $MgSO_4$, which was also observed in the case of the *ugtP* mutant. We conclude that glucolipids synthesized by UgtP are key compounds to maintain normal cell surface structure in *B. subtilils*.

O31

Antibiotic sensitivity reveals that wall teichoic acids mediate DNA binding during competence in *Bacillus subtilis*

Mirouze, N.[1], C. Ferret[2], C. Cornilleau [2], and R. Carballido-Lopez[2].

[1] *Institute for Integrative Biology of the Cell (I2BC), INSERM, CEA, CNRS, Université Paris-Sud, Orsay, 91190, Gif sur Yvette, France.*
[2] *MICALIS, INRA, AgroParisTech, Université Paris-Saclay, 78350, Jouy-en-Josas, France*

Despite decades of investigation of genetic transformation in the model Gram-positive bacterium *Bacillus subtilis*, the factors responsible for exogenous DNA binding at the surface of competent cells remain to be identified. Here, we report that wall teichoic acids (WTAs), cell wall-anchored anionic glycopolymers associated to numerous critical functions in Gram-positive bacteria, are involved in this initial step of transformation. Using a combination of cell wall-targeting antibiotics and fluorescence microscopy, we show that competence-specific WTAs are produced and specifically localized in the competent cells to mediate DNA binding at the proximity of the transformation apparatus. Furthermore, we propose that TuaH, a putative glycosyl transferase induced during competence, modifies competence-induced WTAs in order to promote (directly or indirectly) DNA binding. On the basis of our results and previous knowledge in the field, we propose a model for DNA binding and transport during genetic transformation in *B. subtilis*.

O32

Genus- and genome- wide analysis of natural transformation events

Tanya Falbel, Jonathan Lombardino, and **Briana Burton**

University of Wisconsin – Madison, Wisconsin, USA

Many bacterial strains are known to be naturally transformable. Transformable bacteria can take up segments of donor DNA several kilobases in length from the environment and recombine portions of these into their genomes. What remains largely unknown are the molecular rules governing which sequences are preferentially integrated into a recipient genome for a given donor and recipient pair. We have developed an experimental method and associated informatic analysis tools that allow unbiased interrogation of the landscape of potential donor DNA to a given naturally transformable bacterial species. Using a variety of members of the *Bacillus* genus, we can identify and map thousands of unique transformation events in a single experiment. The transformation data thus acquired enabled us to analyze key features of these horizontal gene transfer events, including parameters for lengths of transferred segments, the minimum degree of homology allowed for efficient donor DNA incorporation, and genome-wide preferences for integration sites in the recipient genome. Analysis of individual transformation sites revealed that, in addition to congression of unlinked genomic regions, many transformation occurrences include multiple crossover events such that long stretches of genome alternate between donor and recipient sequence. Current efforts are focused on analyzing these data to build predictive tools that can identify likely gene transfer events in microbial communities with known microbial constituents.

O33

Insight into the conjugative transfer locus of pXO16 from *Bacillus thuringiensis* sv. *israelensis*

Pauline Hinnekens, Audrey Leprince, and Jacques Mahillon

Laboratory of Food and Environmental Microbiology, Earth and Life Institute, UCLouvain, Louvain-la-Neuve, Belgium.

pXO16, the 350 kb conjugative plasmid from *Bacillus thuringiensis* sv. *israelensis*, is able to transfer itself at high frequencies, to mobilize and retro-mobilize non-conjugative plasmids, including "non-mobilizable" plasmids, and to transfer chromosomal loci. It also induces the formation of macroscopic aggregates during conjugation under liquid conditions. Even though its kinetics of transfer and its host spectrum have been investigated and described, the conjugation mechanism of pXO16 remains poorly understood. However, the "*transfer israelensis plasmid*" (tip) region was recently delimited, based on bioinformatics predictions and experimental data. This region covers 25 kb and includes 16 genes, alphabetically named from *tipA* to *tipP*. Data suggest that the conjugation mechanism used by pXO16 is a novel T4SS-mediated DNA transfer system. Among the 16 tip genes, tipH and tipL have been identified as encoding a motor ATPase and the coupling protein of the T4SS, respectively, both signature proteins of T4SS systems. Bioinformatics analyses also predicted TipB as the conjugative transglycosylase, essential for the insertion of the T4SS into the bacterial cell wall. Knock-out of tipB from pXO16 sequence, recombinant expression of TipB and study of its activity confirmed its role as a transglycosylase and its importance in pXO16 conjugation. Besides ATPases and transglycosylase, T4SS also requires a relaxase and an origin of transfer (oriT), forming the relaxosome machinery, in charge of DNA translocation through the T4SS. Investigations are currently carried around the downstream extremity of tip locus, including tipO - encoding a predicted helicase - and tipP to identify the pXO16 relaxosome components.

O34

Evolution of *Bacilli* on plants roots reveals novel adaptation strategies

Mathilde Nordgaard Christensen, Yicen Lin, and Ákos T. Kovács

Bacterial Interactions and Evolution Group, Technical University of Denmark, Kgs Lyngby, Denmark

The spore-forming bacteria *Bacillus subtilis* and *Bacillus thuringiensis* are naturally found in soil and are known to protect plants against diseases and pests as well as promote plant growth. Strains of these bacteria are already applied as commercialized biocontrol agents in the agriculture. However, the mechanisms responsible for the beneficial effects on the plant remain largely unknown. We studied *B. subtilis* and *B. thuringiensis* root colonization through experimental evolution on *Arabidopsis thaliana* roots. During and at the end of the laboratory evolution, evolved strains were isolated and tested for altered phenotypic properties. Most evolved isolates of *B. thuringiensis* showed improved properties on the plant including enhanced colonization, re-colonization as well as enhanced pellicle formation in response to plant polysaccharides, while certain isolates showed reduced motility. Surprisingly, enhanced plant re-colonization was accompanied by increased hemolytic effect and virulence against *Galleria mellonella* (greater wax moth). Re-sequencing of selected evolved isolates identified a frame-shift mutation in the *rho* gene (a transcription termination factor), while re-introduction this mutation to the ancestor recreated the observed phenotypic changes. Preliminary results of evolved *B. subtilis* strains also indicate gradual adaptation of this species to the *A. thaliana* host.

Our approach of studying bacteria-plant interactions through experimental evolution not only reveals the adaptation potential and allows the identification of bacterial genes and pathways involved in improved root colonization and plant protection, but also represents a novel methodology for improving (already commercialized) plant growth-promoting bacterial strains without the need for genetic engineering.

O35

The impact of a horizontally acquired virulence plasmid on Bacillus cereus G9241, the causative agent of an anthrax-like illness.

Grace Taylor-Joyce, Tom Brooker, and Nick Waterfield
University of Warwick, Coventry, UK

Bacillus cereus, *Bacillus anthracis* and *Bacillus thuringiensis* are members of the *Bacillus cereus* sensu lato complex. The species are highly similar at a chromosomal level, but are phenotypically diverse due to the presence of different plasmids. The anthrax pathogen, *B. anthracis* contains two virulence plasmids; pXO1, carrying the tripartite anthrax toxin genes, and pXO2 carrying capsule production genes. Both plasmids are required to allow *B. anthracis* to act as a highly virulent mammalian pathogen. The pXO1 plasmid also encodes *atxA*, a mammalian virulence transcriptional regulator that controls gene expression from the plasmid and chromosome. It is proposed that AtxA is incompatible with the chromosomally encoded global regulator PlcR, as all *B. anthracis* isolates carry an inactivated *plcR* gene. This is thought to have driven the evolution of high mammalian virulence. Interestingly, there are several *B. cereus* isolates that possess a pXO1-like plasmid, called pBCXO1, which are capable of inducing an anthrax-like illness. Importantly, *B. cereus* G9241, carries intact copies of both *atxA* and *plcR* genes. To understand the impact of pBCXO1 on the biology and lifestyle of *B. cereus* G9241, a pBCXO1-cured strain has been used to study the influence of pBCXO1 on the transcriptome of *B. cereus* G9241. An RNAseq analysis has shown the effect of temperature on pBCXO1 activity and the influence this plasmid has on the cell metabolism, biosynthesis of amino acids and cell membrane. The transcriptome of *B. cereus* G9241 has also revealed the potential importance of a lysogenic bacteriophage on the biology of this pathogen.

O36

Bacterial nanotubes: Genetic determinants, formation dynamics & biological role.

Jiří Pospíšil[1], Olga Kofroňová[2], Katarína Muchová[3], Dragana Vítovská[1], Hana Šanderová[1], Martin Hubálek[4], Michaela Šiková[1], Oldrich Benada[2], Imrich Barák[3], and **Libor Krásný[1]**

[1] *Laboratory of Microbial genetics and Gene Expression, institute of Microbiology of the Czech Academy of Sciences, 14220 Prague 4, Czech Republic.*

[2] *Laboratory of Molecular Structure Characterization, Institute of Microbiology of the Czech Academy of Sciences, 14220 Prague 4, Czech Republic.*

[3] *Department of Microbial Genetics, Institute of Molecular Biology, Slovak Academy of Sciences, Bratislava, Slovakia.*

[4] *Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, 14220 Prague 6, Czech Republic.*

Bacterial nanotubes (NTs) are relatively recently discovered membranous structures that have been reported to connect cells and function as channels for transfer of metabolites, proteins, and nucleic acids. Here, using *Bacillus subtilis* as the main model organism, we present a comprehensive approach to identify genes that play roles in NT formation and describe in detail the dynamics of their extrusion. Importantly, we identify the key environmental triggers that cause bacterial cells to form NTs and this sheds light on their biological role. In summary, this presentation will reveal the ultimate model of the genesis of bacterial NTs.

O37

Small, but mighty: the ecology of *B. subtilis* secondary metabolites

Heiko T. Kiesewalter, Carlos N. Lozano-Andrade, and Ákos T. Kovács

Bacterial Interactions and Evolution group, Technical University of Denmark, KgsLyngby, Denmark

Bacillus subtilis is well known for its ability to produce various secondary metabolites (SM) and to form biofilm in the rhizosphere, the traits that are important for biocontrol. We isolated several *B. subtilis* strains from environmental soil samples and tested the variance in non-ribosomal peptide (NRP) production, including plipastatin and surfactin, using LC-MS. An established NRP mutant library of 22 isolates was used to screen the bioactivity potential of NRPs against plant-pathogenic fungi and bacteria as well as protozoa. Antifungal screening exposed a correlation between fungal inhibition and the production of certain NRPs. Interestingly, isolates from the same soil sample displayed variable NRP production ability. Furthermore, the determination of the closed genomes for 13 *B. subtilis* isolates allowed us to predict the arsenal of biosynthetic gene clusters revealing the lantibiotic subtilomycin to likely determine the ability to inhibit certain plant-pathogenic bacteria.

Finally, to dissect the impact of NRP production on microbial communities, wild type *B. subtilis* or its NRP mutant strains were added into soil-derived semi-synthetic mock communities. 16S amplicon sequencing revealed the influence of wild type *B. subtilis* on community assembly and relative abundance of certain genera, which was reduced in the mutant lacking NRP production. We propose that *B. subtilis* soil isolates are promising targets to understand the ecological role of these small secondary metabolites in nature.

The project is part of the Center for Microbial Secondary Metabolites that is supported by the Danish National Research Foundation (DNRF137).

O38

Role of a *Bacillus subtilis* transglutaminase in the macromolecular assembly of the spore surface layers

Diogo Martins[1], Bruno Gonçalves[1], Catarina G. Fernandes[1], Guillem Hernandez[1], Charles. P. Moran Jr.[2], Tiago N. Cordeiro[1], Mónica Serrano[1], and **Adriano O. Henriques**[1]

[1]Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal; [2]Emory University School of Medicine, Atlanta GA 30322, USA

Bacterial endospores have a peptidoglycan layer, the cortex, surrounded by a protein coat differentiated into an inner and an outer layer. Tgl, a transglutaminase (TGase) that cross-links proteins through the formation of ε-(γ-glutamyl)lysil bonds, is recruited to the cortex and the inner coat through docking interactions with pre-assembled substrates. One of the Tgl substrates, SafA, functions as the hub for inner coat assembly while also promoting attachment of the inner coat to the cortex. One of the two main forms of SafA that accumulates *in vivo* forms a polymer in solution whose basic unit is an oblong hexamer. Tgl cross-links this polymer *in vitro* and *in vivo* into high molecular weight forms. Tgl thus exerts a "spotwelding" activity, which *in vivo* is directed at the covalent fortification of complexes important for the formation of the inner coat and the cortex/inner coat interface. The crystal structure of Tgl reveals a functional core bearing a papain-like catalytic dyad which, as for papain, is partially redundant (Cys116 and Glu187 or Glu115), suggesting that catalysis does not occur from a single steric position. The structure also suggests that a key distinction between TGases and cysteine proteases is the insulation of the active site from water. At only 25 kDa, Tgl approaches the archetypal TGase, illustrating the minimal requirements for protein cross-linking. Unlike other TGases, Tgl is produced in active form, efficiently catalyzing amine incorporation and cross-linking of non-physiological substrates *in vitro*. These features allow a range of *in vitro* and *in vivo* applications.

O39

Global Absolute Quantification of Membrane Proteins in *Bacillus subtilis*

Minia Antelo-Varela[1], Jürgen Bartel[1], Ane Quesada-Ganuza[2], Karen Appel[2], Margarita Bernal-Cabas[3], Thomas Sura[1], Andreas Otto[1], Michael Rasmussen[2], Jan Maarten van Dijl[3], Allan Nielsen[2], **Sandra Maaß[1]**, and Dörte Becher[1].

[1] *University of Greifswald, Institute of Microbiology, Department of Microbial Proteomics, Greifswald, Germany*
[2] *Research & Technology, Novozymes A/S, Bagsværd, Denmark*
[3] *University of Groningen, University Medical Center Groningen, Department of Medical Microbiology, Groningen, The Netherlands.*

Absolute quantification of proteins is a prerequisite to model biological processes in the context of systems biology and to determine protein stoichiometries in a global manner. However, the data produced for membrane proteins by current approaches is lagging behind the requirements of this field. Here we present a novel approach for large-scale absolute quantification of this challenging subset of proteins, which has been established and evaluated using osmotic stress management in *Bacillus subtilis* as proof of principle. Selected membrane proteins were labelled using a SNAP-tag and absolute membrane protein concentrations were determined via shotgun proteomics by spiking crude membrane extracts of chromosomally SNAP-tagged and wild-type *B. subtilis* strains with protein standards of known concentration. Shotgun data was subsequently calibrated by targeted mass spectrometry using the SNAP protein as reference, and an enrichment factor was calculated in order to obtain membrane protein copy numbers/μm^2. Additionally the SNAP-tag allowed for visual inspection of the enrichment of the membrane fraction by immunoassays. Hence, the accurate determination of physiological changes resulting from imposed hyperosmotic stress was possible, which simultaneously allowed for sensitive detection of alterations in membrane protein arrangements and putative membrane complexes. This straightforward and cost-effective methodology for quantitative proteome studies can be applied to the full spectrum of physiologically relevant conditions, ranging from environmental stresses to the biotechnological production of small molecules and proteins.

O40

Molecular mechanisms overcoming ribosomal stalling

Hiraku Takada[1, 2], Caillan Crowe-McAuliffe[3], Daniel N. Wilson[3], and **Vasili Hauryliuk[1, 2, 4]**

[1]Department of Molecular Biology, Umeå University, Sweden, [2]Laboratory of Molecular Infection Medicine Sweden, Sweden, [3]Institute for Biochemistry and Molecular Biology, University of Hamburg, Martin-Luther-King-Platz 6, 20146 Hamburg, Germany [4]University of Tartu, Institute of Technology, Estonia

The processivity of protein synthesis on the ribosome can be interrupted by various hindrances. These include antibiotics that specifically disrupt the workings of the translation machinery, truncated mRNA molecules that encode incomplete messages and problematic amino acid sequences of nascent peptides that inhibit transpeptidation or have difficulties in passing through the ribosomal tunnel. Bacteria have evolved dedicated systems that assist the ribosome in overcoming these challenges. Using a combination of bacterial genetics, ribosomal biochemistry and cryo-electron microscopy, we have characterised several factors that assist the ribosome, including *Bacillus subtilis* antibiotic resistance factor VmlR that mediates resistance to antibiotics that inhibit peptide bond formation by the ribosome.

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RF1 Sven Halbedel

Repressor operator interactions contributing to the ecologic interactions of a human pathogen in its natural habitat

RF2 Martin Fischer

Resistance of clinical L. monocytogenes isolates towards cephalosporins and the role of *pbpB3* encoding a homologue of *Bacillus subtilis pbpC*

RF3 Takashi Inaoka

The impact of an autoinducer neotrehalosadiamine overproduction on central metabolism in *Bacillus subtilis*

RF4 Dita Grinanda

Ethanol production by the metabolically engineered genome-reduced strain of *Bacillus subtilis*

RF5 Jinghui Liang

Tackling carbon catabolite repression in *Parageobacillus thermoglucosidasius*

RF6 Timothy Hoffmann

Genetic optimisation of bacterial-induced calcite precipitation for application in self-healing concrete

RF7 Rocio Aguilar-Suarez

midiBacillus strains for protein production: why to keep it small

RF8 Keitaro Kimura

Bacillus subtilis an edible and beneficial bacterium

RF9 Andreas Knapp

Monitoring secreted protein production by *Bacillus subtilis* using the split GFP assay

RF10 Yoshihiro Toya

Metabolic engineering of *Bacillus subtilis* for enhancing productions of dipicolinate and poly-γ-glutamate

RF11 Satoru Watanabe

Gene expression profile of CyanoBacillus, carrying chimeric genome of *Bacillus subtilis* and cyanobacterium *Synechocystis* sp. PCC 6803

RF12 Rachele isticato

Halophilic Bacilli with plant growth-promoting activity

RF13 Birthe Halmschlag

Tailored poly-γ-glutamic acid production with *Bacillus subtilis* 168

RF14 Loredana Baccigalupi

A probiotic treatment increases the immune response induced by the nasal delivery of spore-adsorbed TTFC

RF15 Mareen Geißler

Exploiting novel strategies to produce surfactin in *Bacillus subtilis* cultures

RF16 Peter Klausmann

High cell density fermentations for the production of surfactin

RF17 Ryuichi Sato

In vivo re-constitution of cyanobacterial RNA polymerase in *Bacillus subtilis*

RF18 Anne-Gaëlle Planson

Genome engineering for the construction of *Bacillus subtilis* chassis

RF19 Shunsuke Yoshimura

Characterization of thermostable β-galactosidase to develop a reporter peptide that functions in *Geobacillus* species

RF20 Ryunosuke Isozaki

Introduction of the exogenous AdoMet transporter in *Bacillus subtilis*

RF21 Shota Suzuki

Compatibility of site-specific recombination units between mobile genetic elements

RF22 Iñigo Urrutia Irazabal

Why does PcrA helicase interact with RNA polymerase?

RF23 Tatsuya Yamamoto

Comparative analysis of phage-like bacteriocin PBSX responsible for membrane vesicles formation in *Bacillus subtilis* and relatives

RF24 Jacques Mahillon

Identification of endolysins encoded by Deep-Blue and Deep-Purple phages infecting *Bacillus weihenstephanensis*

RF25 Mizuki Kimura

Development of a systematic evaluation method of insertion sequences derived from thermophilic bacteria

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Bacillus subtilis HGT: Antagonistic interactions result in increased recombination

RF27 Tasneem Bareia

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RF28 Carlos Lozano-Andrade

Bacillus subtilis persistence and secondary metabolite production in artificial soil

RF29 Valeria Verrone

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RF30 Ryo Nagasawa

Extracellular DNA production within the biofilm formed by *Streptococcus mutans*

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Molecular engineering applied to the directed synthesis of lipopeptides by *Bacillus subtilis*

RF35 Bhagyashree N. Swarge

Transcriptome and proteome changeovers reveal the bacterial strategy of spore awakening

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Interplay between biofilm formation and sporulation in *Paenibacillus polymyxa*

RF37 Anastasia Tolcan

Role of the orphan ribonuclease KapD in *Bacillus subtilis* spore development

RF38 Sema Ejder

Structure-function studies of stressosomes from *B. subtilis* and *L. monocytogenes*

RF39 Yifan Zhang

Bacillus spore response to low energy electron beam: role of different DNA repair mechanisms

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Enhanced metabolism of the gamma-amino acid 3-amino-4-hydroxybenzoic acid under oxygen limitation by recombinant *Corynebacterium glutamicum*

P23 Tatsuya Osamura
Monoacylglycerol lipase production in the eight extracellular proteases deficient *Bacillus subtilis* strain

P24 Hirokazu Suzuki
Specific bioconversion of *myo*-inositol into *scyllo*-inositol using thermophilic cells that produce mesophilic enzymes

P25 Shinya Kaneko
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P26 Margarita Bernal Cabas
In vitro characterization of TatAyCy protein translocase complexes from *Bacillus subtilis*

P27 Yuji Tsujikaw
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Molecular breeding of new *Agrobacterium tumefaciens* for increasing plant transformation

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Application of CRISPR/Cas for generation of *Bacillus* enzyme production strains

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Compatibility of site-specific recombination units between mobile genetic elements

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P35 Genki Akanuma
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P36 Masakazu Kataoka
Optimization of RK2-based gene introduction system for *Bacillus subtilis*

P37 Yuta Fukada
Relationship between chromosome transfer phenomenon and clt

P38 Misaki Ohara
Identification and application of thermophilic insertion sequences functional in *Geobacillus kaustophilus* HTA426

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Social interactions between *Bacillus subtilis* and *Dickeya solani*

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P46 Takahiko Kondo
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P47 Chika Yamamoto
Quorum sensing regulated membrane vesicle production in *Streptococcus mutans*

P48 Shathviga Manoharan
The role of the PlcR-PapR circuit in *B. cereus* G9241, the causative agent of anthrax-like illness

P49 Mitsuo Ogura
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P50 Lars Lilge
Friend or foe: Impact of surfactin production on cell physiology

P51 Quentin Gras
Molecular engineering applied to the directed synthesis of lipopeptides by *Bacillus subtilis*

P52 Frédérique Braun
Rae1 cleaves mRNAs in a translation-dependent manner in *Bacillus subtilis*

P53 Cyprien Guérin
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Abstracts for Poster presentations

P1

Repressor operator interactions contributing to the ecologic interactions of a human pathogen in its natural habitat

Samuel Hauf, and **Sven Halbedel**

FG11 Division of Enteropathogenic bacteria and Legionella, Robert Koch Institute, Burgstrasse 37, 38855 Wernigerode, Germany

The firmicute bacterium *Listeria monocyctogenes* occurs ubiquitously in the environment, but can cause severe invasive disease in humans upon ingestion. We recently identified the *L. monocytogenes* genes *lieAB* and *lftRS*, encoding a multi drug resistance ABC transporter and a regulatory module, respectively. These genes jointly mediate resistance against aurantimycin, an antibiotic produced by the soil-dwelling species *Streptomyces aurantiacus*. Aurantimycin production is advantageous for *S. aurantiacus* when grown in direct contact with *L. monocytogenes*, indicating that aurantimycin resistance contributes to survival of *L. monocytogenes* in its natural habitat, *i. e.* the soil. We here show that expression of the *lieAB* genes is sufficient to confer aurantimycin resistance to naturally sensitive species such as *Bacillus subtilis*. In *L. monocytogenes*, transcription of *lieAB* and *lftRS* is strongly repressed under standard growth conditions by the PadR-type transcriptional regulator LftR, but induced by aurantimycin. A motif in the P_{lieAB} promoter potentially serving as LftR binding site was deduced from a systematic truncation and mutation analysis. This motif was also present in the P_{lftRS} promoter, but was not found elsewhere in the chromosome. Mutational analysis of this putative operator in the P_{lftRS} promoter confirmed its relevance for LftR-dependent repression in *in vivo* and in further *in vitro* experiments. Our results provide more detailed insights into the regulation of aurantimycin resistance in *L. monocytogenes* and may help to characterize the ecological habitat and thus the source of this pathogen more precisely.

P2

Resistance of clinical *L. monocytogenes* isolates towards cephalosporins and the role of *pbpB3* encoding a homologue of *Bacillus subtilis pbpC*

Martin A. Fischer , Antje Flieger, and Sven Halbedel

Robert Koch-Institute (German-Austrian Binational Consiliary Laboratory for Listeria, FG11 - Enteropathogenic Bacteria and Legionella, Wernigerode, Germany)

Bacillus subtilis and *Listeria monocytogenes* share a variety of homologous genes, among them important penicillin binding proteins relevant for the resistance against β-lactam antibiotics. Unlike *B. subtilis*, a common trait of *L. monocytogenes* is the intrinsic resistance towards cephalosporins, a group of frequently applied antibiotics. *L. monocytogenes* is the causative agent of listeriosis, a serious foodborne infection. With a fatality rate between 7 to 30 %, listeriosis is one of the most fatal gastrointestinal infections. Case numbers are increasing during the last decade with almost 700 notified cases in 2018 in Germany. An observation obtained during an ongoing study investigating the antibiotic susceptibilities of clinical isolates was a variation of cephalosporin resistance levels between different phylogenetic clades of the species *L. monocytogenes*. We employed different comparative genomic approaches to identify alleles or mutations explaining the variance in cephalosporin resistance observed between these clades. Among the observations made was a sequence variation of *lmo0441*, a homologue of *pbpC* of *B. subtilis*, encoding penicillin binding protein PBP B3. We found an association of this gene with reduced resistance towards cephalosporins, which would be in good agreement with a role for PBP B3 and PbpC in cephalosporin resistance of *L. monocytogenes* and *B. subtilis*, respectively. In ongoing experiments, we now aim to clarify if and to which extend the *lmo0441* alleles in cephalosporin sensitive clinical isolates are the underlying reason for their reduced cephalosporin resistance.

P3

The impact of an autoinducer neotrehalosadiamine overproduction on central metabolism in *Bacillus subtilis*

Takashi Inaoka[1], Natsumi Saito[2], and Nguyen Minh Huong[1]

[1]*Food Research Institute, National Agriculture and Food Research Organization (NFRI), Tsukuba, Japan*

[2]*National Institute of Technology, Tsuruoka College*, *Tsuruoka*, *Japan*

Neotrehalosadiamine (3,3'-diamino-3,3'-dideoxy-α,β-trehalose; NTD) is a unique secondary metabolite produced by several *Bacillus* species including *B. subtilis*. NTD was originally identified as an amino-sugar antibiotic that inhibits the growth of *Staphylococcus aureus* and *Klebsiella pneumoniae*. We previously reported that it acts as an autoinducer for its own biosynthetic operon *ntdABC* through the function of transcriptional activator NtdR. The expression of *ntdABC* is also regulated negatively in response to the GlcP-mediated glucose transport. It suggests that NTD can function as an extracellular signal in response to glucose. In this study, we investigated the effect of NTD overproduction on central metabolic pathway.

Pentose phosphate (PP) pathway is a major source of reducing power and metabolic intermediates for biosynthetic processes. In *B. subtilis*, disruption of *zwf* gene, which encodes a glucose-6-phosphate dehydrogenase (the first enzyme in PP pathway), resulted in a growth defect phenotype under specific condition. Interestingly, we found that NTD overproduction can restore the growth defect caused by *zwf* disruption. Metabolome analysis showed that NTD overproduction resulted in accumulation of several TCA cycle metabolites and NADPH. Furthermore, the growth defect phenotype of *zwf* mutant was no longer observed by adding malate into the growth medium. These results suggest that NTD overproduction resulted in accumulation of TCA metabolites, leading to an increase in NADPH level through malate-to-pyruvate conversion. Thus, NTD can modulate central metabolic pathway by activating its biosynthesis pathway in response to glucose depletion.

P4

Construction of heterologous expression system of Nocardithiocin and its derivatives

Kanae Sakai, Kazuki Matsumoto, Shunsuke Takahashi, Kenji Tsuge, Jun Ishii, and Akihiko Kondo

Kobe University (Graduate school of Science, Technology and Innovation, Kobe, Japan)

Nocardithiocin is thiopeptide compound produced by pathogenic actinomycetes *Nocardia pseudobrasiliensis* and shows promising activity against Gram-positive bacteria especially *Mycobacterium tuberculosis*. However, the clinical use of nocardithiocin was prevented due to its low productivity and low water solubility. In this study, we expressed nocardithiocin gene cluster in heterologous host to solve these problems.

For the industrial production of nocardithiocin, original producer *N. pseudobrasiliensis* will be undesirable host because of its pathogenicity. So we tried to use *Escherichia coli* and several *Streptomyces* sp. as a host for nocardithiocin production. At first, nocardithiocin gene cluster was cloned and introduced into heterologous host. Codon optimized gene cluster was also prepared for *E. coli* expression. As a result nocardithiocin production was confirmed in *Streptomyces* host but not in *E. coli.* Furthermore, to improve the solubility nocardithiocin derivatives are also tried to express in *Streptomyces* host.

P5

Ethanol production by the metabolically engineered genome-reduced strain of *Bacillus subtilis*

Dita Grinanda, and Takashi Hirasawa

School of Life Science and Technology, Tokyo Institute of Technology, Yokohama, Japan

In the present study, the genome-reduced strain of *Bacillus subtilis* MGB874, lacking 874 kb (20.7 %) of genome DNA regions (Morimoto *et al*., DNA Res. 15: 73-81, 2008) was engineered to produce ethanol and its ethanol production during the stationary phase was compared with those in the parental strain, OA105 (Toya *et al*., Biosci. Biotechnol. Biochem. 79: 2073-2080, 2015). To reduce byproducts formation, we used recombinant strains, where genes encoding the enzymes responsible for formation of acetoin, lactate, and acetate were disrupted in both strains, namely A267 and SA123, respectively, as production hosts, both of which were kindly provided from Kao Corporation (Japan). For engineering ethanol production strains, pyruvate decarboxylase (*pdc*) and alcohol dehydrogenase (*adhB*) genes from *Zymomonas mobilis* under the *spac* promoter were introduced into the A267 and SA123 strains and the resulting strains were designated A267_EtOH and SA123_EtOH, respectively. The engineered strains were cultured on glucose as a carbon source in fed-batch mode under microaerobic conditions at 30˚C. The genome-reduced ethanol producing strain A267_EtOH exhibited higher ethanol production than the control strain SA123_EtOH. Further, the *spac* promoter in the A267_EtOH was replaced with the *lytR* promoter, which is active during the entire growth phase, for expressing the *pdc* and *adhB* genes. Ethanol production in the A267_EtOH was increased approximately 3.7-fold by promoter replacement and its yield reached half of the theoretically maximum yield. Our results indicate that the genome-reduced strain MGB874 is an effective host for producing ethanol during the stationary phase.

P6

Tackling Carbon Catabolite Repression in *Parageobacillus thermoglucosidasius*

Jinghui Liang, David Leak, and Richard van Kranenburg

University of Bath, Bath, UK

The genus Parageobacillus are thermophilic gram-positive bacteria of industrial interest for lignocellulosic biomass fermentation. Though Parageobacillus spp. are facultative anaerobes microbes capable of fermenting a wide range of C6 and C5 sugars, carbon catabolite repression (CCR) has been a bottleneck in the complete utilization of mixed-sugar substrates, constraining microbial growth rate and productivity. This study aims to remove CCR from Parageobacillus thermoglucosidasius DSM2542 with devising methods in order to optimize its industrial performance.

In gram-positive bacteria, CCR is mediated via the PTS system through transcriptional regulation of catabolic gene expression. With a preferred PTS sugar in the substrate, high glycolytic activity leads to the accumulation of glucose-6-phosphate and fructose-1,6-biophosphate, resulting in the regulatory phosphorylation of HPr and Crh on Ser46 residue by HPrK16. The activated HPr (Ser-P) and Crh (Ser-P) bind to CcpA to form a HPr-Ser46-P/CcpA or Crh-Ser46-P/CcpA complex which can bind to CRE located 5' or within the catabolic genes, and CCR occurs when the genes are down-regulated. However, it has been found that it is difficult to generate an HPrS46A and CrhAS46A double mutant, and therefore alternative methods are required to remove CCR from DSM2542.

Secondly, quantitative RT-PCR was used to identify the xylose transportation system, which demonstrated that arabinose transporter might be involved in xylose transportation. Therefore, modifying the CRE within the arabinose transporter could potentially relieve xylose and arabinose utilization from CCR.

Lastly, a cocktail containing glucose analogue was used to remove CCR from P. thermoglucosidasius via adapted evolution within a month.

P7

Genetic Optimisation of Bacterial-Induced Calcite Precipitation for Application in Self-Healing Concrete

Timothy Hoffmann[1], Kevin Paine[2], and Susanne Gebhard[1]

[1] *Department of Biology and Biochemistry, Milner Centre for Evolution, University of Bath, Bath, United Kingdom*

[2] *Department of Architecture and Civil Engineering, University of Bath, Bath, United Kingdom*

Bacteria in our environment contribute to Earth's landscapes through mineral deposits via a process known as bacterial-induced calcite precipitation (BICP). Over recent years, such bacteria have been at the basis of innovative biotechnologies arising within civil engineering sectors, finding application, for example, in self-healing concrete. The technology uses encapsulated, alkali-tolerant, spore-forming bacteria, such as those of the genus *Bacillus*. The bacteria are embedded into concrete to facilitate the repair of cracks that appear during aging of built structures. In BICP, bacterial metabolism creates a microenvironment that favors the precipitation of calcium cations and carbonate anions in the form of mineral calcite. This process is dependent on changes in pH, availability of cell surface nucleation sites, and ion concentrations. Current approaches using this technology require bacteria that are both capable of BICP as well as possessing specific growth characteristics required for the respective application (e.g. pH/salt tolerance). In this project, we explore the genetic optimization of BICP for application in self-healing concrete using *Bacillus subtilis* as a model. Our work offers an approach to identify the molecular components needed for BICP to occur and a way to mobilize these into better-suited chassis organisms for applications. Our results show that upregulating the ureolytic pathway, and modulating biofilm production offer mechanisms whereby BICP can be engineered into a non-precipitating strain. The ultimate goal is to create bio-concrete that increases the lifespan of cementitious structures and consequently decreases the maintenance costs and carbon dioxide release associated with concrete production and building.

P8

midi*Bacillus* strains for protein production: why to keep it small

Rocio Aguilar Suarez[1], Minia Antelo-Varela[2], Michael Kohlstedt[3], Josef Altenbuchner[4], Dörte Becher[2], Christoph Wittmann[3], and Jan Maarten van Dijl[1].

[1]University Medical Center Groningen-University of Groningen, Groningen, the Netherlands. [2]Centre of Functional Genomics of Microbes, Institute of Microbiology, University of Greifswald, Greifswald, Germany. [3]Institute for Systems Biotechnology, Saarland University, Saarbrücken, Germany. [4]Institute of Industrial Genetics, University of Stuttgart, Stuttgart, Germany.

Genome-reduction offers the possibility to create completely novel chassis for use as cell factories in the production of recombinant proteins. In particular, this strategy allows the removal of redundant, unnecessary and potentially detrimental genes that could hinder the production of valuable proteins. Recently, a genome reduction by 36% was shown to allow highly enhanced secretion of staphylococcal antigens by *B. subtilis* 168 (*Aguilar Suarez et al. ACS Synth Biol. 2019)*. However, the mechanisms behind such improvements were not revealed. In the present study, landmark strains in the phylogeny of the genome-engineered *Bacillus* were used to disclose major cellular adaptations. First, an in-depth proteomic analysis was performed in 'midi*Bacillus'* during the production of the immunodominant staphylococcal antigen A, in order to define its protein hardware and to compare it to the parental strain. Additionally, batch-cultivation in bioreactors was performed to map important physiological changes as evidenced by growth and energy parameters, amino acids levels and organic acids formation. The insights obtained are valuable to adjust and improve the next-generation *Bacillus* chassis. Altogether, our results identify critical steps and the main adaptations in terms of protein production, secretion, energy use, overflow metabolism and product stability, and they highlight the potential benefits of genome-reduced *B. subtilis* derivatives over the parental strain.

P9

Bacillus subtilis an edible and beneficial bacterium

Keitarou Kimura

Food Research Institute, National Agriculture and Food Research Organization (NFRI), Tsukuba, Japan

In Asian and West African countries *Bacillus* species such as *B*. *subtilis* and *B. amyloliquefaciens* are used to produce fermented foods from legumes. Principle fermentation process (non-salted alkaline fermentation) is commonly observed for the *Bacillus*-fermented foods, for examples, natto (Japan), chungkookjang (Korea), kinema (Nepal), thua-nao (Thailand), dawadawa (Ghana and Nigeria), and sumbara (Burkina Faso). Recently, a comparative genome study was performed for soybean-fermenting strains, which revealed genotypes related to the soybean fermentation process.

For Japanese natto, extracellular amino-acid polymer (poly-γ-glutamic acid, γPGA) produced by *B. subtilis* (natto) is important to provide the sticky texture to natto. In addition, γPGA is used as a food additive to enhance thickness of drinks (thickener). Synthesis of γPGA is regulated by quorum sensing that governs cell physiology in the stationary phase. To find regulatory genes for the γPGA synthesis and cultivate γPGA over-producing strains, mutants that can produce γPGA bypassing the quorum-sensing signal pathway were screened. As a result, an amino-acid substitution (S103F) in the *yabJ* gene was found to be a dominant suppressor of loss of cell-density signal. *degU-lacZ* fusion gene was hyper-expressed in cells carrying the *yabJ*S103F, but disruption of *yabJ* did not affect the transcription level of the *degU-lacZ*. These observations suggested that YabJ acquired a function to stimulate *degU* expression through the S103F mutation. The *yabJ* is annotated as a deaminase that detoxify enamine and imine metabolic intermediates. YabJ might be involved in the γPGA synthesis in response to stresses including those of enamine/imine toxic metabolic intermediates.

P10

Monitoring secreted protein production by *Bacillus subtilis* using the split GFP assay

Andreas Knapp [1], Marzena Malek[1], Patrick Lenz[1], and Karl-Erich Jaeger[1,2]

[1]*Institute of Molecular Enzyme Technology, Heinrich Heine University Düsseldorf, Germany,*
[2]*Institute of Bio- and Geoscience IBG-1: Biotechnology, Forschungszentrum Jülich GmbH, Germany.*

Approaches to optimize protein production and secretion often require high-throughput screening of large libraries. Detection methods make use of enzymatic activity, preferably with photometric or fluorimetric methods. However, activity assays often cannot be adapted to a high-throughput format, and assays for non-enzyme proteins are difficult and laborious to perform. We have developed an activity-independent method based on the split GFP assay to enable the activity-independent detection and quantification of proteins secreted by *Bacillus subtilis*. Here, the C-terminal, eleventh β-sheet of superfolder GFP (sfGFP11) is fused to a target protein. Upon addition of the non-fluorescing detector protein containing the β-sheets 1-10 (sfGFP1-10) the amount of protein can be quantified by monitoring fluorescence.

In the course of this study, we observed that the detector is prone to degradation in the supernatant of *B. subtilis*. Interestingly, degradation starts at the beginning of the early stationary growth phase and is slightly reduced, but not avoided by using different protease-deficient strains. We optimized the cultivation conditions using a BioLector micro fermentation system by testing different growth temperatures and concentrations of tryptone, yeast extract and glucose in the growth media. In this way, we identified conditions allowing a prolonged growth phase of up to 20 hours at 30 °C in batch mode resulting in an increase in cell density and in product yields of GFP and a fungal cutinase by a factor of 4.

Currently, we evaluate whether addition of the detector protein directly to the culture broth allows online monitoring of a split GFP signal.

P11

Metabolic engineering of *Bacillus subtilis* for enhancing productions of dipicolinate and poly-γ-glumate

Yoshihiro Toya[1], Takashi Hirasawa[2], Shenghao Liu[3], Kenta Masuda[3], Takuya Morimoto[3], Shu Ishikawa[4], Onuma Chumsakul[4], Yasushi Kageyama[3], Fumio Matsuda[1], Hiroshi Hagihara[3], Katsuya Ozaki[3], Ken-ichi Yoshida[5], Naotake Ogasawara[4], and Hiroshi Shimizu[1]

[1]Osaka Univ, Suita, Japan, [2]Tokyo Inst Tech, Yokohama, Japan, [3] Kao Corp Tochigi, Japan, [4]NAIST, Ikoma, Japan, [5]Kobe Univ, Kobe, Japan

Bacillus subtilis is one of the promising host microorganisms for bio-production because of the easy genetic manipulation, high cell growth rate, well characterized metabolism, and safety. Its genome-reduced strain from which non-essential genomic regions were deleted has been developed. This strain showed a great potential as a cell factory that produced high levels of heterologous proteins even in stationary phase. In this study, we demonstrated metabolic engineering of the genome-reduced strain for enhancing productions of useful chemicals such as dipicolinate (DPA) and poly-γ-glutamate (PGA) during the stationary phases. After improved their synthesis pathways, we evaluated the flux distributions of the central carbon metabolism with ^{13}C-metabolic flux analysis, and predicted next engineering target reactions. In the DPA production, deletion of the acetoin synthesis pathway (*alsSD*) and overexpression of pyruvate carboxylase (*pycA*) were predicted to enhance the productivity. In the PGA production, in addition to the above modifications, overexpression of citrate synthase (*citZ*) was predicted to enhance the productivity. Finally, we successfully demonstrated that these predicted modifications enhanced the DPA and PGA productions in genome-reduced *B. subtilis*.

P12

Gene expression profile of CyanoBacillus, carrying chimeric genome of *Bacillus subtilis* and cyanobacterium *Synechocystis* sp. PCC 6803

Satoru Watanabe[1], Tomoya Mita[1], Masataro Hosomura[1], Yu Kanesaki[2], Hirofumi Yoshikawa[1], Mitsuhiro Itaya[3], and Kei Asai[1]

[1] *Department of Bioscience, Tokyo University of Agriculture, Tokyo, Japan.*
[2] *Institute of Green Science, Shizuoka University, Shizuoka, Japan.*
[3] *Technology Research Association of Highly Efficient Gene Design (TRAHED), Tokyo, Japan.*

Bacillus subtilis BEST 7613 (CyanoBacillus), carrying the chimeric genome of *B. subtilis* BEST 7003 and photosynthetic cyanobacterium *Synechocystis* sp. PCC 6803, has been established by the mega-cloning technology (Itaya, et al., 2005 PNAS, 102, 15971-15976). Whole-genome sequence analysis of the isolate and parental B. subtilis strains provided clues for identifying single nucleotide polymorphisms (SNPs) in the 2 complete bacterial genomes in one cell (Watanabe et al., 2012 J. Bacteriol., 194, 7007). The behavior of CyanoBacillus is just *B. subtilis*: this strain sustains heterotrophic growth and rod-shaped morphology. In order to reveal the transcriptome in CyanoBacillus, we carried out RNA-seq analysis. Interestingly, *Synechocystis* genes were hardly expressed in CyanoBacillus.

The chimeric genome strain CyanoBacillus has raised a number of poorly argued issues. CyanoBacillus does not contain the ribosomal RNA (*rrn*) operon of *Synechocystis*, because the *rrn* operon inhibits the further cloning of other genomic regions of *Synechocystis* to *B. subtilis* genome. RNA-seq and northern analysis of the genealogy of CyanoBacillus revealed that the expression of *Synechocystis rrn* operon disturbed the cloning of Synechocystis genome. In this poster presentation, the barriers relying in between *B. subtilis* and *Synechocystis* will be discussed.

P13

Halophilic Bacilli with *Plant Growth-Promoting* activity

Stefany Castaldi[1], Claudia Petrillo[1], Mariamichela Lanzilli[2],Vladimir Totev Valkov[2], Maurizio Chiurazzi[2], Ezio Ricca[1], and **Rachele Isticato[1]**

[1]Dept Biology, Federico II Univ., [2]Institute of Biosciences and Bioresources, CNR, Naples, Italy

Plant Growth-Promoting Bacteria (PGPB) are soil bacteria that colonize the rhizosphere and exert direct or indirect beneficial effects on plant growth. Direct effects are generally played by bacterial secreted molecules that act as plant hormones, nutrients or regulatory factors controlling, for example, the plant stress responses. The protection against pathogens is, instead, an example of indirect beneficial effects exerted by PGPB. Plant-PGPB interactions are of particular interest in environments with sub-optimal growth conditions, such as high or low temperatures, high salinity, and nutrient starvation, and are likely to become even more relevant in the future, as agriculture faces climate change and soil degradation issues.

To identify potential PGPB, rhizosphere samples were collected from plants growing in high salinity conditions and used to isolate a collection of halophilic bacteria. As part of their characterization, we also assessed traits of relevance as PGPB, such as phosphorus solubilization, nitrogen fixation, biofilm production and antimicrobials secretion. Selected strains were then used for an *in vivo* assessment of their effects on the growth of *Lotus japonicus* plants. Most strains did not affect *L. japonicus* growth in standard conditions while some strains reduced the negative effects of high salinity on plant growth. In particular, a halophilic member of the *B. subtilis* species allowed an optimal *L. japonicus* growth in the presence of 150 mM NaCl, a salinity condition that drastically reduced plant growth. Our results indicate that halophilic *Bacillus* cells might be useful in formulating new bio- fertilizers to improve cropping systems.

P14

Tailored poly-γ-glutamic acid production with *Bacillus subtilis* 168

Birthe Halmschlag[1], Kyra Hoffmann[2], René Hanke[2], Sastia P. Putri[3], Eiichiro Fukusaki[3], Jochen Büchs[2], and Lars M. Blank[1]

[1]*Institute of Applied Microbiology-iAMB, RWTH Aachen University, Germany*
[2]*AVT - Biochemical Engineering, RWTH Aachen University, Germany*
[3]*Department of Biotechnology, Graduate School of Engineering, Osaka University, Japan*

Poly-γ-glutamic acid (γ-PGA) is a water-soluble, non-toxic, edible, hygroscopic, and biodegradable polymer with various potential industrial applications. While the applications are manifold, γ-PGA of defined molecular structure is costly. We aimed at cost reduced selective synthesis of tailored γ-PGA by strain and process optimization.

To enable γ-PGA synthesis based on biomass-derived carbon sources, a derivative of the reference strain *B. subtilis* 168 was used as host. Specifically, the native γ-PGA operon was activated by activating transcription, enabling the strain to produce γ-PGA from glucose as sole carbon source. To efficiently use the hemicellulose-derived substrate xylose, a *B. subtilis* strain harboring the heterologous Weimberg pathway was engineered. In bacteria using the Weimberg pathway, xylose is converted to 2-oxoglutarate without carbon loss. Hence, the Weimberg pathway offers a more carbon-efficient alternative for γ-PGA production compared to the xylose isomerase pathway that is native in *B. subtilis*. The engineered *B. subtilis* strain harboring the Weimberg pathway allowed resource efficient, high yield γ-PGA production from xylose/glucose mixtures.

The γ-PGA synthesized by different *Bacillus* strains exhibits great structural diversity. We combined PGA synthetase and glutamate racemase genes from several *Bacillus* strains to synthesize tailor-made γ-PGA with *B. subtilis* as production host. The tailored γ-PGA produced with the same microbial chassis covered a broad range of stereochemical composition and molecular weight.

The results demonstrated the huge potential of *B. subtilis* as host organism for sustainable designer-γ-PGA production. The developed strain and cultivation conditions enhance the potential of γ-PGA for the use in industrial applications.

P15

A probiotic treatment increases the immune response induced by the nasal delivery of spore-adsorbed TTFC

Santos, F.D.S. [1,2], A.R. Maia[1], A. Saggese[1], R. Isticato[1], F. Leite[2], E. Ricca[1], and **L. Baccigalupi**[3]

[1] Dipartimento di Biologia, and [3] Dipartimento di Medicina Molecolare e Biotecnologie Mediche, Università di Napoli Federico II, Italy; [2] Centro de Desenvolvimento Tecnológico, Núcleo de Biotecnologia, Universidade Federal de Pelotas, Brazil.

Bacterial spores are widely used as commercial probiotic preparations and are known to exert their beneficial effects also through the stimulation of the host immune response. We used the C fragment of the tetanus toxin of *Clostridium tetani* (TTFC) as a model antigen to evaluate whether a treatment with probiotic spores increased the immune response elicited by a mucosal immunization. TTFC was efficiently displayed on *B. subtilis* spores and given by the nasal route to mice treated or not with the probiotics. Spore-adsorbed TTFC was more efficient than the free antigen in inducing an immune response and the probiotic treatment improved the response, increasing the production of TTFC-specific sIgA and causing a faster production of serum IgG. The analysis of the induced cytokines indicated that also the cellular immune response was increased by the probiotic treatment. A 16S RNA-based analysis of the gut microbial composition did not show dramatic differences due to the probiotic treatment. However, the abundance of members of the *Ruminiclostridium* 6 genus was found to correlate with the increased immune response of animals immunized with the spore-adsorbed antigen and treated with the probiotic. Our results indicate that probiotic spores significantly contribute to the humoral and cellular responses elicited by a mucosal immunization with spore-adsorbed TTFC, pointing to the probiotic treatment as an alternative to the use of adjuvants for mucosal vaccinations.

P16

Exploiting novel strategies to produce surfactin in *Bacillus subtilis* cultures

Mareen Geissler, Katharina Rief, Lars Lilge, Marius Henkel, and Rudolf Hausmann

Institute of Food Science and Biotechnology, Department of Bioprocess Engineering, University of Hohenheim, Fruwirthstrasse 12, 70599 Stuttgart, Germany

Bacillus subtilis synthesizes cyclic lipopeptides, with surfactin being the most abundant. Surfactin is often reported as promising biosurfactant for different industrial fields. However, while much research focuses on physico-chemical properties and potential applications, only little research aims at developing processes to pave the way for a large-scale production. Three strategies can be applied to increase the overall low yields of wild-type strains and to overcome issues such as strong foam formation, namely media optimization, strain engineering and establishing novel process approaches.

Previous research has demonstrated that the anaerobic cultivation is an elegant way to avoid foam formation, but still poses several bottlenecks such as reduced growth rates and overall low cell densities and surfactin titers. Hence, switch processes were examined, where the first part of cultivation was performed under aerobic conditions and then oxygen was allowed to be depleted. Accordingly, the biomass can reach higher values in a much shorter time before cells enter anaerobic conditions. To improve the process, different ammonium and nitrate ratios were tested and the switch was performed at different time points. However, running a bioreactor cultivation, issues due to foaming were still present during the first aerobic cultivation. Consequently, two further approaches will be examined: (1) An aerobic cultivation shall be realized employing nanoporous membranes to introduce a bubble-free aeration unit. (2) A strain shall be engineered where surfactin synthesis is activated under anaerobiosis. This project demonstrates that all three strategies go hand in hand to approach a large-scale production of surfactin.

P17

High Cell Density Fermentations for the Production of Surfactin

Peter Klausmann, Kambiz Morabbi, Marius Henkel, and Rudolf Hausmann

University of Hohenheim, Department of Bioprocess Engineering, 70599 Stuttgart, Germany

Surfactin is one of a variety of biosurfactants produced by various *Bacillus* species. It is considered one of the most powerful biosurfactants yet discovered, being able to reduce the surface tension of water from 72N m^{-1} to 27 N m^{-1}. It is also biodegradable, which is an important factor considering its wide application potential, from detergents to microbial enhanced oil recovery. Until now, little to no data exist on the actual industrial application potential. This is due to low titers in most fermentation processes of 1 to 4 g/L and the requirement of high amounts of product for many application studies. To overcome this issue, a high cell density fermentation process was developed with non-sporulating *B. subtilis* strains. This new production process achieved yields of 23,65 g/L of Surfactin in fed-batch fermentations after 28 h of fermentation, with a biomass concentration of 41,3 g/L. For product purification, a two-step ultrafiltration process was utilised in combination with acid precipitation and freeze drying, which yielded a product with up to 97 % purity. Further process and strain optimisation will be required to achieve an industrially lucrative bioprocess for large scale surfactin production. However, high cell density fermentations of *B. subtilis* can be used to provide enough Surfactin for studies on applications for lipopeptides.

P18

In vivo re-constitution of cyanobacterial RNA polymerase in *Bacillus subtilis*

Ryuichi Sato[1], Tomoya Mita[1], Satoru Watanabe[1], Yu Kanesaki[2], Mitsuhiro Itaya[3], Hirofumi Yoshikawa[1], and Kei Asai[1].

[1]*Department of Bioscience, Tokyo University of Agriculture, Tokyo, Japan.*, [2]*Research Institute of Green Science and Technology, Shizuoka University, Shizuoka, Japan.*, [3]*TRAHED, Tokyo, Japan*.

The *Bacillus subtilis* strain, in which the genome of cyanobacteria, *Synechocystis* sp. PCC6803, was integrated in its genome, had been constructed and the resultant strain was designated as CyanoBacillus. (Itaya, et al., 2005 PNAS, 102, 15971-15976). In this CyanoBacillus strain, transcripts derived from cyanobacteria genome were undetectable compared with that of *B*. *subtilis* genome. We assumed that this deficiency was caused by absence of active cyanobacterial RNA polymerase (RNAP) holoenzyme and inability of *B*. *subtilis* RNAP holoenzyme to transcript genes encoded on cyanobacteria genome. Therefore we decided to re-constitute an active cyanobacterial RNAP in *B*. *subtilis*.

The cyanobacteria RNAP-encoding genes (including *rpoD* gene) followed by ideal ribosome binding sequence were put under the control of IPTG-inducible promoter and integrated into *B*. *subtilis* genome. We detected the formation of cyanobacteria RNAP core enzyme but not core enzyme. Instead of cyanobacteria RpoD, B. subtilis SigA was bound to cyanobacteria RNAP core enzyme. It was suggested that RpoD might be excluded out from RNAP because of its lower affinity to RNAP than SigA. Additionally we put the *sigA* gene under the control of xylose-inducible promoter in the *B*. *subtilis* strain harbouring cyanobacteria RNAP genes. The growth of this strain was retarded and RpoD was accumulated within the cells in the presence of IPTG, when the expression of the *sigA* gene was reduced by decreasing xylose addition, suggesting that active cyanobacteria RNAP holoenzyme was formed and competed with *B*. *subtilis* RNAP.

P19

Genome engineering for the construction of *Bacillus subtilis* chassis

Planson, A.G., C. Wolfender, and M. Jules

Université Paris-Saclay, INRAE, AgroParisTech, Micalis Institute, 78350, Jouy-en-Josas, France

Efficient genome engineering methodologies for high-throughput genetic modifications (such as MAGE) were built upon a very small set of domesticated, laboratory organisms, primarily utilizing *Escherichia coli* and *Saccharomyces cerevisiae* because they were highly adapted to laboratory conditions. However, it is becoming increasingly clear that these are not the universal, ideal chassis. To move from laboratory demonstrators to fieldable technology, it is imperative to create a panel of robust chassis with valuable properties. Our project aims at providing a novel multiplex-assisted, high-throughput genome engineering methodology for the Gram-positive soil bacterium *Bacillus subtilis* through the development of a 'CRISPR' DNA-targeting toolbox. We first identified the limitations to the use of CRISPR-based methodologies for high-throughput genetic modifications in *B. subtilis*. The major limitations are that (i) the experimental procedure for each genetic modification is highly time-consuming and labor-intensive as it relies on the use of numerous plasmids containing the CRISPR-^{Sp}Cas9 nuclease encoding gene, the single guide RNA (sgRNA) and the repair DNA template; (ii) the iterative genetic modifications require to get rid of the plasmids carrying the sgRNA and the repair DNA template before each novel experimental iteration; and (iii) the Cas9-induced lethality imposes to transform bacteria with both the repair DNA and sgRNA. We herein propose an experimental pipeline that overcomes the experimental bottlenecks. Although currently under development, we expect our experimental strategy to significantly accelerate synthetic design implementation in *B. subtilis* genome.

P20

Characterization of thermostable β-galactosidase to develop a reporter peptide that functions in *Geobacillus* species

Shunsuke Yoshimura,[1,2] Takashi Ohshiro,[1,3] and Hirokazu Suzuki[1,3]

[1]Faculty of Engineering; [2]Department of Engineering, Graduate School of Sustainability Science; and [3]Center for Research on Green Sustainable Chemistry, Tottori University, Tottori, Japan

Reporter proteins facilitate detection of promoter expression and tracing target proteins via fusion. Although several proteins have been used as reporters to detect promoter expression in thermophiles, these are not suitable to trace targets because of large size. This study aimed to develop a thermostable small peptide that serves as a reporter in thermophiles. To this end, we focused on LacZ (GH2 β-galactosidase) because the enzyme can be split into small (α peptide) and large (ω peptide) fragments. Both fragments are catalytically inactive but spontaneously reconstitute an active form when mixed together. We cloned a possible gene for LacZ from *Geobacillus stearothermophilus* 10 and prepared the gene product ($LacZ_{GS10}$). The protein was produced as a thermostable dimer and efficiently hydrolyzed *p*-nitrophenyl-β-D-galactopyranoside (specific activity at 37°C, 22.9 ± 0.6 unit/mg protein; k_{cat}, 47.3 ± 0.4 s^{-1}; K_m, 215 ± 10 μM). The enzyme was relatively stable at pH 6.0–8.0 and below 60°C. It was resistant to diverse organic solvents (10%) and surfactants (0.1%). An ω peptide of $LacZ_{GS10}$ was produced as inactive dimer (ω_2); however, when mixed with α peptide fused with venus (venus-α), it formed a heterotetramer (venus-α_2/ω_2) and exhibited β-galactosidase activity (specific activity at 37°C, 65.1 ± 0.7 unit/mg protein; k_{cat}, 171 ± 4 s^{-1}; K_m, 160 ± 24 μM). As expected, venus-α was stable for 1 h even at 80°C. The results suggest that the α peptide may serve as a thermostable reporter in thermopiles that can be detected using ω peptide and chromogenic substrates.

P21

Introduction of the exogenous AdoMet transporter in *Bacillus subtilis*

Ryunosuke Isozaki, Natsuki Osaka, and Kei Asai.

Department of Bioscience, Tokyo University of Agriculture, Tokyo, Japan.

In every living organism, S-Adenosyl methionine (AdoMet), which is indispensable for life, is a common and important donor involved in methyl group transfers. It is synthesized from adenosine triphosphate and methionine by S-adenosylmethionine synthetase. However, the relationship between intracellular concentration of AdoMet and cellular metabolisms, and the detailed mechanisms remain to be elucidated. Therefore, we aimed to construct artificially altering system of intracellular concentration of AdoMet in *B*. *subtilis*.

In almost all eubacteria, S-adenosylmethionine synthetase, which is encoded by the *metK* gene in *B*. *subtilis*, is essential and cannot be inactivated. Although AdoMet is not incorporated within a cell, some gram-negative pathogenic bacterium like *Rickettsia* possesses a membrane protein, AdoMet transporter, involved in AdoMet uptaking. We introduced this AdoMet transporter in the *B*. *subtilis* strain, in which *metK* is under the control of IPTG. Without the addition of IPTG, this strain could not grow in LB medium. On the other hand, growth was slightly recovered when the AdoMet transporter was strongly expressed and AdoMet was added to the medium, suggesting that the exogenous AdoMet transporter was working in *B*. *subtilis*. In gram-positive bacteria, AdoMet is bound to so-called S-box riboswitch, which is located upstream of the genes involved in methionine and cysteine synthesis. We constructed the fusion gene of the S-box and *lacZ* gene, and monitored the change of intracellular concentration of AdoMet by using this fusion gene in *B*. *subtilis*.

P22

Enhanced metabolism of the γ-amino acid 3-amino-4-hydroxybenzoic acid under oxygen limitation by recombinant *Corynebacterium glutamicum*

Hideo Kawaguchi[1], Shoko Miyazaki[1], Tomohisa Hasunuma[1], Yasuo Ohnishi[2], Takashi Sazuka[3], Chiaki Ogino[1], and Akihiko Kondo[1]

[1]*Kobe University, Kobe, Japan,* [2]*The University of Tokyo, Tokyo, Japan,* [3]*Nagoya University, Nagoya, Japan*

Microbial production of aromatic compounds is of great interest as alternatives of commercial available aromatics, such as BTEX, that depend exclusively on unsustainable use of fossil resources. A γ-amino acid, 3-amino-4-hydroxybenzoic acid (3,4-AHBA), serves as a precursor for the synthesis of a thermostable super-engineering plastic, polybenzoxazole, and can be biologically produced from fermentable sugars. In our previous study, a recombinant *Corynebacterium glutamicum* strain expressing *griH* and *griI* derived from *Streptomyces griseus* successfully produced 3,4-AHBA from sugars, but the bottlenecks for enhanced 3,4-AHBA production have not been identified.

In order to improve 3,4-AHBA production from glucose, effects of cultivation conditions, particularly in dissolved oxygen (DO) concentrations, on the γ-amino acid metabolism were investigated in a jar fermenter using a DO-stat program. 3,4-AHBA production was significantly increased with reduced levels of DO. Under the DO-stat conditions, metabolome analysis clearly revealed a metabolic shift in both the central metabolic pathway and amino acid metabolism in response to reduced oxygen levels. Based on the results of metabolome analysis, a metabolic pathway was engineered to relieve the bottleneck for improved 3,4-AHBA production.

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P23

Monoacylglycerol lipase production in the eight extracellular proteases deficient *Bacillus subtilis* strain

Tatsuya Osamura, Mitsuyoshi Okuda, Fumikazu Takahashi, and Yasushi Takimura

Biological Science Laboratories, Kao Corporation, Wakayama, Japan

Monoacylglycerol (MAG) is commonly used in various industry. MAG is produced by chemical reaction and the high-purity MAG is obtained by molecular distillation process for removing diacylglycerol and triacylglycerol.

MAG lipase (MGLP) selectively catalyses the hydrolysis of MAG to free fatty acid (FFA) and glycerol, with less activity toward triacylglycerol or diacylglycerol. We found that the high purity MAG was efficiently synthesized from FFA and glycerol by EstGtA2, which was MGLP from *Geobacillus thermodenitrificans*. EstGtA2 is potentially catalyst for the high-purity MAG production without molecular distillation process.

We chose *Bacillus subtilis* as the host for EstGtA2 production. However, EstGtA2 is known as the intracellular enzyme, so the cell disruption is needed to produce this enzyme. The cell disruption process in industrial production makes the cost higher, so we studied how to produce EstGtA2 in supernatant without the cell disruption.

We found that Dpr8, which was the eight extracellular proteases deficient strain, could produce EstGtA2 in supernatant. It was known that some proteases affected the stability of cell wall lytic enzymes, so Dpr8 were more prone to lysis and released EstGtA2 in supernatant. On the other hand, Dpr7, which had not disrupted *aprE*, could not produce EstGtA2 in supernatant. Moreover, production examination with each protease deficient mutant showed that EstGtA2 was accumulated well in the supernatant by only Δ*aprE*.

Our data show that Dpr8 is suitable for EstGtA2 production, because not only this strain lysis more than the parent strain, but also do not produce protease that hydrolyses EstGtA2.

P24

Specific bioconversion of *myo*-inositol into *scyllo*-inositol using thermophilic cells that produce mesophilic enzymes

Ryota Kurashiki,[1] Yuta Okumura,[1] Masahiro Takahashi,[2] Kohei Makino,[3] Ken-ichi Yoshida,[3] Takashi Ohshiro,[4,5] and **Hirokazu Suzuki**[4,5]

[1]Department of Engineering, Graduate School of Sustainability Science; [4]Faculty of Engineering; and [5]Center for Research on Green Sustainable Chemistry, Tottori University, Tottori, Japan. [2]IMRA Japan Co., Ltd., Sapporo, Japan. [3]Graduate School of Science, Technology and Innovation, Kobe University, Kobe, Japan

Because thermophilic enzymes are generally less active at moderate temperatures, mesophilic enzymes could dominate in thermophiles to catalyze specific reactions under the conditions. Aiming to establish an approach for readily constructing microorganisms that activate specific metabolic pathway, the study was designed to examine production of mesophilic enzymes in a thermophile, *Geobacillus kaustophilus* HTA426. To this end, we first explored promoters that were highly expressed at 40°C in *G. kaustophilus* using transcriptome analysis. The candidates identified were then analyzed in terms of their expression profiles using reporter strains that expressed a *venus* gene (encoding for yellow fluorescent protein) under the control of respective promoters. The analysis revealed that the *gk0647* promoter was expressed at 40°C more efficiently than at 60°C. The promoter was cloned in an *Escherichia coli*–*Geobacillus* shuttle plasmid to construct pGKE120, which directed substantial expression of *venus* at 40°C for 24 h in *G. kaustophilus* and *G. stearothermophilus* strain 10, although not in other three strains. To examine whether pGKE120 can direct the production of mesophilic enzymes, we constructed a pGKE120 derivative that carried *iolG* and *iolX*, which are responsible for conversion of *myo*-inositol to *scyllo*-inositol in *Bacillus subtilis* 168. *G. kaustophilus* harboring the plasmid successfully converted *myo*-inositol to *scyllo*-inositol at <37°C following culture at 40°C, although the reaction required the addition of NAD^+ and cell permeabilization by surfactants. The result demonstrates that pGKE120 can be used to produce mesophilic enzymes in *G. kaustophilus*. The productivity of *scyllo*-inositol will be discussed in more detail on our presentation.

P25

Application for delivery of Large-sized DNA from *Bacillus subtilis* using Horizontal Gene Transfer (HGT) system.

Shinya Kaneko[1], and Mitsuhiro Itaya [2]

[1]*School of Life Science and Technology, Tokyo Institute of Technology, Yokohama, Japan.*
[2]*Center for Genome Synthesis, TRAHED, Tokyo Japan*

Application of *Bacillus subtilis* 168 as the initial host for DNA synthesis and subsequent delivery of the engineered DNA to other cells appears attractive. The expected system should cover efficient genome DNA synthesis for various host cells. In previous studies, the unique DNA assembly technology, named as Ordered Gene Assembly in *Bacillus subtilis* (OGAB) and Domino method using *B. subtilis* have been reported, by which DNA over 50 kb to 3,000 kb can be synthesized in *B. subtilis* cell.
The constructed large-sized DNA should be delivered into other cells including beneficial host cells for not only metabolic engineering for material production but also basic research. If DNA size increase, however, they become less stable due to nature of DNA as a long polymer in liquid. Handling of large-sized DNAs in liquid generally suffers from shearing and results in breakdown to small pieces.
To avoid the intrinsic physicochemical features of large-sized DNA, we have developed two DNA delivery techniques. Cell Lysis Technology to provide Transformable Extra-cellular DNA; CELyTED' enables introduction of over 50 kb sized plasmid DNA into the other host *E. coli* or *Saccharomyces cerevisiae,* etc. using lysate of donor *B. subtilis* host cells. Another T4SS conjugational plasmid systems can transfer over several hundred-kb sized DNA to the other *B. subtilis.* These procedures are simple protocol and don't require the extraction and purification step for the transfer DNAs. Our application for delivery of large-sized DNAs should be useful in the synthetic genome area in future.

P26

In vitro characterization of TatAyCy protein translocase complexes from Bacillus subtilis

Margarita Bernal-Cabas[1], Marcus Miethke[1], Minia Antelo[2], Rocío Aguilar Suárez[1], Jolanda Neef[1], Andreas Otto[2], Dörte Becher[2], Diana Wolf[3], and Jan Maarten van Dijl[1#]

[1]*University of Groningen, University Medical Center Groningen, Department of Medical Microbiology, Hanzeplein 1, P.O. box 30001, 9700 RB, Groningen, The Netherlands*
[2]*Institut für Mikrobiologie, University of Greifswald, Friedrich-Ludwig-Jahn-Str. 15, D-17489 Greifswald, Germany.*
[3]*Institute of Microbiology, Technische Universität (TU) Dresden, Zellescher Weg 20b, 01217 Dresden, Germany*

The Tat pathway in *Bacillus subtilis* stands out for its minimalist nature with two independently acting translocases being composed of dedicated TatA and TatC subunits only. Here we addressed the question whether TatAyCy recruits additional cellular components for activity. To this end, TatAyCy was purified by affinity chromatography and size exclusion chromatography, and interacting proteins were identified by mass spectrometry. This revealed the cell envelope stress responsive LiaH protein as an appendix to the TatAyCy complex. Our functional studies show that Tat expression is tightly trailed by LiaH induction, and that LiaH determines the capacity and quality of Tat-dependent protein translocation. Altogether, our observations show that protein translocation by the minimal Tat translocase TatAyCy is tightly intertwined with an adequate bacterial response to cell envelope stress. This is consistent with a critical need to maintain cellular homeostasis, especially when the membrane is opened to permit passage of fully-folded proteins via Tat.

P27

Investigation of mechanism how *Lactobacillus delbrueckii* JCM 1002^T directly transports inulin-type fructans

Yuji Tsujikawa[1,2], Masahiko Suzuki[2], Iwao Sakane[2], Shu Ishikawa[3], Ken-ichi Yoshida[3], and Ro Osawa[1]

[1] *Department of Bioresource Sciences, Graduate School of Agricultural Sciences, Kobe University, Rokko-dai 1-1, Nada-ku, Kobe, Hyogo 657-8501, Japan;* [2] *Cectral Research institute, Itoen Ltd, 21 Megami, Sagara-cho, Haibara-gun, Shizuoka 421-05, Japan;*
[3] *Graduate School of Science, Technology and Innovation, Kobe University*

Inulins are fructans comprising glucose and fructose units with degrees of polymerization of 2-65, which have been reported to selectively stimulate, as prebiotic substances, growth and activity of certain strains in *Lactobacillus* and *Bifidobacterium* bacteria. Inulins are generally imported by specific transporters after degradation by extracellular enzymes to oligofructoses. However, interestingly, we previously discovered that *Lactobacillus delbrueckii* JCM 1002^T strain transported inulin directly without degradation as a carbon source. The purpose of this study is to find out genes responsible for the novel inulin transporter to elucidate this unique transporting mechanism.

Based on the result that the inulin transporter was induced by inulin, inulin-induced genes were detected by RNA-seq and confirmed by quantitative RT-PCR. To examine whether the candidate genes worked as the inulin transporter, wide regions containing transcriptional units of the candidates were introduced into *amyE* region of *Bacillus subtilis* 168 that doesn't grow in a medium with inulin as the only carbon source (inulin medium), and then growth of the transformants on the inulin medium was examined.

22 candidate genes that were specifically induced by inulin were detected by RNA-seq analysis. Amongst them, Ldb0442 and Ldb1384 were the most up-regulated genes as the result of quantitative RT-PCR. While the Ldb0442-introduced 168 strain did not grow on the inulin medium, Ldb1384-introduced strain grew well. These results suggested that gene cluster of the Ldb1384 would encode the novel inulin transporter which directly transports inulin in the *L. delbrueckii* JCM 1002^T strain.

P28

Molecular Breeding of new Agrobacterium tumefaciens for increasing Plant transformation

Satoko Nonaka[1,2], Someya Tatsuhiko[2], Yasuhiro Kadota[3], Kouji Nakamura[1,2], and Hiroshi Ezura[1,2]

[1] *Tsukuba Plant Innovation Research Center, Gene Research Center, University of Tsukuba, Tsukuba, Japan.*
[2] *Faculty of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Japan.*
[3] *RIKEN Center for Sustainable Resource Science, Plant Immunity Group, Yokohama, Japan.*

Agrobacterium tumefaciens has a unique ability to mediate inter-kingdom DNA transfer. This ability of *A. tumefaciens* has been utilized in fields such as functional analysis of genes and breeding of Generic Modified (GM) crops. However, in some plants, transformation frequency is still low. To increase transformation frequency in wide variety of plant species, the transformation process is focused. The transformation process comprise the following three steps: the first step is T-DNA transfer, second is transgenic cell selection, and third is transgenic cell regeneration.
To improve T-DNA transfer frequency, we tried to modify *A. tumefaciens*, focusing on the T-DNA transfer, which is the first step of transformation and is the most important process. Several studies showed that plant release several compounds during T-DNA transfer process. The compounds include not only positive factors (i.e. acetosyringon, phenolic) but also negative factors such as ethylene, phytohormone, and gamma aminobutyric acid (GABA), a non-proteomic amino acid, in plant–*Agrobacterium* interactions. Thus, we hypothesized that the removal of these negative factors would be effective in improving the T-DNA transfer frequency. Based on this hypothesis, we tried to introduce the removal ability of negative factors into *A. tumefaciens*. And four types of Super-*Agrobacterium* were created and analyzed. In this presentation, we will evaluate these Super-Agrobacterium with five kinds of plants.

P29

Application of CRISPR/Cas for generation of Bacillus enzyme production strains

Felle, M.[1], N. Welsch[2], M. Appelbaum [2], and T. Schweder [2]

[1]*BASF SE, Ludwigshafen, Germany*
[2]*Institute of Marine Biotechnology, Greifswald, Germany*

CRISPR/Cas9-based genome editing technology has been successfully used for many different organisms. Most advantages have been in the broad spectrum of application and high efficiency of introducing gene deletions, gene integration or point mutations.
For the microbial enzyme production workhorse Bacillus, a variety of different CRISPR/Cas9-based genome editing approaches have been applied – single plasmid and two plasmid systems, the Cas9 endonuclease integrated into the genome and working with both active endonuclease, nicking endonuclease variant nCas9 or for gene silencing the defective dCas9 endonuclease.
We have implemented the single plasmid CRISPR/Cas9 genome editing approach as routine tool for genomic manipulation of various Bacillus species. Moreover, by combination of a 'smart' automated workflow it is now possible to allow genome editing of multiple strains in parallel and to construct combinatorial strain deletions.
By this means, genes identified from modern *OMICS* based approaches as targets for potential strain improvement can be efficiently constructed.

P30

Compatibility of site-specific recombination units between mobile genetic elements

Shota Suzuki[1], Miki Yoshikawa[2], Kimihiro Abe[1], Daisuke Imamura[2], Patrick Eichenberger[3], and Tsutomu Sato[1,2]

[1]*Research Center of Micro-Nano Technology (Hosei university, Tokyo, Japan)*
[2]*Department of Frontier Bioscience (Hosei university, Tokyo, Japan)*
[3]*Center for Genomics and Systems Biology (New York university, New York, United States of America)*

Lysogenic phage is one of mobile genetic elements (MGEs) which are crucial agents for gene transfer and bacterial evolution. Recent reports show that the site-specific recombination (SSR) of lysogenic phages are adequately regulated by a site-specific integrase (*int*), a recombination directionality factor (*rdf*) and *attL*/*attR* sequences (*attL-int-rdf-attR*; SSR unit), and the SSR is induced not only under stress-condition but also in cell differentiation process. Therefore, SSR unit is crucial component for the coexistence of lysogenic phages and host bacteria. Similarly, the excision of integrative conjugative elements (ICEs), one of MGEs, are also controlled by the SSR unit. However, there is little attention on the similarity of the excision mechanisms between lysogenic phages and ICEs. In this study, we demonstrate that the SSR units are compatible and can functionally substitute between phage and ICE and that of defective phage is also exchangeable with active phages by using lysogenic phage, a defective phage, and an ICE existing on *B. subtilis* 168. Specifically, when SP□ prophage is turned into a defective prophage by deletion of its SSR unit, introduction of the SSR unit of *skin* (*sigK* intervening element: a defective prophage) or ICE*Bs*1 converts it back to an active prophage. We also show the clues to the conversion of SSR unit in lysogenic phages in the nature. Our data suggest that the compatibility of the SSR unit between MGEs, which will provide us a better understanding of the evolution of SSR mechanism of MGEs.

P31

Why does PcrA helicase interact with RNA polymerase?

Iñigo Urrutia-Irazabal[1], Frank Sobott[2], Nigel J Savery[1], and Mark S. Dillingham[1]

[1]*School of Biochemistry, Medical Sciences Building, (University of Bristol, Bristol, UK)*
[2]*Astbury Centre for Structural Molecular Biology (University of Leeds, Leeds, UK)*

(Abstract is not available.)

P32

Comparative analysis of phage-like bacteriocin PBSX responsible for membrane vesicles formation in *Bacillus subtilis* and relatives.

Tatsuya Yamamoto[1], Masanori Toyofuku[1,2], and Nobuhiko Nomura[1,2]

[1] Faculty of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Japan
[2] Microbiology Research Center for Sustainability, Tsukuba, Japan

Bacteria release membrane vesicles (MVs) that range from ten to hundreds nanometers in diameter. MVs are involved in intercellular communication, horizontal gene transfer, transport of toxins to host cells. We reported that the phage lysis mechanism holin-endolysin is involved in MV production. Holin-endolysin is conserved in the genomes of various bacteria as a lysis mechanism that phages and phage-like bacteriocins use to be released from the host. MV production by holin-endolysin is considered as a universal MV production pathway and how this system that have multiple roles has evolved is of interest.

In *Bacillus subtilis*, a phage-like bacteriocin, PBSX, is involved in MV production. In the PBSX region, a gene encodes phage particles and the holin-endolysins system, but has lost recombinase that are essential to pack the phage genes in the phage particle. When the genomes of *B. subtilis* and similar species were investigated, PBSX was found to be preserved in all strains of *B. subtilis*, *Bacillus amyloliquefaciens,* and *Bacillus velezensis* strains. Genes encoding the phage particles were lost in some strains, however, holin-endolysin systems were found to be conserved. This suggests that while losing the function to release bacteriocins, the holin-endolysin systems have other important roles including MVs formation.

P33

Identification of endolysins encoded by Deep-Blue and Deep-Purple phages infecting *Bacillus weihenstephanensis*

Audrey Leprince [1], Manon Nuytten [1], Annika Gillis[1,2], and **Jacques Mahillon** [1]

[1] Laboratory of Food and Environmental Microbiology, Earth and Life Institute, UCLouvain, Louvain-la-Neuve, Belgium, and [2] Imperial College, London, UK.

Since several years, there is a renewed interest in phages and their derived proteins. Among phage proteins, endolysins are peptidoglycan degrading enzymes synthetized at the end of the lytic cycle. In combination with holins, they are responsible for the lysis of the bacterial host allowing the release of newly synthetized virions. Endolysins from phages infecting Gram-positive bacteria are organized into two domains: the C-terminal Cell Wall Binding domain (CBD) binds specifically bacteria, whereas the N-terminal part contains the Enzymatically Active Domain (EAD) that cleaves conserved bonds in the peptidoglycan. This study focuses on endolysins encoded by Deep-Blue [1] and Deep-Purple [2], two phages infecting *B. weihenstephanensis*. Deep-Blue PlyB221 has a Peptidase_M15_4 conserved domain from the VanY superfamily as EAD, which is also present in the endolysins of phages B4 [3] and Phrodo [4] infecting members of the *Bacillus cereus* group. Deep-Purple PlyP32 possesses a GH25_PlyB-like domain belonging to the GH25_muramidase superfamily. As for the CBD, two SH3 domains were detected at the C-terminus end of PlyB221 and one at that of PlyP32. Both putative endolysins were cloned and expressed to assess their lytic activity. Each CDB was also fused to a GFP to evaluate their binding capacities. Both endolysins displayed a broader spectrum than their parental phage and their CBD were able to bind the surface of several strains of the *B. cereus* group. Characterization studies of both endolysins, including their host spectrum and optimal pH, temperature and salt concentration will be presented and discussed.

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P34

Development of a systematic evaluation method of insertion sequences derived from thermophilic bacteria

Mizuki Kimura[1], Wataru Katano[2], Hirofumi Yoshikawa[2], Kei Asai[2], Ken-ichi Yoshida[1], and Shu Ishikawa[1]

[1] *Graduate School of Science, Technology and Innovation, Kobe University, Rokko-dai 1-1, Nada-ku, Kobe, Hyogo 657-8501, Japan;* [2]*Department of Bioscience, Tokyo University of Agriculture, 1-1-1 Sakuragaoka, Setagaya-ku, Tokyo 156-8502, Japan*

Insertion sequence (IS) is a member of mobile genetic elements that transposes on the genome by the action of a transposase and distributed widely in prokaryotes; however, knowledge of IS of thermophilic gram-positive bacterium is still limited. To develop the systematic evaluation method, *Bacillus subtilis* was used as a host because the genetic manipulation is very easy. As is important for enzymatic activity of transposase, *B. subtilis* grows even at 49°C which is higher than low permissive temperature for many thermophilic gram-positive bacteria. To measure frequency of transposition of IS, "jumping *cat* assay" system, which had been developed in *B. subtilis*, was utilized. As a model case, four ISs that have a complete DDE transposase gene with terminal inverted repeats in *Geobacillus kaustophilus* HTA426 were selected and the transposal activities were examined. Transpositions of all ISs were detected as chloramphenicol resistant colonies, but the frequencies were considerably different. The most efficiently transposed IS was IS*Gk03*, whose transpositional events were exponentially increased during exponential growing phase while close to ceased upon entry into stationary phase, suggesting transposed by a copy-and-paste mechanism in a DNA replication dependent manner. The transpositional frequency was the highest at 49°C, indicating that the IS*Gk03* is thermophilic. Interestingly, although at much lower frequency, the transposition was also significantly detected at 37°C. This result suggests that the transposase would have wide ranging enzymatic activity, which may be related to the wide distribution of this IS not only in thermophilic bacteria but also in hyperthermophilic and methophilic bacteria.

P35

Diversity of the 30S subunit resulting from the evolution of ribosomal protein S14

Genki Akanuma[ab], Fujio Kawamura[a], Satoru Watanabe[c], Shota Suzuki[b], Kei Asai[c], Taku Chibazakura[c], Hirofumi Yoshikawa[c], and Yasuyuki Kato-Yamada[a].

[a]*Department of Life Science, College of Science, Rikkyo University*
[b]*Department of Life Science, Graduate School of Science, Gakushuin University*
[c]*Department of Bioscience, Tokyo University of Agriculture*

Ribosomal protein S14 can be classified into three types. The first, C+, is the ancestral type and has a Zn^{2+} binding motif. The second and third types, C- short and C- long, are ca. 90 and 100 residues in length, respectively, but lack Zn^{2+} binding motifs. In the present study, the C+ type of S14 found in *Bacillus subtilis* was replaced by the heterologous C- long types of either *Escherichia coli* (S14Ec) or *Synechococcus elongatus* (S14Se). Surprisingly, both S14Ec and S14Se were efficiently incorporated into the 70S ribosomes of *B. subtilis*. However, the growth rates and sporulation efficiencies of the mutants harbouring the heterologous S14 proteins were significantly decreased. In these mutants, the polysome fraction was decreased while there was an increase in free 30S and 50S subunits, indicating that their translational activity was decreased. The abundance of ribosomal proteins S2 and S3, which are located in the 30S subunit near the S14 protein, were reduced in the mutants, while that of the heterologous S14 proteins was not significantly affected. These results suggest that the incorporation of heterologous S14 proteins changes the structure of the 30S subunit which, in turn, causes a decrease in the incorporation efficiency of ribosomal proteins S2 and S3. We also found that ribosomal protein S3Se functioned in ribosomes containing S14Se, but not in ribosomes containing S14Bs. It is therefore likely that coevolution of S3 and S14 is necessary for their functionality in the ribosome.

P36

Optimization of RK2-based gene introduction system for *Bacillus subtilis*

Takahiro Yokoi[1], Mitsuhiro Itaya[2], Hirotada Mori[3], and **Masakazu Kataoka**[1,]

[1] Interdisciplinary Graduate School of Science and Technology, Shinshu University, Nagano, Japan. [2] TRAHED, Chuo-ku, Tokyo, Japan. [3] Graduate School of Biological Sciences, Nara Institute of Science and Technology, Ikoma, Nara, Japan

The gram-positive bacterium *Bacillus subtilis* has important roles in both industrial applications and basic research. However, transformation of competent *B. subtilis* cells is more difficult to achieve compared to that of *Escherichia coli*. It has been reported that the conjugative broad host range plasmid RK2 can be transferred to various organisms, including *B. subtilis*. Nevertheless, the protocol for conjugation from *E. coli* to *B. subtilis* has not been properly established. Thus, we optimized interspecies conjugation from *E. coli* to *B. subtilis* using the RK2 system. We constructed mobilizable shuttle and integrative vectors pEB1 and pEB2, respectively. pEB1 was used to evaluate the effect of mating media, time, temperature, and genetic background of the recipient and donor strains. We found that conjugation was not significantly affected by the conjugation time or genetic background of the recipient and donor strains. Conjugation on agar was more efficient than that in liquid medium. Low temperature (16 °C and lower) drastically decreased conjugation efficiency. When using the optimized protocol for homologous recombination after conjugation, we could not obtain double crossing over mutants, as only single cross over mutants were observed in the initial selection. We then established a two-step homologous recombination method whereby positive colonies were cultivated further, which finally allowed efficient yield of double cross over recombinants. The optimized conjugation method described here allowed facility and efficient gene introduction into *B. subtilis* from *E. coli*.

P37

Relationship between chromosome transfer phenomenon and *clt*

Yuta Fukada[1], Loghapirya Syvasamy[1], Haruo Ikeda[2], and Masakazu Kataoka[1]

[1]Shinshu Univ., Nagano, Japan, [2]Kitasato Univ., Tokyo, Japan.

In the genus *Streptomyces*, it has been confirmed that the chromosomes are transferred along with the transfer of the conjugative plasmid. This phenomenon is quite unique in the microbaial world. It has been found that the gene responsible for plasmid transmission is deeply involved in this phenomenon. In this study, we focused on the relationship between the conjugative transfer factor *clt* of *S. lividans*-derived plasmid pSN22 and highly efficient chromosome transfer. It has been reported that the efficiency of the chromosome translocation phenomenon is improved by incorporating the pSN22-derived conjugation-related genes *traA*, *traB*, *traR*, and *clt* into the chromosome. Among these genes, *clt* is a gene that functions as a *cis* factor in pSN22 transmission, and is a sequence for the *tra* gene involved in transmission to recognize the target DNA. We predicted that the *clt* integrated into the chromosome would be recognized by *tra* genes and causes highly efficient transmission. To confirm this prediction, we compared the efficiency of chromosome transfer with and without *clt* integration into the chromosome under conditions where plasmid-related genes exist. As a result, chromosome transfer efficiency increased when *clt* was located in the chromosome. This is probably because the *tra* genes recognize the chromosomes as target DNA for transmission due to the presence of *clt*.

P38

Identification and application of thermophilic insertion sequences functional in *Geobacillus kaustophilus* HTA426

Misaki Ohara,[1,2] Tatsunari Taketani,[2] Misaki Tanabiki,[3] Jyumpei Kobayashi,[3] Takashi Ohshiro,[1,4] and Hirokazu Suzuki[1,4]

[1]Faculty of Engineering; [2]Department of Engineering, Graduate School of Sustainability Science; [3]Department of Engineering, Graduate School of Sustainability Science; and [4]Center for Research on Green Sustainable Chemistry, Tottori University, Tottori, Japan

Geobacillus kaustophilus HTA426 is a thermophilic bacterium that harbors numerous insertion sequences (IS) in the genome. This study was originally designed to examine thermoadaptation-directed evolution of *T7* RNA polymerase in *G. kaustophilus*; however, the experiment unexpectedly revealed that the strain induced transposition of several IS elements in response to ribonucleotide deficiency. Transposition occurred at 50–70°C and was more efficient at 65°C. IS elements that performed transposition were categorized into three groups, IS*Gka0169*, IS*Gka0302*, and IS*Gka0015*, which belong to IS*4*, IS*701*, and IS*Lre2* families, respectively. IS*Gka0302* more frequently transposed while generating direct repeats of 4–9 bp. Southern blot analysis showed that IS*Gka0302* transposition used a copy and paste mechanism and was induced by antibiotics and starvation of carbon or nitrogen in addition to ribonucleotide deficiency. The observations suggest that *G. kaustophilus* induces IS transposition when faced with growth inhibition to generate derivative cells that have genome diversification and thereby may be able to overcome the inhibition. It is noteworthy that few IS elements have been demonstrated to function in thermophiles and that there is no genetic tool for random gene disruption and/or multiple gene integration using transposition in thermophiles. To develop such a tool, we constructed a plasmid that contained inverted repeats and transposase gene from IS*Gka0169* in tandem. The plasmid was ligated with a marker gene (*venus*) between the inverted repeats and then introduced to *G. kaustophilus* to examine *venus* transposition from the resultant plasmid. We will show transposition profiles from the plasmid on the presentation.

P39

Bacillus subtilis HGT: Antagonistic interactions result in increased recombination

Polonca Stefanic[1], Katarina Belcijan[1], Barbara Kraigher[1], Rok Kostanjšek[1], Joseph Nesme[2], Jonas Madsen[2], Jasna Kovac[3], Søren Sørensen[2], Michiel Vos[4], and Ines Mandic-Mulec[1]

[1]*Biotechnical Faculty, University of Ljubljana, 1000 Ljubljana, Slovenia*
[2] *Department of Biology, University of Copenhagen, 2100 København Ø, Denmark.*
[3] *Department of Food Science, The Pennsylvania State University, University Park, PA, United States*
[4] *European Centre for Environment and Human Health, University of Exeter, Penryn, UK.*

B. subtilis is a soil dwelling bacterium with a diverse social life including quorum sensing-regulated interactions. These interactions can lead to horizontal gene transfer, and thus have a profound influence on bacterial evolution. Sexual isolation in *B. subtilis* predicts for more frequent uptake of DNA isolated from closely related microorganisms, but the DNA exchange between two interacting *B. subtilis* strains has never been studied before. Recently we discovered kin discrimination among highly related strains of *B. subtilis*, where less related strains exhibited antagonistic behaviour in the form of killing. Here we show that antagonistic interactions between two less closely related *B. subtilis* strains lead to increased recombination, in contrast to current dogma. We show that the induction of competence between non-kin strains is responsible for the observed increased DNA uptake, which can increase the adaptation rate through increased genetic variation. Our results demonstrate an important evolutionary mechanism of ''promiscuous but safe sex'': a type of bacterial cell-contact dependent DNA exchange that could promote the diversification of conspecifics and exclude non-specific and potentially incompatible DNA of other species. Our findings may help to understand the immense diversity of *B. subtilis* species at the genomic level despite existing mechanisms limiting less-related DNA integration during transformation. It is possible that this (or a similar) mechanism could be responsible for the diversification of many other bacterial species capable of natural transformation.

P40

Variation of quorum-sensing signal uptake systems

Tasneem Bareia, Shaul Pollak, and Avigdor Eldar

Tel-Aviv University, Tel-Aviv, Israel

Quorum sensing (QS) is a bacterial cell-cell communication system which relies on the secretion and density-dependent group-wide detection of diffusible molecules. The NRPP family of QS systems is perhaps the most prevalent in Gram-positive bacteria. In this family, the signal molecule is a peptide which is expressed from a specific gene and undergo a series of cleavages during and after secretion. The mature signaling peptide is then transported into the cytoplasm through oligopeptide permeases to interact with a cytoplasmic receptor. Intriguingly, many Gram-positive species code for two, seemingly redundant, oligopeptide permease systems named Opp and App. Here, we explore the role of these two oligopermeases in a wild isolate of Bacillus subtilis. A phylogenetic analysis of the two systems show that while both are present in all B. subtilis strains, the Opp system is conserved through evolution, while the App system is horizontally transferred. To further explore the difference between the permease systems we analyzed their deletion mutants for their effect on biofilm formation and quorum-sensing response to a Rap-Phr system and to an exogenous NRPP system called PlcR-PapR. We show that Opp deletion reduces Surfactin production and represses biofilm formation whereas the App deletion had no effect. We further show that the App permease has higher affinity to the PapR signal peptide than the Opp, but a lower maximal transport rate. In contrast, the App system was unable to import the endogenous Phr signal we studied. These preliminary results start to elucidate the possible functional differences between the two transporters.

P41

Bacillus subtilis persistence and secondary metabolite production in artificial soil

Carlos N. Lozano-Andrade, Heiko T. Kiesewalter, and Ákos T. Kovács

Bacterial Interactions and Evolution Group, Technical University of Denmark, Kgs Lyngby Denmark

Bacillus subtilis strains have been proposed as excellent agents to control several plant pathogens. This beneficial activity is based on its ability to produce a vast array of secondary metabolites (SM) and colonize actively different niches. Those traits have shown to play a pivotal role in plant growth promotion and biocontrol of phythopathogens. However, the fundamental question about the natural role of SM in the lifestyle of the producing bacteria in soil has been less explored due in part to technical limitations imposed by the complexity of the soil matrix. Therefore, in this work, we explore the use of an artificial soil made of a polymer matrix to describe the population dynamics and secondary metabolite production by *B. subtilis* growing in an artificial soil, either in axenic conditions or under a synthetic bacterial community. This approach allows us to track down the growth of *B. subtilis* by plate count and fluorescence microscopy, likewise, surfactin and pliplastatin were detected *in situ* by targeted LC-MS analysis. Our results showed that artificial soil is suitable to study, under controlled conditions, the factors that modulate the lifestyles of *B. subtilis* and the SM production in a soil-like system.

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P42

Engineering the plant microbiome to improve crop quality and yield

Valeria Verrone[1], James Skelton[1], Kotaro Mori[4], Isha Hashmi[2], Saskia Bindschedler[2], Pilar Junier[2], Ken-ichi Yoshida[4], Sanjay Swarup[3], and Anil Wipat[1]

[1] *School of Computing, Newcastle upon Tyne, Newcastle University*
[2] *Institute of Biology, Neuchâtel, University of Neuchâtel*
[3] *Department of Biological Science, Singapore, National University of Singapore*
[4] *Graduate School of Science, Technology and Innovation, Kobe, Kobe University*

This research seeks to establish whether the microorganisms associated with plants, the plant microbiome, can be engineered using the natural process of bacterial conjugation in order to improve crop quality and yield. The overall aim of the project is to contribute to an alternative and more sustainable future for agriculture.

In order to provide a tractable model for engineering rhizosphere bacteria, we are working with a simplified, plant growth promoting *Bacillus* community. This community is composed of three soil strains (*Bacillus licheniformis*, *Bacillus thuringiensis* Lr 3/2 and *Bacillus thuringiensis* Lr 7/2), that, in combination, have been shown to promote plant growth.

The genomes of these strains have been sequenced and analysed in an effort to understand the mutualistic nature bacterial of the consortium and in to try to identify the genetic traits responsible for the growth promotion phenotype in plants. In particular, genome scale metabolic pathway reconstructions are being used to analyse metabolic interdependency between the strains and the plant. The identification of key genetic traits for mutualistic behavior and plant growth promotion, will, in turn, lead to the identification of potential targets for genetic modification and optimization.

We are also developing a genetic delivery system for the *in-situ* modification of the consortium and other soil *Bacillus* species. A conjugation system, based on the plasmid pLS20, has been developed to deliver the new genetic traits into the genomes of genetically intractable rhizobacteria. This will be also applied to modify the metagenome of the rhizosphere population.

P43

Extracellular DNA production within the biofilm formed by *Streptococcus mutans*

Ryo Nagasawa[1], Nozomu Obana[2,3], Andrew S. Utada[3,4], and Nobuhiko Nomura[3,4]

[1]*Graduate School of Life and Environmental Sciences, University of Tsukuba, Ibaraki, Japan*

[2]*Transborder Medical Research Center, Faculty of Medicine, University of Tsukuba, Ibaraki, Japan*

[3]*Microbiology Research Center for Sustainability, University of Tsukuba, Ibaraki, Japan*

[4]*Faculty of Life and Environmental Sciences, University of Tsukuba, Ibaraki, Japan*

Streptococcus mutans, an etiological agent of dental caries, causes tooth decay through biofilm formation on tooth surfaces. Extracellular DNA (eDNA) is an important factor for the biofilm formation that mechanically enhances the biofilm structure. The eDNA is believed to be released from dead cells as a consequence of cell lysis. However, no direct observation of eDNA production in *S. mutans* is documented, and how cells release eDNA within the biofilm remains unclear. In this study, we focus on the cell death induced by quorum sensing (QS) and analyze the eDNA production at a single-cell and subpopulation level.

Since secretory QS signal (competence-stimulating peptide, CSP) was known to induce cell death in *S. mutans*, we added to CSP the medium to induce cell death and eDNA release. Time-lapse imaging at a single-cell level revealed that a subpopulation of cells induced cell death and released DNA to the extracellular milieu. The cell death and eDNA production was reduced in the deletion mutant of *lytF*, a QS-controlled putative autolysin. In addition, we found that *lytF*-expressing cells and dead cells were localized at the bottom of the biofilm by using the promoter reporter strain of *lytF*. Accordingly, our results suggest that *S. mutans* produces eDNA by cell lysis, which controlled by QS-dependent autolysin, at the bottom of the biofilm. This can contribute to attachment to the surfaces and mechanical stability of the biofilm.

P44

Social interactions between *Bacillus subtilis* and *Dickeya solani*

Roberta Gatta[1], Andrzej Wiese[1], Michał Obuchowski[2], and **Adam Iwanicki[2]**

[1]*Intercollegiate Faculty of Biotechnology UG&MUG, University of Gdańsk, Gdańsk, Poland*
[2]*Intercollegiate Faculty of Biotechnology UG&MUG, Medical University of Gdańsk, Gdańsk, Poland*

The ability of bacteria to survive different environmental conditions depends on tight cooperation between members of the same species. In practice it means such social behaviours as coordinated movement, biofilm formation, or cannibalism. In this study we show that an environmental isolate of *Bacillus subtilis* MB73/2 exhibits antagonistic effect towards potato plant pathogen *Dickeya solani* IFB0102, causing its coordinated directional movement. The observed phenomenon is, for the most part, dependent on large amounts of surfactins produced by *B. subtilis* MB73/2. Nevertheless, a surfactin-negative mutant version of this strain does also exhibit antagonistic effects towards *Dickeya solani* IFB0102.

The presented study is part of a larger project aimed on identification of factors and processes responsible for antagonism between members of *B. subtilis* and *Dyckeya* sp. species. Obtained results, apart from undoubted cognitive value of knowledge about mechanisms of antagonisms between investigated bacterial species can in the further perspective contribute to designing new methods of plant protection against phytopathogens.

P45

Quorum sensing controlling heterogeneity and biofilm morphology

Yoshihiko Tabushi [1], Nozomu Obana [2,3], and Nobuhiko Nomura [3,4]

[1] *Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Japan*

[2] *Transborder Medical Research Center, Faculty of Medicine, University of Tsukuba, Tsukuba, Japan*

[3] *Microbiology Research Center for Sustainability (MiCS), Tsukuba, Japan*

[4] *Faculty of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Japan*

Clostridium perfringens is known as a causative agent of gas gangrene. Quorum sensing (QS) regulates the toxin production in this organism. Besides, *C. perfringens* has the biofilm-forming ability. In the previous study, we reported that the threadlike extracellular matrix production increases at 25°C, and the biofilm morphology changes compared to 37°C (Obana et al. (2014) J. Bacteriol). The morphological change of biofilms could be an environmental adaptation between the inside and outside of the host. Thus, both QS and biofilm formation plays an important role in the pathogenesis of this organism. However, the involvement of QS on biofilm formation is poorly understood in *Clostridium*. Therefore, we started to analyze the relationship between QS and biofilm formation.

We constructed a fluorescent reporter strain of threadlike extracellular matrix-producing gene cluster (*sipW* operon). The *sipW* promoter displays bimodal expression in the wild type strain population. In contrast, in the mutant strain of *agrBD* genes, which is responsible for QS signal peptide synthesis, the *sipW* promoter expression is activated in the whole population. Moreover, Δ*agrBD* loses the ability for the morphological change of biofilms in response to temperature. External addition of QS signal to Δ*agrBD* restores the bimodal expression of the *sipW* promoter. These results suggest that the intercellular delivery of QS signals regulates the morphological change of biofilms by suppressing the expression of *sipW* operon. Therefore, we suggest that *Clostridium* QS plays a vital role in their pathogenesis through the regulation of biofilm formation as well as toxin production.

P46

Bacillus velezensis S141 enhancing symbiotic nitrogen fixation of *Bradyrhizobium diazoefficiens* USDA110 was stimulated by soybean root exudate.

Takahiko Kondo[1], Surachat Sibponkrung[2], Panlada Tittabutr[2],Nantakorn Boonkerd[2], Shu Ishikawa[1], Neung Teaumroong[2], and Ken-ichi Yoshida[1]

[1]*Graduate School of Science, Technology and Innovation, Kobe University, Kobe, Japan.*
[2]*Suranaree University of Tech., Nakhon Ratchasima, Thailand.*

Soil bacteria in rhizosphere that promote plant growth are referred to as Plant Growth Promoting Rhizobacteria (PGPR). *Bacillus velezensis* S141 was isolated as one of the PGPR from a soybean field in Thailand. It was shown to enhance the process of nodulation and symbiotic nitrogen fixation of *Bradyrhizobium diazoefficiens* USDA110 with soybean(*Glycine max*) and thereby to contribute to the better growth. However, the mechanisms underlying the beneficial effects on soybean remain to be elucidated.
Co-inoculation experiment with USDA110 and S141 to soybean suggested that S141 may not elevate the frequency of USDA110 infection, but facilitate nodulation and maturation after infection. Moreover, this promoting effect tended to be noticeable during the later stages of soybean growth. However, when GFP-labeled S141 was inoculated to soybean with USDA110, no clear localization was observed in plant tissues, on the surface of roots, or in the rhizosphere. The results suggested that S141 does not need to locate in a specific region of the plant or rhizosphere for its effect on rhizobium-legume symbiont.
On the other hand, it was found that S141 and USDA110 grew in the nitrogen-limited medium respectively for legume cultivation. When S141 was grown in the nitrogen-limited environment supplemented with or without soybean root exudate, they exhibited their characteristic growth profile suggesting its specific behavior reacting to the root exudate. The results implied that S141 could proliferate even in the poor soil to be stimulated by the root exudate of soybean.

P47

Quorum sensing regulated membrane vesicle production in *Streptococcus mutans*

Chika Yamamoto[1], Ryo Nagasawa[1], Nozomu Obana[2,3], Nobuhiko Nomura[4,5], and Masanori Toyofuku[4,5]

[1]*Graduate School of Life and Environmental Sciences, University of Tsukuba, Ibaraki, Japan* [2]*Transborder Medical Research Center, University of Tsukuba, Ibaraki, Japan*
[3]*Faculty of Medicine, University of Tsukuba, Ibaraki, Japan*
[4]*Microbiology Research Center for Sustainability, University of Tsukuba, Ibaraki, Japan*
[5]*Faculty of Life and Environmental Sciences, University of Tsukuba, Ibaraki, Japan*

Most bacteria release membrane vesicles (MVs) into the extracellular environment, which diameter range from 20 to 400 nm. In *Bacillus subtilis*, we reported that cell wall damage accompanying cell death (bubbling cell death) is the main route for MV production under DNA damaging conditions. In *Streptococcus mutans* it has been reported that quorum sensing (QS) induces cell death by upregulating autolysin. Still, whether QS can induce MV production in this bacterium has not been investigated, and the role of MVs in QS of Gram-positive bacteria remains largely unexplored. In this study, we examined the involvement of QS in MV production of *S. mutans.*
S. mutans utilize two peptide signals called competence-stimulating peptide (CSP), and *comX*-inducing peptide (XIP). In this study, QS was induced in *S. mutans* UA159 by adding exogenous CSP and XIP, and MVs production was examined. MVs were isolated by ultracentrifugation and quantified by staining with a membrane staining fluorescent dye. Our results show that MV production is triggered by CSP and XIP. We further examined the mechanisms by using gene deletion mutants. MV production was hardly induced in a *lytF* mutant, suggesting that autolysin is involved in the MV induction by the QS systems. Further studies are carried out to understand the role of MVs in QS and competence in this bacterium.

P48

The role of the PlcR-PapR circuit in *B. cereus* G9241, the causative agent of anthrax-like illness.

Shathviga Manoharan[1], Thomas Brooker[1], Victoria Baldwin[2], Petra Oyston[2], and Nick R. Waterfield[1]

[1] *Division of Biomedical Sciences, Warwick Medical School, University of Warwick, Coventry, United Kingdom*
[2] *Defence Science and Technology Laboratory (Dstl), Porton Down, Salisbury, Wiltshire, United Kingdom.*

Bacillus cereus G9241, a member of the *Bacillus cereus* sensu lato complex, was isolated from a Louisiana welder with a pulmonary anthrax-like illness and is closely related to *B. anthracis*.

Most members of the *B. cereus* group express PlcR, a pleiotropic transcriptional regulator of secreted toxins and enzymes allowing insect infection, which is activated by the peptide PapR at stationary phase of growth. However, in all *B. anthracis* isolates, the *plcR* gene contains a point mutation, which frame-shifts the gene, thus inactivating it. It has been proposed that the acquisition of AtxA, the mammalian responsive transcriptional regulator, was incompatible with the activity of PlcR, leading to selection for PlcR inactivation. Interestingly, G9241 encodes intact copies of both *atxA* and *plcR*. Preliminary data has shown that *B. cereus* G9241 has a temperature dependent haemolytic activity, possibly caused by haemolysins that are regulated by PlcR. We hypothesise that a change in the PlcR-PapR regulatory network in G9241 has allowed the co-existence of *plcR* and *atxA* through temperature dependent suppression of the PlcR-PapR circuit at the time AtxA becomes active.

Here we investigated whether the activity of the PlcR-PapR circuit and PlcR regulated toxins in *B. cereus* G9241 are temperature dependent (25 °C and 37 °C), in order to accommodate the activity of AtxA. Plasmid based transcription-translation GFP reporter of PlcR, PapR and PlcR-regulated toxins have been used to study translational activity.

P49

Glucose-mediated transcriptional control of protein arginine phosphorylation kinase/phosphatase regulates transcription of *ylxR* encoding NAP-like protein and cell growth in *Bacillus subtilis*

Mitsuo Ogura

Institute of Oceanic Research and Development, Tokai University, Shizuoka, Japan

Glucose is the most favorable carbon source for many bacteria, and these bacteria have developed several glucose-responsive networks. We proposed a new glucose-responsive system that includes protein acetylation and probable translation control through TsaEBD, a tRNA modification enzyme required for the synthesis of threonylcarbamoyl adenosine (t^6A)-tRNA. The system also includes the nucleoid-associated-like protein YlxR, which regulates more than 400 genes including many metabolic genes, and the *ylxR*-containing operon, which is driven by P*ylxS* and induced by glucose. Thus, transposon mutagenesis was performed to search for regulatory factors of *ylxR* expression, resulting in the identification of *ywlE*. McsB kinase phosphorylates arginine (Arg) residues of proteins, whereas YwlE phosphatase counteracts McsB activity through Arg-dephosphorylation. Phosphorylated Arg is known to function as a tag for ClpCP-dependent protein degradation. A previous study identified TsaD as an Arg-phosphorylated protein. Our results showed that the McsB/YwlE system regulates *ylxR* through ClpCP-mediated protein degradation of TsaD. We also observed that glucose induced *ywlE* expression and repressed *mcsB* expression, resulting in glucose induction of P*ylxS* according to western blot analyses of TsaD-FLAG. These observations and previous findings showing that many glycolytic and TCA-cycle enzymes are Arg-phosphorylated suggest that the McsB/YwlE system is involved in cell growth in glucose-containing medium. Disruption of *mcsB* and *ywlE* resulted in an increase in the cell mass and delayed growth, respectively, in semi-synthetic medium. These results provide insight into the physiological roles of the McsB/YwlE system and protein Arg-phosphorylation.

P50

Friend or foe: Impact of Surfactin production on cell physiology

Lars Lilge, Jens Pfannstiel, Marius Henkel, and Rudolf Hausmann

University of Hohenheim, Department of Bioprocess Engineering, 70599 Stuttgart,

The cyclic lipopeptide Surfactin is described as a biosurfactant with antibacterial characteristics. In the case of *B. subtilis* 168, the Surfactin synthetase-activating enzyme *sfp* is inactive (*sfp*-). Consequently, cell growth proceeds without Surfactin production. By repairing this pseudogene (*sfp*+), lipopeptide production is restored.
Accordingly, the impact of Surfactin on cell physiology could be analyzed by comparing protein compositions and quantities in both strains (*sfp*+ and *sfp*-). To get an idea about the cellular adaptations during the cultivation process, multiple time points from exponential as well as stationary phase were investigated. The protein expression profiles were determined by mass spectrometry resulting in a promising list of proteins, which exhibited remarkable differences between these strains.
Apart from different transporters, regulatory systems for iron homeostasis, sporulation and biofilm formation were identified. In this way, several bacterial strategies could be revealed to deal with Surfactin accumulation in physiological concentrations. Based on these insights, novel targets were conceived for strain engineering to obtain an optimized cell growth for best possible Surfactin production.

P51

Molecular engineering applied to the directed synthesis of lipopeptides by *Bacillus subtilis*

Quentin Gras[1,2], Frédérique Gancel[1], François Coutte[1], Valérie Leclere[1], and Philippe Jacques[2]

[1] *University Lille, INRA, ISA, University Artois, University Littoral Côte d'Opale, EA 7394 – ICV – Institut Charles Viollette, Lille, France*
[2] *Terra Teaching and Research Centre, Microbial Processes and Interactions, Gembloux Agro-Bio Tech, University of Liege, Gembloux, B-5030 Liège, Belgium*

Lipopeptides produced by *Bacillus subtilis*, like surfactin or fengycin can be an alternative to chemical pesticides in agriculture. Nevertheless, fengycin productions by wild strains are low and this prevents it to be an economically viable solution to the massive use of chemical pesticides. Genetic enhancement of lipopeptides production has gained attention in the last years and for that, different strategies can be followed.

In this study, the native promoter of fengycin synthetase operon has been substituted by a panel of 6 strong promoters. The results have shown that the production can be multiplied up to 6 times thanks to a partial promoter of the surfactin synthetase operon compared to the mother strain.

Global fengycin regulation has also been studied: many regulators control the fengycin synthetase. The genes coding for regulators AbrB, CodY, SinR and ScoC were knocked-out and the lipopeptides productions of the respective mutants has been studied. Δ*abrB* and Δ*codY* increase 3 folds the surfactin productions but the two others reduce by a factor of 2 the productions. For fengycin, productions have been increased by 1,6 folds for Δ*codY* mutants and 2,4 folds for Δ*sinR* or Δ*scoC*. The greatest increase in the production of fengycin has been obtained with the Δ*AbrB* mutant that produced 3,6 folds more than the mother strain.

Those results shown that fengycin synthetase native promoter is partially responsible for the low productions of this lipopeptide by *B.subtilis*. However, substitute it by a strongest one or acting on the global regulation system with gene knocking-out can improve those productions.

P52

Rae1 cleaves mRNAs in a translation-dependent manner in *Bacillus subtilis*

Fréderique Braun, Aude Trinquier, Jeremie Piton, Magali Leroy, Laetitia Gilet, and Ciarán Condon

UMR8261 CNRS - Université de Paris
Institut de Biologie Physico-Chimique, 13 rue Pierre et Marie Curie, 75005 Paris, France

We have recently identified the function of the YacP protein in *B. subtilis* that we renamed Rae1 for Ribosome associated endoribonuclease 1, because it cleaves RNA in a translation dependent manner. We solved its crystal structure and modelled Rae1 into the A-site of the ribosome with the catalytic site appropriately positioned for cleavage (C. Condon *et al* RNA Biol, 2018; M. Leroy *et al*, EMBO.J 2017).
Rae1 is the only identified endoribonuclease in *B. subtilis* that cuts mRNA within the A-site of the ribosome. We postulate that Rae1 might access the A-site of paused ribosomes and might play a role in a process similar to the eukaryotic No-Go decay pathway.
Rae1 cleaves within two small ORFs encoding peptides that both belong to large polycistronic transcripts: the *yrzI* and the *bmrBCD* operon mRNAs. We have determined the degradation pathway of these two polycistronic transcripts targeted by Rae1. Strikingly, Rae1 cleaves within unrelated nucleotide sequences but similar amino acid context: EKI and EKD, respectively. To define the modality of Rae1 cleavage, we analyzed the ability of Rae1 to cleave mRNAs that are mutated around the cleavage site. We also mutated residues of Rae1 predicted to interact with these nucleotides and our results support our model of Rae1 docked in the A site.
Interestingly, the two transcripts cleaved by Rae1 are both upregulated upon chloramphenicol (Cm) treatment and the *rae1* deleted strain appears more resistant to Cm. Because Cm affects ribosome pausing, we hypothesize that it modulates Rae1 access to the A-site.

P53

Online exploration of transcription profiles along *B. subtilis* and *S. aureus* genomes

Sandra Dérozier[1], Pierre Nicolas[1], Ulrike Mäder[2], and **Cyprien Guérin**[1]

[1] *Université Paris-Saclay, INRAE, MaIAGE, 78350, Jouy-en-Josas, France.*

[2] *Interfaculty Institute for Genetics and Functional Genomics, University Medicine Greifswald, Greifswald, Germany.*

There is a continued interest in visualizing and exploring large and complex collection of transcription profiles available from the literature along annotated reference genome for the study of specific regulatory mechanisms. Allowing experts to superimpose multiple selected transcription profiles on the genome annotation and to customize these views is the key in this context. Here, we developed a new genome and transcriptome online browser to meet this demand. As a proof of concept we propose two different websites that allow the users to fully explore the original tiling array transcriptomic data, respectively from *Bacillus subtilis* and *Staphylococcus aureus* transcriptome architecture annotation projects (Nicolas & al. 2012; Mäder & al. 2016). Highly interactive user interfaces allow visualization of hundreds of transcription profiles with selection of samples and associated colours. Personalised genomic views can be shared with other experts through simple web links. These two websites aim to replace those disclosed in the original publications to offer the Gram+ community a new and improved experience of transcriptome data browsing. Use of the framework developed here to represent other data sets in also envisioned.

P54

The effects of mutations in *greA* in *Bacillus subtilis*

Chiaki Fujita, Kazuki Fukamachi, Yoko Kusuya, and **Taku Oshima**

Toyama Prefectural University, Toyama, Japan

When RNA polymerase (RNAP) faces obstacles inhibiting RNA polymerase progression, the RNA polymerase moves to the opposite direction. This reverse movement of RNAP results in the protrusion of 3' end of the synthesizing RNA into secondary channel and the RNAP-arrest on the genome, which may cause the transcription-replication collision and lead genome instability. To salve the RNAP-arrest, RNAP cut the protruded 3'-end of RNA and removed RNA from the active centre in RNAP and resume the RNAP progression. This activity of RNAP is known to be activated by Gre factors in *E. coli*. GreA, the unique Gre factor in *B. subtilis*, may have the similar activities of *E. coli* Gre factors, while the investigation for *B. subtilis* Gre factor has been limitedly performed so far. In this study, we replaced the amino acids which are indispensable to activate the RNase activity of RNAP in *B.subtilis* GreA with alanine. These mutations of GreA caused the growth impairment under stress condition and morphology change of the *B. subtilis* cells. In addition, the additive mutations of the enzymes of DNA repair enhanced those defects. With these observations, we are going to discuss the relations of genome instability with the RNAP-arrest in the *B. subtilis* cells.

P55

Transcriptional analysis of carbon catabolite repression (CCR) in an efficient L-(+)-lactic acid-producing bacterium *Enterococcus mundtii* QU 25 grown in media with combinations of cellobiose, xylose, and glucose

Yuh Shiwa[1,2], Haruko Fujiwara[3], Mao Numaguchi[3], Mohamed Ali Abdel-Rahman[3,4], Keisuke Nabeta[5], Yu Kanesaki[2], Yukihiro Tashiro[3], Takeshi Zendo[3], Naoto Tanaka[1], Nobuyuki Fujita[1], Hirofumi Yoshikawa[5,2], Kenji Sonomoto[3], and Mariko Shimizu-Kadota[6,5]

[1]Department of Molecular Microbiology, [2]NODAI Genome Research Center, [5]Department of Bioscience, Tokyo University of Agriculture, Tokyo, Japan. [3]Division of Systems Bioengineering, Department of Bioscience and Biotechnology, Faculty of Agriculture, Graduate School, Kyushu University, Fukuoka, Japan. [4]Botany and Microbiology Department, Faculty of Science for boys, Al-Azhar University, Cairo, Egypt. [6]Department of Environmental Systems Sciences, Musashino University, Tokyo, Japan.

Enterococcus mundtii QU 25 is a non-dairy lactic acid bacterium isolated from ovine feces that can ferment cellobiose, xylose, and glucose to produce optically pure L-lactic acid (≥99.9%) via homofermentation. In our previous study, the QU 25 strain exhibited apparent CCR, in which glucose is assimilated faster than xylose; however, replacement of glucose with cellobiose led to simultaneous consumption of both sugars without CCR. Although non-inhibition of xylose metabolism in the presence of cellobiose in QU 25 has been demonstrated, the mechanism(s) is not known, especially at the transcriptional level.

In this study, we performed RNA-seq analysis during the exponential growth phase of cells grown in glucose, cellobiose, and xylose as either sole or co-carbon sources. The gene expression pattern in cells grown in the cellobiose-xylose mixture was closer to that of xylose-grown cells than to cells grown in the glucose-xylose mixture. The expression levels of the xylose operon were approximately 100-fold lower in the cells grown in the glucose-xylose mixture than in the xylose-grown cells, whereas the expression levels of the above genes were approximately 7-fold lower in the cells grown in the cellobiose-xylose mixture than in xylose-grown cells. These results demonstrated that in glucose-xylose grown cells, strong transcriptional repression occurred in the xylose operon, whereas the co-presence of cellobiose and xylose relieved the transcriptional repression of the xylose operon. Because the decreased transcription of the xylose operon genes was likely to be sufficient for xylose metabolism in the cellobiose-xylose mixture, we could not observe the phenotypic CCR.

P56

Escherichia coli RNase E can efficiently replace RNase Y in *Bacillus subtilis*

Sylvain Roque, Marina Cavaiuolo, Carine Chagneau, Harald Putzer, and **Soumaya Laalami**

CNRS, Université de Paris, Institut de Biologie Physico-Chimique, Paris, France

The instability of mRNA is fundamental to controlling gene expression. Most fundamental knowledge in this respect stems from studies in *E. coli* and *B. subtilis*, two organisms separated by an evolutionary stretch of several billion years. For many years, mRNA decay in these two model organisms was thought to be radically different, but now can probably best be resumed by “different enzymes – similar strategies”.

The primordial role of endoribonucleases with relaxed sequence specificity producing short-lived decay intermediates is now clearly recognized even in Gram positive organisms. In *B. subtilis,* RNase Y is the major enzyme initiating global mRNA decay and bulk mRNA is stabilized in its absence. In *E. coli,* RNase E plays a comparable role. The similar *in vitro* activity between *B. subtilis* RNase Y and *E. coli* RNase E despite a complete absence of sequence similarity incited us to perform complementation studies.

We used *B. subtilis* RNase Y knockout mutants to analyze whether and to what extent *E. coli* RNase E can substitute for the *Bacillus* enzyme *in vivo.*

Our results suggest a surprisingly efficient « complementation » between these two enzymes, including cellular localization and cleavage of several individual substrates and shed light on an impressive case of convergent evolution. They highlight the importance of low-specificity endonucleases for initiating bacterial mRNA decay.

P57

The transcription elongation factor, GreA, suppresses transcriptional attenuation of RNA polymerase in *Bacillus subtilis* cells that have the potential to develop competence

Yoko Kusuya[1], Daisuke Shiomi[2], Shu Ishikawa[3, 4], Naotake Ogasawara[3], Hiroki Takahashi[1], and Taku Oshima[3, 5]

[1] Chiba Univ, Chiba, Japan., [2] Rikkyo Univ, Tokyo, Japan., [3] NAIST, Nara, Japan., [4] Kobe Univ, Kobe, Japan., [5] Toyama Prefectural Univ, Toyama, Japan

The bacterial transcription elongation factor GreA rescues backtracked RNA polymerase (RNAP), thereby allowing RNAP to complete transcription along the length of a transcription unit. However, the physiological importance of Gre in many bacteria remains unknown. We previously showed that deletion of *greA* increases the pausing of promoter-proximal RNAP on DNA of *B. subtilis* cultured in LB (*Luria-Bertani*) medium, whereas the transcriptome composition was hardly altered. Herein, we show that GreA transcriptionally regulates development of competence in *B. subtilis*. We found that deletion of *greA* reduced transformation efficiency and the number of cells expressing late-competence genes. A ChIP-chip analysis of RNAP binding revealed that deletion of *greA* enhanced transcriptional attenuation in many *B. subtilis* operons involved in the development of competence. One such operon was that of *srf*, which also encodes *comS*, the protein product of which is required for expression of late-competence genes. A complementation study showed that expression of a plasmid-derived ComS maintained the number of cells that expressed the late-competence genes even when *greA* had been deleted. Our results indicate that GreA suppresses transcriptional attenuation and is necessary for regulation of the transcriptional network involving late-competence genes in *B. subtilis*.

P58

In vivo and in vitro characterization of a PDH-ODH hybrid complex from *Corynebacterium glutamicum*

Hirokazu Kinugawa, Naoko Kondo, Ayano Komine-Abe, Makoto Nishiyama, and **Saori Kosono**

Biotechnology Research Center, The University of Tokyo (Tokyo, JAPAN)

Pyruvate dehydrogenase (PDH) and 2-oxoglutarate dehydrogenase (ODH) are members of 2-oxoacid dehydrogenase family and have central roles in carbon metabolism. PDH and ODH are composed of three subunits (E1, E2, and E3) and usually exist as independent complexes. Meanwhile, in the famous L-glutamate producer *Corynebacterium glutamicum*, PDH and ODH exist as a hybrid complex, in which E1p (E1 subunit of PDH) and E1o (E1 subunit of ODH) associate onto the E2-E3 subcomplex. By ultracentrifuge analysis using *C. glutamicum* lysates, we found that a PDH-ODH hybrid complex were sedimented in fractions of lower molecular mass compared to those of PDH (4.8 MDa) and ODH (2.4 MDa) from *E. coli*. We were successful to reconstitute PDH and ODH activities in vitro using the recombinant E1p, E1o, E2, and E3 proteins. The increasing amount of E1p inhibited ODH activity and vice versa, suggesting that E1p and E1o are likely compete for association onto the E2-E3 subcomplex. Gel-filtration chromatography analysis using the reconstituted PDH and ODH samples showed that the binding of E1o to the E2-E3 were stable, while that of E1p were likely weak to be maintained during gel-filtration chromatography. Our study will provide a molecular basis to understand the central carbon metabolism of *C. glutamicum* which is important for the production of useful compounds.

P59

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 J. M. Harmsen[1], and Jan Maarten van Dijl[1]

[1] *University of Groningen, University Medical Center Groningen, Groningen, the Netherlands*
[2] *Shanghai Medical College, Fudan University, Shanghai, China*
[3] *University of Bath, Bath, United Kingdom*
[4] *Institute for Microbiology, University of Greifswald, Greifswald, Germany*
[5] *Institute of Biology, Freie Universität Berlin, 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 σ^B-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.

P60

Transcriptome and proteome changeovers reveal the bacterial strategy of spore awakening

Bhagyashree N. Swarge[1, 2], Wishwas Abhyankar[1, 2], Martijs Jonker[3], Huub Hoefsloot[4], Gertjan Kramer[1] , Stanley Brul[2], and Leo de Koning[1]

[1]Department of Mass Spectrometry of Bio macromolecules, [2]Department Molecular Biology & Microbial Food Safety, [3]RNA Biology & Applied Bioinformatics Research Group, [4]Biosystems Data Analysis, Swammerdam Institute for Life Sciences, University of Amsterdam, The Netherlands

Bacterial endospores pose challenges to the food and medical sectors. Spores reactivate their metabolism upon contact with germinants and develop into vegetative cells. The molecular machinery that triggers the progress of germination is still unsettled. To gain insights in the germination and outgrowth processes, the progressions of the transcriptome and proteome changeover during spore germination and outgrowth to vegetative cells, are mapped. *B. subtilis spores* are sampled at regular time-intervals from germination initiation to the stage of vegetative cell outgrowth. RNA's are purified from spores at each time-point. This transcriptome analysis allows us to trace the different functional groups of genes expressed. For each time-point sample, the change in the spore proteome is quantitatively monitored with ^{15}N metabolic labeling and a SILAC approach. Until the phase bright spores turn phase-dark, which indicates completion of germination, no significant change in the proteome is detected. Thereafter, the observed diverse timing of the synthesis of different protein sets reveals the strategy of spore revival. Proteins of fundamental metabolic pathways are identified in the dormant spores suggesting that these 'dormant' metabolic pathways can kick-off spore revival. Correlation of the transcriptomics and proteomics data allows us to understand the planning and execution of the developmental steps crucial to and characteristic for spore awakening.

P61

Interplay between biofilm formation and sporulation in *Paenibacillus polymyxa*

Yuta Hasegawa[1], Hiroko Kato[1], Nozomu Obana[2,3], and Nobuhiko Nomura[3,4]

[1]*Graduate school of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Japan*

[2]*Faculty of medicine, University of Tsukuba, Tsukuba, Japan*

[3] *Microbiology Research Center for Sustainability, University of Tsukuba, Tsukuba, Japan*

[4] *Faculty of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Japan*

Biofilms are complex multicellular communities of microorganisms that consist of cells and self-produced extracellular polymeric substances (EPS). The EPS matrix is necessary for structural biofilm development. In the previous study, we reported that several *Paenibacillus* species form biofilms and that spores derived from biofilm cells show a higher tolerance to sporicide peracetic acid than spores derived from planktonic cells. This indicates that biofilm formation influences the sporulation in *Paenibacillus* that are recognized as a cause of the spoilage of dairy products. However, the factors required for the biofilm formation are not well characterized in *Paenibacillus*. Thus, we aim to elucidate the mechanism of biofilm formation in *P. polymyxa*.

Here, we show that biofilm formation of *P. polymyxa* ATCC39564 requires the sporulation processes. The deletion mutant of a sporulation master regulator *spo0A* gene completely abolished the biofilm formation. In addition, forespore-specific sigma factor genes, *sigF* and *sigG* were necessary for the 3D structure of the biofilm. These results suggest that a sporulation factor *spo0A* regulates the initial cell attachment to the surface and that the forespore-specific sigma factors are responsible for biofilm development. Moreover, the enzymatic treatments of *P. polymyxa* biofilm indicate that extracellular proteins are the main components of the biofilm EPS. Proteinase K treatment, as well as the *sigF* gene deletion, inhibited the 3D structure of the biofilm, suggesting that the sporulation cascade controls EPS production composed of proteins. These results imply biofilm formation and sporulation are reciprocally associated in *P. polymyxa*.

P62

Functional characterization of germination receptors in *Bacillus subtilis* spores

Jin J. Sakmaoto, Ryoko Asada, Masakazu Furuta, and Tetsuaki Tsuchido

Research Center of Microorganism Control, Osaka Prefecture University, 1-2Gakuen-cho, Naka-ku, Sakai, Osaka, Japan.

Bacterial spores have often contaminate foods and cause food-poisoning and spoilage They can germinate when some germinant is added, but the mechanism of spore germination is still unclear. To investigate the function of germination receptors, the GerA in L-Ala germination system, the GerB and GerK in L-Asn, D-Glucose, D-fructose, and KCl (AGFK) system, we constructed the series of germination receptors encoding genes multiple knockout mutants on the basis of a cortex-lytic enzymes mutant defective in *cwlJ sleB ydhD* and *yaaH* genes. Although the optical density (OD) of spore suspension was significantly reduced with these germinant in the presence of cortex-lytic enzymes, the OD decrease of and the release of dipicolinic acid (DPA) caused in the presence of the germination receptors but without cortex-lytic enzymes. According to the amino acid sequence, GerAA, GerBA, and GerKA subunits had a common conserved region with SpoVAF encoding a DPA transporter. It is suggested that the germination receptors may possess a function of DPA release. The OD decrease is probably involved in the following four germination processes, core swelling due to water penetration; leakage of solubilized DPA; cortex degradation; water intrusion due to cell volume increase. In other words, different factors cause the same phenomenon in OD measurement. Although no experimental system taking the above questions into account has been developed in germination research. Such an assay system may be expected to be used for the functional analysis of germination receptors without background noise involved in this system.

P63

Analysis of oxidation-reduction balance at the initiation of sporulation in *Bacillus subtilis*.

Michiru Yabe, Masashi Yamaguchi, and Kei Asai.

Department of Bioscience, Tokyo University of Agriculture, Tokyo, Japan.

Bacillus subtilis ceases growth and initiates spore formation, with sensing stress such as nutrient starvation or an increase of a cell density. However, because the detailed mechanism of sporulation initiation has not yet become clear, we decided to analyse it paying our attention to intracellular metabolism and redox state.

The amount of glucose and acetate dissolved in medium during culturing of the cells was measured. While the amount of glucose decreased, that of acetic acid increased at the initiation of sporulation, and then we assumed that metabolic switch from glycolytic pathway to TCA cycle would be associated with sporulation initiation. Next, we measured an intracellular redox state by using bacterial luciferase so as to monitor metabolic changes in wild type strain and the strains, in which TCA cycle- or sporulation-related genes were defected, during sporulation. In the wild type strain and also sporulation-deficient strains, a reduced state was maintained during the sporulation processes. On the other hand, in the strain, in which TCA cycle-related genes were defected, the reduced state was not recovered. It was suggested that maintenance mechanism of oxidation-reduction balance is associated with very early event of sporulation initiation.

P64

Spo0A-dependent membrane vesicle production in *Bacillus subtilis*

Kimihiro Abe[1], Nozomu Obana[2,3], Masanori Toyofuku[1,3], and Nobuhiko Nomura[1,3]

[1]*Faculty of Life and Environmental Sciences, University of Tsukuba, Ibaraki, Japan*
[2]*Faculty of Transborder Medical Research Center, University of Tsukuba, Ibaraki Japan*
[3]*Microbiology Research Center for Sustainability, University of Tsukuba, Ibaraki, Japan*

Bacteria release small membranous spherical structures called “membrane vesicles (MVs)”, which are derived from the cellular membrane components. MVs contain and convey various biomolecules including nucleic acids, proteins, metabolites, and signal molecules, thereby participating in many biological processes. Yet, the molecular mechanism of formation of MVs still remains to be elucidated, in particular, in Gram-positive bacteria. To date, it is known that *Bacillus subtilis* can be induced to produce abundant MVs by treatment with a DNA-damaging agent, mitomycin C, or by long-term cultivation in Brain-Heart Infusion (BHI) medium. We have previously reported that PBSX prophage-encoded lytic enzymes trigger cell lysis and the MV production upon DNA damage (Toyofuku *et al*., 2017). In this study, we aim to clarify the mechanism of the MV production in the BHI medium. In *Clostridium perfringens*, MV production depends on Spo0A (Obana *et al*., 2017), which is the highly conserved master regulator of sporulation and biofilm formation in Gram-positive spore formers. We confirmed the involvement of Spo0A in the MV production in *B. subtilis*. Moreover, we show that a global transcriptional regulator AbrB, which acts downstream of Spo0A, negatively regulates the MV production. Out of a number of genes controlled by the Spo0A-AbrB regulatory cascade, we found that the biosynthesis genes for sublancin is required for the MV production. Because sublancin has a bactericidal activity, the MV production is inferred to be associated with sibling killing by the antimicrobial and the consequent cell lysis. We will also discuss the mechanism of action of sublancin.

P65

A novel conserved protein complex controls sporulation in *Clostridium perfringens*

Nozomu Obana, Naoki Muto, and Nobuhiko Nomura

University of Tsukuba, Tsukuba, Japan

Pathogenic *Bacillus* and *Clostridium* bacteria form spores to acquire a high tolerance to heat, oxygen, and antibiotics, causing infectious diseases such as food poisoning. *Clostridium perfringens* is a causative bacterium of food poisoning and gas gangrene, and sporulation is recognized as an essential process for the pathogenesis of this bacterium. However, the detailed regulatory mechanisms of sporulation by *Clostridium* are poorly understood compared to *Bacillus*. In this study, we identified a novel regulator of sporulation and toxin production.

Here, we show that the CtrA and CtrB proteins form a stable heteromeric complex within the cells. This protein complex is indispensable for sporulation, biofilm formation, and membrane vesicle formation. We reveal that the *ctrB*-disrupted mutant of food poisoning strain of *C. perfringens* is unable to sporulate. The cell morphology of the Δ*ctrB* mutant was arrested at the early sporulation stage (stage I), suggesting that CtrB regulates an initial step of sporulation. Spo0A is known to be a primary regulatory protein of sporulation in *Bacillus* and *Clostridium*. Transcriptome analysis of *ctrAB* and *spo0A* mutant strains shows that more than 200 *ctrAB* regulons overlap with spo0A regulons, which implies Spo0A is involved in CtrAB-mediated regulation of sporulation. We show that the amount of phosphorylated Spo0A, which is necessary for sporulation, is reduced in the *ctrB*-disrupted strain. Since the *ctrAB* operon is highly conserved in several Clostridium species, our data demonstrate that the CtrAB protein complex is a crucial regulator of initiation of sporulation in *Clostridium*.

P66

The role of metabolites produced before nutritional depletion on *Bacillus velezensis* 83 sporulation

Esmeralda Y. Soriano-Peña, Enrique Galindo-Fentanes, and Leobardo Serrano-Carreón

Universidad Nacional Autónoma de México, Instituto de Biotecnología. Departamento de Ingeniería Celular y Biocatálisis. Cuernavaca, Morelos, 62210

Bacillus velezensis 83 spores are the key ingredient of Fungifree AB®, the first biological fungicide developed and marketed in Mexico. Spore phenotype importance relapse on their strength and stability, which allow producing effective formulations for field applications. It is known that sporulation is a complex process that depends on the nutritional depletion and *quorum sensing*, but little is known about the impact of molecules produced before nutritional depletion on the sporulation. We identify three molecules groups that could affect sporulation: lipopeptides (surfactin / bacillomycin), which are involved in antagonist activity and biofilm formation; carbon overflow metabolites (acetoin /2,3-butanediol) probably acting as carbon reserve source that is consume during sporulation, and the peptide CSF (Competence and Sporulation Factor) as a *quorum sensing* molecule. Batch cultures and exogenous additions in the flask cultures were used as experimental strategies.

First we found that nutritional depletion is the main factor that triggers *Bacillus velezensis* 83 sporulation. The molecules produced before glucose depletion affect sporulation efficiency and synchronization and showing complex interactions. There seems to be a balance between the number of spores and the synchronization of the sporulation process since carbon overflow metabolites increase sporulation efficiency but desynchronize the sporulation while in presence of lipopeptides the opposite behavior is observed. Otherwise, CSF has a positive effect in both sporulation responses.

P67

Role of the orphan ribonuclease KapD in *Bacillus subtilis* spore development

Anastasia Tolcan[1], Laetitia Gilet[1], Magali Ventroux[2], Olivier Pellegrini[1], Sylvain Durand[1], Mónica Serrano[3], Adriano Henriques[3], Rut Caraballido-Lopez[2], and Ciarán Condon[1]

[1]*UMR8261 (CNRS Univeristé de Paris), Institut de Biologie Physico-Chimique, Paris, France*
[2]*Micalis Institute, INRA, AgroParisTech, Université Paris-Saclay, Jouy-en-Josas, France.*
[3]*Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal*

When facing severe nutritional stress, the Gram-positive model bacterium *Bacillus subtilis* forms highly resistant spores. The pathway to sporulation is probably the best-studied developmental program in bacteria. Yet, the function of many genes expressed during this process remains a mystery. We have identified a new 3'-5' exoribonuclease KapD that is expressed specifically in the mother cell during sporulation. Remarkably, in addition to its presumed role as an RNase in the mother cell, 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* co-purification assays show that KapD interacts with the major spore crust protein, CotY. This interaction is further supported by the observation that KapD is no longer specifically localized in the spore coat in a Δ*cotY* deletion strain, but rather accumulates in the mother cell. Although we could detect no major impact of a *ΔkapD* deletion on spore survival or germination, we have identified a visible morphological defect by transmission electron microscopy (TEM), that seems to be correlated with the RNase activity of KapD. Our findings show that the 3'-5' exoribonuclease KapD has an important role in correct spore coat assembly.

P68

Structure-function studies of stressosomes from *B. subtilis* and *L. monocytogenes*

Sema Ejder, Jon Marles-Wright, and Richard J. Lewis

Newcastle University, Newcastle Upon Tyne, UK

Bacillus subtilis (*Bsu*) is widely used as a model organism for Gram-positive pathogens such as *Listeria monocytogenes* (*Lmo*). Both *Bsu* and *Lmo,* for instance, have the ability to resist and overcome environmental fluctuations, such as high salinity levels and alcohol stress. This stress resistance is a consequence of the general stress response regulon, regulated by the alternative sigma factor σ^B, whose activation in response to environmental stressors is regulated by a supramolecular protein complex called the stressosome. The stressosome detects and integrates environmental signals to activate σ^B through a partner-switching cascade. The stressosome comprises of RsbR and a number of distinct paralogues, the scaffold protein RsbS, and the kinase RsbT. The N-terminal domains (NTDs) of RsbR paralogues have been presumed to act as the stress sensors as they project from the core of the stressosome. However, the mechanism by which signals are perceived and transmitted is still unknown.

To understand how the stressosome is activated on a structural level, the structures of the NTDs of the *Lmo* RsbR paralogues were determined by X-ray crystallography. Moreover, interaction assays were performed with the membrane associated miniprotein Prli42, which is a putative interaction partner of RsbR. To better understand the native organisation of the stresssosome, we analysed by single-particle cryo-EM reconstruction, the native stressosome complex purified by affinity pulldowns from *Bsu.* Mass spectrometry analysis of these native stressosomes confirms the presence of each of the RsbR paralogues, with RsbS and RsbT.

P69

Bacillus spore response to low energy electron beam: role of different DNA repair mechanisms

Yifan Zhang[1], Nina Huber[1], Ralf Moeller[2], Barbora Dubovcova[3], Georgios Akepsimaidis[3], Nicolas Meneses[3], Jörg Stülke[4], David Drissner[5], and Alexander Mathys[1]

[1] ETH Zurich, Zurich, Switzerland, [2] German Aerospace Center, Cologne, Germany, [3] Bühler AG, Uzwil, Switzerland, [4] Georg-August-University Göttingen, Göttingen, Germany, [5] Albstadt-Sigmaringen University, Sigmaringen, Germany

Bacillus spores are main targets of sterilization due to their extreme resistance. In order to inactivate them, conventionally intensive thermal treatments are applied. However, thermal decontamination is not suitable for all substances, e.g. heat sensitive ingredients, materials and devices. Therefore, low energy electron beam (LEEB, ≤300 keV), which is chemical- and water-free and easily scalable, was investigated as a non-thermal decontamination alternative.
Our studies revealed that wildtype *Bacillus* spores could be efficiently inactivated by LEEB, with D-values between 2.3-2.9 kGy (the dose required for one $\log_{10}$ reduction). Interestingly, *Bacillus pumilus* DSM492, which is the biological indicator for irradiation-based sterilization, did not show the highest resistance among relevant strains. Six *Bacillus subtilis* DNA repair-deficient mutants showed decreased resistance towards LEEB compared to their wildtype, indicating DNA is the main target. The mutants responded similarly at 80 and 200 keV, indicating the inactivation mechanism is not dependent on kinetic energy levels. Mutants lacking either homologous recombination (Δ*recA*) or non-homologous end joining (Δ*Ku* Δ*ligD*) showed the lowest resistance, with D-values of 1.3 and 1.6 kGy. Mutants lacking base excision repair (Δ*exo* Δ*nfo*) or nucleotide excision repair (Δ*uvrAB*) also showed largely increased sensitivity. This indicates those DNA repair pathways are essential for *Bacillus* to survive LEEB treatment. Furthermore, a continuous industrial LEEB system was tested and 4.3 $\log_{10}$ *B. subtilis* spore inactivation was achieved at applied parameters.

This study revealed how *Bacillus* spores response to LEEB, which will contribute to the development and application of LEEB as future non-thermal decontamination technology for sensitive substances.

P70

Reporter Systems for Load Stress in *Bacillus subtilis*

Wendy Smith, Yiming Huagh, Bradley Brown, David Markham, Jaume Bacardit, and Anil Wipat

School of Computing, Newcastle University, UK

Hosting synthetic genetic circuits imposes a load on a host. This load is in the form of a complex stress responses and can result in detrimental effects on the growth of the host, and function of the introduced system (Ceroni, 2015; Lo, 2016). This phenomenon is particularly pronounced for heterologous protein and metabolite production where potentially large amounts of the cells available resources are consumed. Natural, bacterial gene regulatory systems continuously monitor complex environmental/cellular conditions and respond by adjusting gene expression as needed. In this project we aim to develop synthetic load stress monitoring and feedback control circuits to alleviate load stress by controlling the expression of the heterologous protein stressor.

This work focuses on building reporter systems for complex stresses and the corresponding feedback control circuitry by i) Defining biomarker signatures of gene expression that are uniquely indicative of important types of stresses such as load stress. ii) Developing genetic reporter systems for the variety of different types of stresses which define load stress. iii) Developing regulatory circuits to allow compensatory measures to be implemented.

This project is part of a larger project, Portabolomics, which aims to develop a technology that allows the same synthetic devices and systems to be moved seamlessly between a set of different standardised chassis. Reporter systems developed here will form part of the Portabolomics interface in these strains. Logic circuits, based on dual inputs (AND gate) will be implemented to monitor the host stress profile and allow regulatory and reporter circuits to be developed.

P71

Ribosome association primes Rel stringent factor for recruitment of deacylated tRNA to ribosomal A-site

Hiraku Takada[1, 2] and Vasili Hauryliuk[1, 2, 3]

[1]Department of Molecular Biology, Umeå University, Sweden, [2]Laboratory of Molecular Infection Medicine Sweden, Sweden, [3]University of Tartu, Institute of Technology, Estonia

In the Gram-positive Firmicute bacterium *Bacillus subtilis*, amino acid starvation induces synthesis of the alarmone (p)ppGpp by the multi-domain RelA/SpoT Homolog factor Rel. This bifunctional enzyme is capable of both synthesizing and hydrolysing (p)ppGpp. To detect amino acid deficiency, Rel monitors the aminoacylation status of the ribosomal A-site tRNA by directly inspecting the tRNA's CCA end with conserved H420 histidine residue of its TGS domain. Here we uncover the catalytic cycle of this enzyme. Off the ribosome, Rel assumes a 'closed' conformation which has predominantly (p)ppGpp hydrolysis activity. This state does not specifically inspect tRNA: the interaction is only moderately affected by tRNA amino acylation and the H420E substitution. On the ribosome, Rel assumes an 'open' conformation, which primes the TGS and Helical domains for specific recognition and recruitment of cognate deacylated tRNA to the ribosome. The tRNA locks Rel on the ribosome in a hyperactivated state that processively synthesises (p)ppGpp while the hydrolysis activity is suppressed. In stark contrast to non-specific tRNA interactions off the ribosome, both the H420 mutation and tRNA aminoacylation abrogate tRNA-dependent Rel locking on the ribosome and activation of (p)ppGpp synthesis.

P72

The proteomic analysis of transport systems in *Bacillus subtilis*

Jennifer Wright[1], Martyn Dade-Robertson[2], Anil Wipat[2], Gary Black[1], and Meng Zhang[1]

[1] *Northumbria University, Newcastle Upon Tyne, UK,*
[2] *Newcastle University, Newcastle Upon Tyne, UK*

How cells adapt and respond to nutrient limitation are basic questions in microbial physiology. Numerous global regulatory systems that allow bacteria to preferentially assimilate compounds of Carbon and Nitrogen now been described. *Bacillus subtilis*, in particular, has evolved to adapt to stress and nutrient limitation by a variety of mechanisms.

In this work cultivation of *B. subtilis* in minimal media with a controlled nitrogen source enabled the study of the molecular mechanisms of nutrient stress under nitrogen limitation. A proteomics approach was used to investigate the differentially expressed proteins in nitrogen limited conditions. The function and interactions of those proteins were analysed, and a number of transport systems have shown significant changes.

This work reveals that *B. subtilis* transport systems such as Opp play an important role in survival during nitrogen limitation. The Opp system is an ATP-binding cassette transporter formed by five membrane associated proteins. The *opp* transport system is already understood to regulate the developmental processes of sporulation and competence. Knockouts of specific genes of the oligopeptide transport system Opp were investigated using a proteomics approach. The *opp* knockouts identified the upregulation of the zinc ABC transporter system *znuABC* which was increased in the peptide transport knockouts. Zinc is a necessary metal and serves as a co-factor and structural constituent for numerous enzymes and regulatory proteins. Knockouts of the high affinity zinc transporter *znuABC* were also investigated, also using a proteomics approach to further understand the role in nitrogen limiting conditions in *B. subtilis.*

P73

Preadaptation of *Bacillus subtilis* to mild osmostress contributes to increased antibiotic resistance

Luiza P. Morawska[1], Ruud G.J. Detert Oude Weme[1], Elrike Frenzel[1], Tamara Hoffmann[2], Erhard Bremer[2], and Oscar P. Kuipers[1]

[1]*Molecular Genetics Group, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Nijenborgh 7, 9747 AG Groningen, Netherlands*
[2]*Philipps University Marburg, Department of Biology, Laboratory for Microbiology, Karl-von-Frisch-Str.8, D-35032 Marburg, Germany*

Bacterial communities, exposed to rapid changes in their habitat, encounter various forms of stress. Fluctuating conditions of the microenvironment drive microorganisms to acquire a number of stress responses to sustain efficient growth and division, like altering gene expression and changing the physiology of the cell. It is known, that these protection systems may give rise to heteroresistant populations and indirectly have an impact on bacterial susceptibility to antimicrobials. One condition that bacteria are routinely exposed to is oxidative stress that can emerge from general stress response mechanisms and directly from antimicrobial exposure. In our study, we focused on osmotically-induced oxidative stress and its contribution to antibiotic resistance in the soil-dwelling bacterium *Bacillus subtilis*.

To study the adaptive response and antibiotic resistance development in *B. subtilis* we determined pretreatment conditions for the preadaptation of cells to lethal doses of antibiotics. To find these conditions we followed the expression of osmoprotective proline biosynthesis pathway genes – *proHJ* at the single-cell level under 0.6M NaCl salt stress by time-lapse microscopy. Under these conditions, we observed a switching point and heterogeneous proline biosynthesis, where the subpopulation of cells showing active *proHJ* transcription is able to continuously divide and the residual cells remain dormant. We demonstrate that preadapted cultures are more resistant to certain classes of antibiotics that are also known to elevate levels of reactive oxygen species. Moreover, we show that the tested antibiotics significantly upregulate *proHJ* transcription, suggesting that the osmo-specific proline biosynthesis pathway could be also involved in protection mechanisms during antibiotic exposure and ROS formation.

P74

Synthetic Biology for the Built Environment: A Pressure Sensing and Responsive *Bacillus subtilis*

Pollyanna E.J. Moreland, Mingpei Li, Martyn Dade-Robertson, and Anil Wipat

Newcastle University, Newcastle upon Tyne, UK

Hydrostatic pressure is an important stimulus to which bacteria react in various ways; simply put, a change is sensed and response coordinated. Very little is known about how microorganisms respond to high pressure and so pressure remains one of the few stimuli left unexploited in synthetic biology systems.

The Thinking-Soils project aims to design engineered bacteria to respond to a change in pressure by inducing bio-mineral crystal formation. A proof of concept project has been previously demonstrated using hydrogels to mimic a soil environment . In this system the formation of calcium carbonate crystals increases the strength of the hydrogel matrix and resists the pressure change (Christgen et al., 2019, unpublished). The ability to control bio-mineralisation to only occur when an increase in pressure is sensed presents a potentially useful biomaterial for the prevention of subsidence, or the construction of large-scale structures which would otherwise be impossible.

To achieve this goal, we must first increase our understanding of the genetics underpinning the bacterial response to pressure. With *Bacillus subtilis* as our target organism, we are using both a transcriptomic and synthetic biology approach to identify gene promoters which exclusively respond to pressure. RNASeq has been carried out on Bacillus subtilis 168 grown in pressure chambers. A novel machine learning approach has been developed to identify sets of genes whose expression is unique to the pressure conditions used. Synthetic promoters are also being screened for those whose expression is regulated specifically by pressure stress. These promoters will then be used to control the regulation of bio-mineralisation genes to result in a pressure sensing and responsive *B. subtilis*. This project aims to demonstrate this principle by creating a novel, responsive, living material.

P75

Bacterial Sterilization Mechanism by Citrate

Jun-ya Yamamoto[1], Yoshikazu Kawai[2], Ken-ichi Yoshida[1], and Shu Ishikawa[1]

[1] *Graduate School of Science, Technology and Innovation, Kobe University*
[2] *Centre for Bacterial Cell Biology, Institute for Cell and Molecular Biosciences, Medical School, Newcastle University*

It is known that citrate has strong activity to some pathogenic bacteria, and thus is used as an antimicrobial agent for catheter locking, and preservative in foods and beverage; however, its detailed mechanism has not been well understood. Here, we tried to elucidate how citrate kills bacteria, by use of Bacillus subtilis as model bacteria. In spontaneous citrate-tolerant mutants which proliferated even in the presence of lethal concentration of citrate, one of the strains had single mutation in PonA (PonA*) that is a class A Penicillin-binding protein and plays a major role in cell wall assembly. On the other hand, interestingly, a Δ*ponA* mutant was more citrate-sensitive than wild-type. Observation of morphological change in the presence of lethal citrate suggested that citrate would chelate Mg^{2+}, resulting in blockage of cell wall synthesis. It has been reported that blocking of cell wall synthesis stimulates glycolytic flux, leading to increased generation of reactive oxygen species (ROS) as a by-product of the metabolism of molecular oxygen in the respiratory chain pathway. Thus, we hypothesized that the lethal effect would be resulted from ROS generated in the same way. Based on this hypothesis, we expected that PonA* may have higher cell wall assemble activity to compensate the negative effect caused by chelation of Mg^{2+} whilst lowered assemble activity in the Δ*ponA* mutant may be further inhibited by the Mg^{2+} depletion. Consistent with our model, reduction of glycolysis activity by inhibition of sugar uptake and suppression of ROS damage restored cell growth even at lethal citrate level.

P76

Investigation of a physiological role of the ferredoxin-NADP$^+$ oxidoreductase paralog found in *Bacillus subtilis*

Daisuke Seo[1], Hirofumi Komori[2], and Kazuhito Inoue[3]

[1] *Kanazawa University, Kanazawa, Japan,* [2]*Kagawa University, Takamatsu, Japan,* [3]*Kanagawa University, Hiratsuka, Japan*

In *Bacillus subtilis* genome, one genuine ferredoxin-NADP$^+$ oxidoreductase (FNR) gene *yumC* and one FNR paralog *ycgT* have been annotated. Although the amino acid sequence identity between YcgT and YumC is rather high (~ 0.5), several residues indispensable for the FNR activity are substituted in YcgT. Our previous works on recombinant YcgT revealed that FNR activity of YcgT was significantly lower than YumC suggesting YcgT plays another physiological role in *B. subtilis* cells. In this presentation we report a potent physiological role of YcgT examined by biochemical approaches using purified YcgT protein.

Photoreducibility of YcgT was decreased when phosphate buffer was utilized in place of HEPES-NaOH buffer. Addition of HEPES-NaOH buffer as well as EDTA increased the photoreducibility indicating some amine compounds can act as an electron donor to the photoexcited YcgT. On INRA Expression data browser, the expression of the *ycgT* gene is reported to be enhanced under the iron limiting conditions. In other bacteria such as *E. coli*, FNR has been reported to participate in the reduction of Fe^{3+} bound to the siderophore molecules. Based on these information, we assessed the Fe^{3+} reduction activity bound to siderophore molecules. Obtained results indicated that YcgT utilizes 2,3-dihydroxybenzoate-glycine(DHB-Gly) as an electron donor and photoreduces Fe^{3+} bound to DHB-Gly, suggesting YcgT can function as a Fe^{3+} reductase utilizing siderophores as an electron donor.

P77

Effect of Galactolipids from *Arabidopsis thaliana* on activities of ECF sigmas in *Bacillus subtilis ugtP* mutant

Manami Kawakami, and Satoshi Matsuoka

Saitama University, Saitama, Japan

Glucolipids in *Bacillus subtilis* are synthesized by UgtP, processively transferring glucose from UDP-Glc to diacylglycerol (DG). Consequently, three spices of glucolipids, monoglucosyldiacylglycerol (MGlcDG), diglucosyldiacylglycerol (DGlcDG), and TGlcDG are produced in *B. subtilis*. Glucose moiety of these glucolipids is beta configuration. A *ugtP* mutant, which lacks glucolipids, shows abnormal morphology and activation of ECF sigmas(*sigM*, *sigX*, *sigV*). Conversely the expression of MGlcDG synthase from *Acholeplasma laidlawii* almost completely suppressed these phenotypes, therefore glucolipid molecules are considered to be important for maintenance of cell shape and regulation of ECF sigmas.

In this study, we focused on slight differences of the structure of glycolipids to clarify the correlation between structures and functions of glycolipids based on activity levels of ECF sigmas. Chroloplasts contain monogalactosyldiacylglycerol (MGalDG) and digalactosyldiacylglycerol (DGalDG). *MGD1* and *DGD2* involves in the synthesis of galactolipids in *Arabidopsis thaliana*. Galactose moiety of MGalDG and DGalDG is beta configuration, and these are C4 epimer of glucose of glucolipids produced by UgtP. We introduced *MGD1* into the *ugtP* mutant. Activation of *sigX* was supressed by the expression of *MGD1*, while the activity of *sigM* was unchanged. We are now analysing the effect of DGalDG on ECF sigmas activity of *ugtP* mutant, and further analysis should reveal the effect of galactolipids on cell membranes of *B. subtilis*.

P78

Comprehensive evaluation of overall injury of stressed *Bacillus subtilis* spores by the double subculture method

Asada, R.[1, 2], H. Den[1], J. Sakamoto[2], R. Kuwana[3], H. Takamatsu[3], T. Tsuchido[2], and M. Furuta[1 2]

1 Graduate School of Engineering, Osaka Prefecture University, Sakai, JAPAN

2 Research Center of Microorganism Control, Organization of Research Promotion, Osaka Prefecture University, Sakai, JAPAN

3 Faculty of Pharmaceutical Sciences Setsunan University, Hirakata, JAPAN

Bacterial spores survived sterilization process in the fields of food and medical industries may be generally sublethally injured and recover during the post-processing storage. However, the evaluation method for those injured spores seems to have not been reported. In the present study, we proposed an evaluation method for overall injury in heated and gamma-irradiated spores, including damages to germination and the subsequent development systems. This method is based on the double subculture (DS) technique, proposed previously in this laboratory, in which the injured population is estimated from the differential survival rate of between CFU by plate count method and the integrated viability by the growth delay analysis method. We applied this DS method to heat-treated and gamma ray-irradiated spores of *Bacillus subtilis* 168 wild-type and its mutant lacking small acid-soluble protein structural genes (*sspA*, *sspB*). As a result, for heated spores, the relative size of injured population was larger in the wild type strain than in the mutant, whereas the reverse situations was obtained for gamma ray-irradiated spores. The relationship between the generation of injured spores and spore death is discussed in relation to the difference in the possible intrasporal targets of both physical treatments.

P79

Metabolism and function of Magic spot III (pGpp) in *Bacillus subtilis*

Yuzuru Tozawa[1], Yuhta Nomura[2], and Dominik Rejman[3]

[1]Saitama University, Saitama, Japan, [2]RIKEN, Wako, Japan, [3] Institute of Organic Chemistry and Biochemistry CAS v.v.i., Prague, Czech Republic

It has been previously demonstrated that *Bacillus subtilis* accumulates guanosine 3'-diphosphate 5'-monophosphate (pGpp) upon stringent response together with guanosine penta- and tetra-phosphate, (p)ppGpp. We observed profound accumulation of a unique nucleotide, which was assumed to be pGpp or ppGp, in *B. subtilis* upon alkaline response. Genetic analysis confirmed that the alkaline-induced accumulation of the nucleotide depends on the activity of (p)ppGpp synthetase, RelA. In order to clarify the nucleotide, we synthesized both pGpp and ppGp, and used each nucleotide as an external standard for HPLC analysis. We have confirmed that the accumulated nucleotide is pGpp but not ppGp upon the alkaline shock. Further, we observed that Gram-positive soil bacterium, *Corynebacterium glutamicum*, likewise accumulates significant amount of pGpp in the cell upon alkaline shock. Based on biochemical analysis, we revealed that pGpp has potent inhibitory effect on the activity of *B. subtilis* guanylate kinase (GK) as well as ppGpp. On the other hand, ppGp was not recognized as a substrate by GK. We also demonstrated that pGpp is hydrolyzed by the Mn^{2+}-dependent pyrophosphatase activity of RelA, resulting in GMP, whereas ppGp is not affected by the hydrolase activity in vitro. Our study thus revealed the metabolism and function of pGpp and its significant roles in stress responses of Gram-positive bacteria.

P80

Phase variation-derived functional differentiation in biofilm of *Lactobacillus plantarum*

Kaori Shibusawa[1], Yuka Ehashi[1],Kimihiro Abe[4],Nozomu Obana[2,3], and Nobuhiko Nomura[3,4]

[1]Sch. Life Environ. Sci., Univ. Tsukuba, [2]Fac. TMRC, Medicine, Univ. Tsukuba, [3]MiCS, Univ. Tsukuba, [4]Fac. Life Environ. Sci., Univ. Tsukuba, Tsukuba, Japan.

In a natural environment, bacteria survive in biofilm, a bacterial multicellular community, resulting in higher tolerant to various stresses. *Lactobacillus plantarum* is a lactic acid bacterium (LAB) that has a higher tolerance to ethanol and organic acid stresses. Biofilms formed by LAB is one of the causative agents of food spoilage. Thus, understanding the mechanism of stress tolerance through the biofilm formation is fundamental to prevent food spoilage by these bacteria.

We found that two types of the colony with different morphologies, compact colony (Cc) and mucoid colony (Mc), emerge from environmental isolates of *L. plantarum*. Phase variation of the capsular polysaccharide (CPS) gene controls the colony morphology. Here, we show *L. plantarum* form the mixed biofilm of Cc and Mc that contributes to increasing the stress tolerance.

We compared the stress tolerance of Cc, and Mc and found that Mc has a higher tolerance to acetic acid. In Cc-Mc mixed biofilm, Cc showed a higher stress tolerance to acetic acid than that in monoculture biofilm. We observed the biofilm structure of Cc and Mc biofilm by CLSM. Cc cells predominantly attached to the surface and densely packed in the biofilm. Meanwhile, the cell density in the Mc biofilm was lower than the Cc biofilm. This suggests that Cc cells located in the bottom is covered with Mc cells in the mixed biofilm, which protects Cc from the environmental stresses in the mixed biofilm. We presume that the phase variation of CPS preserves the cells with different properties in the biofilm and serves as a survival strategy in actual environments where the cells are exposed to various stresses.

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